ARTICLES

Impact of *Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam. on Leaf Physiological Parameters in Pedunculate Oak (*Quercus robur* L.) Saplings

PREDRAG PAP[1], SRĐAN STOJNIĆ[1], NATAŠA NIKOLIĆ[2], SAŠA ORLOVIĆ[1], MIROSLAV MARKOVIĆ[1], VERICA VASIĆ[1] AND MIRJANA STEVANOV[1]

[1] *Institute of Lowland Forestry and Environment, University of Novi Sad, Antona Čehova 13, 21000 Novi Sad, R Serbia*

[2] *Faculty of Agriculture, University of Novi Sad, Dositeja Obradovića square 8, 21000 Novi Sad, R Serbia*

Corresponding author: pedjapap@uns.ac.rs

Pap, P., Stoinić, S., Nikolić, N., Orlović, S., Marković, M., Vasić, V. and Stevanov, M. 2014. Impact of *Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam. on Leaf Physiological Parameters in Pedunculate Oak (*Quercus robur* L.) Saplings. *Baltic Forestry* 20(1): 2–9.

Abstract

The effect of oak powdery mildew (*Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam.) on the most important plant physiological processes, photosynthesis, transpiration, and stomatal conductance, was studied on pedunculate oak saplings, depending on leaf age and fungal infestation intensity. The intensity of net photosynthesis declined progressively with mycelial development on the leaf surface, regardless of leaf age. The reaction to the presence of pathogenic fungi was more intensive on the young leaves. Net photosynthesis was practically interrupted (0.02 μmol m^{-2} s^{-1}) under the highest degree of young leaf infection (>75%). Powdery mildew exerted a moderate effect on transpiration rate, and a decreasing trend was observed with an increase in leaf infection. The effect of powdery mildew on the leaf stomatal conductance was not significant, and the values were, on average, higher in older leaves. The concentration of photosynthetic pigments decreased linearly with an increase in leaf infection regardless of the age. At the same time, the leaf dry matter content increased with higher leaf infection and, on average, it was higher in older leaves. The results of the study point to the adverse effect of powdery mildew on net photosynthesis and other physiological processes in pedunculate oak leaves, allowing them to be used as an important indicator of plant vitality and vulnerability.

Key words: oak powdery mildew, pedunculate oak saplings, net photosynthesis, transpiration, stomatal conductance

Introduction

Oak powdery mildew (*Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam.) has been a chronic disease throughout the oak forest range in Europe for more than a century. This obligate plant pathogenic fungus is most harmful to juvenile plants in the early ontogenetic development stages (Pap 2012, Pap et al. 2012). Young leaves and shoots are especially vulnerable, so under a high infection level, the fungus causes deformations and retards the development. In older stands, only in the cases of defoliation, the fungus becomes an important causal factor which diminishes the vitality of oak forests (Thomas et al. 2002, Marçais and Breda 2006). In his study on physiological processes in trees damaged by defoliators and powdery mildew, Ierusalimov (1979) found that during leaf regeneration, respiration was intensified and the consumption of stored nutrient supplies was increased. Respiration returns to normal two years after defoliation and leaf regeneration. In addition to leaf damage, powdery mildew can lower the increment and lead to all accompanying adverse consequences. The experience of forestry experts and the research undertaken on pedunculate oak regeneration areas emphasise that oak seedlings and saplings are at the highest risk of powdery mildew (Novak-Agbaba and Liović 1998, Glavaš 1999, Bobinac 1999, 2000, Liović and Županić 2006, Liović 2011, Pap 2012, Pap et al. 2012). The impact of powdery mildew on the development and survival of oak seedlings and saplings in the process of forest regeneration and the justification of investments in pedunculate oak protection have been dealt with in only a small number of studies (Soutrenon 1998, 2003, Liović 2011, Pap 2012, Pap et al. 2012). The above authors tried to calculate, as accurately as possible, the values of the effect of powdery mildew on height and diameter increment and plant survival in different production and ecological units of pedunculate oak stands. Their results suggested that the best results in seedling protection can be obtained by minimal application of fungicides during the early development the seedlings whereas application in later development stages is biologically and economically questionable.

Plant increment (diameter and height), as an indicator adverse effects of powdery mildew, is often insufficiently reliable, because it depends on numerous external and internal factors (presence or absence of nutrients and water in the soil, availability of space and light, effect of weeds, etc.). The above factors can have both negative and positive effects on the host plant, and in this way mask the actual effect of powdery mildew. Therefore, it is very important to study and correlate the fungus and its effect on major physiological processes (photosynthesis, transpiration, stomatal conductance). The level of the harmful effects of the pathogen can be evaluated and explained based on the degree of disturbance of physiological processes. These parameters are important indicators of the plant physiological condition on the one hand, and the effect of the pathogen on the other hand. Even the slightest change in any of the analysed parameters can lower, and in extreme cases discontinue, the plant life processes.

The effects of pathogens on plant physiological processes have been studied by numerous authors to date (Ploetz and Schaffer 1989, Bowden and Rouse 1991, Niederleitner and Knoppik 1997, Lopes and Berger 2001, Bassanezi et al. 2002, Moriondo et al. 2005, Aldea et al. 2006, Desprez-Loustau et al. 2006). Depending on the investigated plant species and pathogen, it is reported that plant infection leads to a decrease in the yield and biomass of individual organs, reduced chlorophyll fluorescence, concentration of photosynthetic pigments, and the reduction in overall physiological activity of infested plants. The changes in physiological processes of plants infected with powdery mildew have been studied by a relatively small number of authors (Hewitt and Ayres 1975, 1976, Brüggemann and Schnitzler 2001, Hajji et al. 2009, Liović 2011, Marçais and Desprez-Loustau 2012). All of them emphasise that the assimilation intensity decreases with the advanced leaf infection, but their opinions differ regarding other physiological parameters (respiration, transpiration, stomatal conductance), which is primarily the consequence of different methodological procedures and applied equipments. In the above studies, the effect of leaf age on the changes in some physiological parameters has not been investigated in detail, however in our studies this factor is of decisive significance. Regardless of different results obtained by these studies, the main advantage of this kind of research lies in the fact that it could provide an insight into the mechanisms involved in the pathogenesis at the level of different organs. The results of investigations on the impact of pathogens on leaf gas exchange could be used in further studies, as a nondestructive means in determination of incidence and/or severity of infection by *E. alphitoides.*

This study deals with the effect of oak powdery mildew on basic plant functions: net photosynthesis, transpiration, stomatal conductance, content of chloroplast pigments. The variability of the studied parameters was measured on pedunculate oak saplings depending on leaf age and infestation intensity. Because the leaf physiological parameters are dependent on the level of infection, the results obtained on infected leaves were compared with the results of measurements on healthy leaves in order to quantify the impact of disease on investigated parameters. Also, because oak plants were sampled randomly in the field, the significance of *E. alphitoides* was analysed independently of other biotic and abiotic factors.

Material and Methods

Net photosynthesis, transpiration, stomatal conductance

Net photosynthesis, transpiration and stomatal conductance were measured using portable equipment ADC Bioscientific Ltd. LCpro+ with the system for gas exchange monitoring in plants. The physiological parameters were measured on pedunculate oak saplings in the first half of July 2009 (Forest Administration Morović, loc. Blata, Compartment 6). Pedunculate oak saplings developed under the protection of crop trees (after regeneration felling, 50–55 pedunculate oak and narrow-leaved ash trees were left per hectare on the regeneration area). The analysed leaves belonged to two age classes: young, half expanded leaves 7–10 days old, and fully expanded leaves aged 20 days or more. The selected leaves were under different degrees of fungal infection, and based on their coverage with epiphytic mycelia, they were classified into 6 groups: (1) uninfected leaves (0%), (2) mildly infected leaves (< 10%), (3) moderately infected leaves (10-30%), (4) moderately severely infected leaves (30-50%), (5) severely infected leaves (50-75%) and (6) very severely infected leaves (> 75%) (Pap 2012, Pap et al. 2012).

The leaves, which were not separated from the plants, were measured in sunny and clear weather between 07:00 – 09:00 a.m. Each measurement included 25 leaves from the selected plants in three replicates (25 leaves × 3 replicates × leaf age × infestation intensity). Each leaf was carefully placed in the chamber of internal dimensions 6 cm² (2 × 3 cm). Measurements were made under constant light conditions (PAR 1,000 µmol m^{-2} s^{-1}) and CO_2 concentration (350 µmol mol^{-1}), and the temperature (25°C) and relative air humidity (80%) were measured during the experiments. The intensity of photosynthesis, transpiration, and stomatal conductance was calculated based on gas exchange and other determined parameters.

Concentration of photosynthetic pigments

The concentration of photosynthetic pigments was measured on the leaves taken from oak seedlings in late June 2009 in the area of Forest Administration Klenak, loc. Grabovačko-Vitojevačko Ostrvo, Compartment 86. The plants including the roots were pulled out of the ground and brought to the laboratory of the Department of Biology and Ecology – Novi Sad. The leaf ages and the degrees of infection were the same as in the previous experiment. The contents of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids (Car) were determined according to Wettstein method (1957) in acetone solution. Leaf samples were finely cut and then homogenised with a pestle, with the addition of 5-10 ml acetone. Pigment concentration was determined in acetone extract using BECKMAN spectrophotometer: the absorbances were measured at the appropriate wavelengths (chlorophyll a – 662 nm, chlorophyll b – 644 nm, carotenoids – 440 nm) and expressed in mg g^{-1} of dry matter.

Content of dry matter

Plant material was dried at 80°C to constant mass, and the dry matter percentage (Dm) was calculated using the formula:

% moisture = $(m - n) \times 100/\mathrm{m}$,

where: m (g) is the mass before drying, and n (g) is the mass after drying.

% dry matter = 100-% moisture

The data in all experiments were processed using the analysis of variance and the differences in significance between variants were evaluated using Duncan's test at $p = 0.05$ significance level (StatSoft Inc. STATISTICA). The estimation of statistical importance of different sources ("leaf age", "infestation intensity" and interaction "leaf age × infestation intensity") was conducted by Two-way ANOVA procedure. Data regarding content of dry matter was subjected to the *arcsine* transformation for statistical analysis, but actual percentages are given in the tables and figures. Linear regression analysis was applied in order to examine the relationship between infestation intensity and investigated physiological parameters. Probability value of 0.05 was used as the benchmark of significance.

Results

Based on two-way ANOVA, it is observed that impact of oak mildew changed with leaf age and infestation intensity (Table 1). Also, interaction "leaf age × infestation intensity" was statistically significant for all investigated leaf parameters. The rate of net photosynthesis declined progressively with mycelium development on the leaf surface, regardless of leaf age. A noticeable decrease in net photosynthesis was especially substantial in the leaves infected above 30%. However, new leaves were more susceptible and more threatened by fungal presence and nourishment than older leaves (Figure 1). Net photosynthesis was decreased approximately 85% on the young leaves infected between 50–75% and was practically interrupted on the leaves infected above 75% (Table 2). A decrease in net photosynthesis also occurred in older leaves, but to a lower degree. On the leaves infected above 75%, net photosynthesis decreased for about 70%. Nevertheless, it is evident that net photosynthesis was considerably higher in older leaves under the same intensities of leaf infection (Table 2; Figure 1). The effect of powdery mildew on transpiration rate was moderate. A trend of mild decrease in transpiration occurred in parallel with an increase in mycelium area. In young leaves, significant decrease in transpiration rate was observed only in most severely infected leaves (> 75%) (Table 2). Similarly, there were no major changes in transpiration rate of older leaves, although the differences between some categories of leaf infection were significant. The effect of powdery mildew on stomatal conductance had similar pattern as in the case of transpiration rate. On average, stomatal conductance was lower in young leaves, even though the differences were not significant, except in the case when this parameter was compared for the leaves with the highest rate of infection (Table 2). In older leaves, the differences were significant, with the clear decreasing trend of the observed values (Figure 1). The concentration of chlorophyll a, chlorophyll b, total chlorophyll (a + b) and carotenoids decreased linearly with an increase in the degree of infestation intensity, in the leaves of both ages (Figure 1). Concentration of photosynthetic pigments decreased approximately by 70% in the young leaves infected above 75%, compared to the 56% recorded on older leaves. An in-depth analysis of data showed that the concentration of photosynthetic pigments was higher in older leaves

(Table 2). Opposite to investigated physiological parameters, the content of dry matter increased with an increase in leaf infection, regardless of the leaf age (Tables 2; Figure 1). However, this increase was significant only in the case of leaves aged 7–10 days ($r^2 = 0.85$; $p < 0.01$).

Table 1. Two-way ANOVA for observed leaf parameters depending on leaf age and *E. alphitoides* infestation intensity

Source of variation	*df*	*F* values							
		A[1]	E	gs	Chl a	Chl b	Chl a+b	Car	Dm
Leaf age	1	6066.4 ***	4.0 *	56.5 ***	255.5 ***	16.8***	186.9 ***	47.9 ***	2459.4 ***
Infestation intensity	5	813.2 ***	18.9 ***	11.6 ***	84.5 ***	17.8***	73.8 ***	41.4 ***	302.0 ***
Leaf age x Inf. intensity	5	106.3 ***	6.1 ***	16.1 ***	11.3 ***	8.4***	12.8 ***	4.4 **	167.3 ***

Level of signifficance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

[1] - The parameter acronyms are defined in Material and Methods

Table 2. Median (m_e) and standard deviation (*SD)* of observed leaf parameters depending on leaf age and *E. alphitoides* infestation intensity

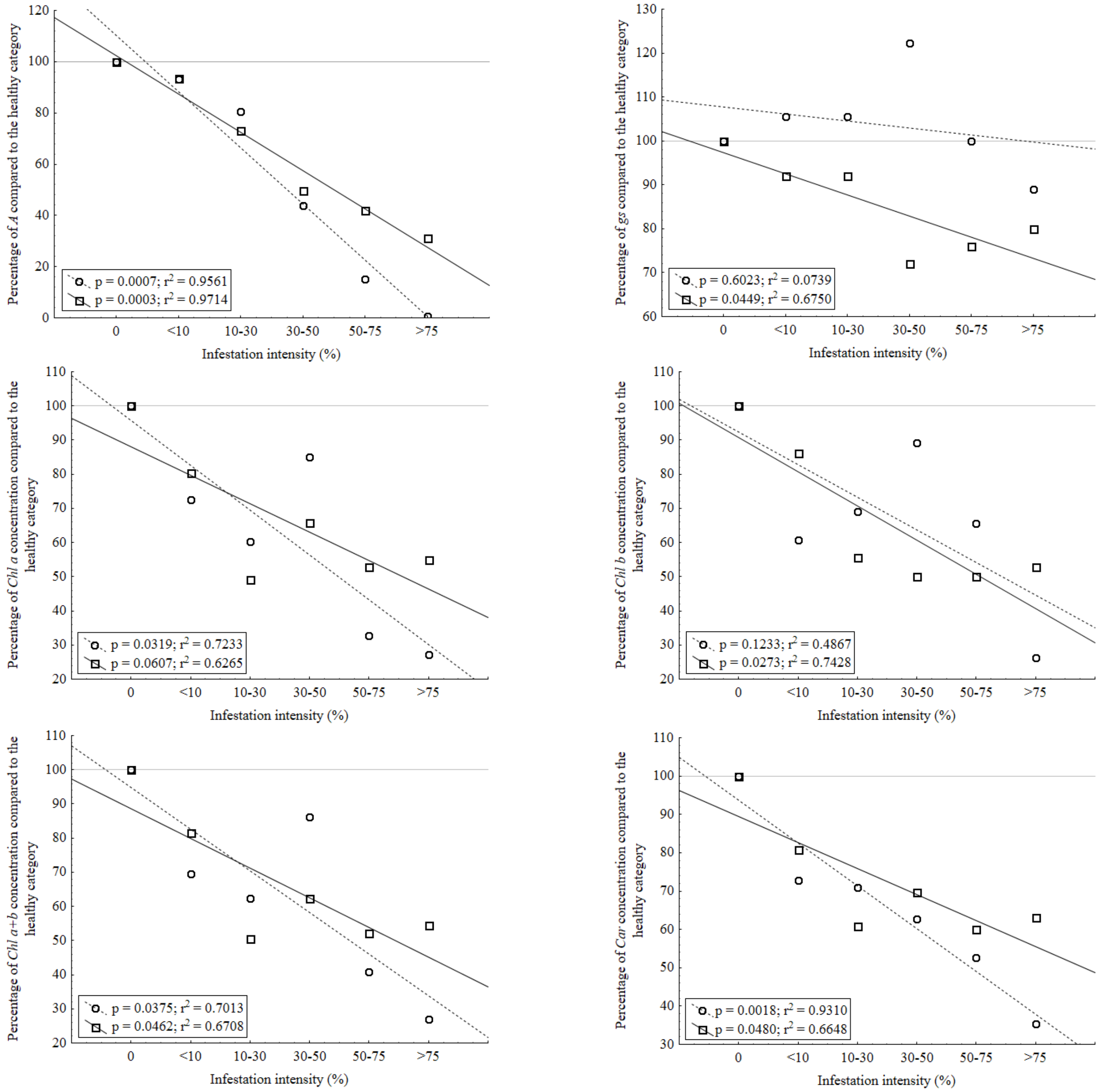
Infestation intensity (%)	A[1] (μmol m^{-2} s^{-1})		E (mmol m^{-2} s^{-})		gs (mol m^{-2} s^{-1})		Chl a (mg g^{-1})		Chl b (mg g^{-1})		Chl a+b (mg g^{-1})		Car (mg g^{-1})		Dm (%)	
	m_e	SD	m_e	SD	m_e	SD	m_e	SD	m_e	SD	m_e	SD	m_e	SD	m_e	SD
Leaves aged 7 – 10 days																
0	2.63^{a}	0.28	4.10^{a}	0.16	0.18^{ab}	0.03	$2.53^{\bullet}$	0.13	0.88^{a}	0.14	3.41^{a}	0.25	1.12^{a}	0.08	28.2^{e}	0.66
<10	2.45^{a}	0.18	4.00^{a}	0.24	0.18^{ab}	0.02	1.81^{bc}	0.32	0.52^{bc}	0.18	2.33^{bc}	0.48	0.76^{ab}	0.20	32.5^{d}	0.74
10 – 30	2.19^{b}	0.18	4.21^{a}	0.24	0.19^{ab}	0.03	1.49^{c}	0.51	0.55^{ab}	0.24	2.01^{c}	0.75	0.80^{ab}	0.20	37.5^{b}	1.65
30 – 50	1.10^{c}	0.15	4.23^{a}	0.28	0.21^{a}	0.03	2.17^{ab}	0.31	0.75^{ab}	0.08	2.92^{ab}	0.35	0.67^{bc}	0.17	35.8^{c}	1.02
50 – 75	0.37^{d}	0.08	4.15^{a}	0.19	0.18^{ab}	0.02	0.81^{d}	0.24	0.56^{ab}	0.12	1.42^{d}	0.22	0.59^{bc}	0.11	42.7^{a}	1.01
>75	0.02^{d}	0.02	3.68^{b}	0.15	0.16^{b}	0.01	0.65^{d}	0.10	0.20^{c}	0.08	0.92^{d}	0.07	0.39^{c}	0.12	41.7^{a}	0.11
Leaves aged > 20 days																
0	8.18^{a}	0.36	4.25^{a}	0.26	0.25^{a}	0.01	3.81^{a}	0.47	1.08^{a}	0.25	4.97^{a}	0.35	1.23^{a}	0.14	43.1^{cd}	0.51
<10	7.67^{b}	0.55	4.14^{ab}	0.19	0.23^{ab}	0.02	3.20^{b}	0.28	0.88^{ab}	0.25	4.06^{ab}	0.32	0.97^{b}	0.15	44.6^{b}	0.12
10 – 30	5.97^{c}	0.36	4.33^{ab}	0.24	0.23^{ab}	0.01	1.90^{c}	0.26	0.52^{bc}	0.23	2.39^{c}	0.48	0.76^{c}	0.10	42.8^{d}	0.45
30 – 50	4.03^{d}	0.21	3.75^{cd}	0.24	0.18^{c}	0.03	2.25^{bc}	0.39	0.44^{c}	0.21	2.69^{c}	0.59	0.82^{bc}	0.15	41.6^{e}	0.19
50 – 75	3.42^{e}	0.17	3.93^{bc}	0.17	0.19^{c}	0.01	1.97^{c}	0.25	0.55^{c}	0.19	2.45^{c}	0.41	0.72^{c}	0.08	44.2^{bc}	0.79
>75	2.49^{f}	0.18	3.69^{d}	0.13	0.20^{bc}	0.01	2.15^{c}	0.32	0.59^{bc}	0.14	2.62^{c}	0.43	0.80^{c}	0.07	47.3^{a}	0.86

[1] The parameter acronyms are defined in Material and Methods

Generally, the results of linear regression showed that most of the investigated parameters were significantly affected by infestation intensity. In order to shed a different light on the results, we correlated different degrees of fungal infection with relative values of observed leaf parameters, represented through the percentages compared to the values of healthy category (Figure 1). Statistically significant decreases in net photosynthesis and stomatal conductance, as well as significant decreases in the concentration of chlorophyll a + b and carotenoids, were recorded in the leaves of both ages. The relationship between infestation intensity and transpiration rate was not statistically significant, neither in young nor in old leaves and therefore was not presented in Figure 1 (see p. 6).

Discussion

The results of our study showed that increase in leaf infection caused by *Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam. led to disturbances in leaf gas exchange, concentration in photosynthetic pigments and leaf dry matter in *Quercus robur* L. saplings. These disturbances were manifested through the declining in net photosynthesis, transpiration rate and stomatal conductance, as well as concentration of photosynthetic pigments with increasing of infestation intensity. Also, water loss from the leaves, caused by water uptake by the fungus, led to an increase in the content of leaf dry matter. Further, our results indicate that the reaction of young leaves colonised by powdery mildew was more intensive (i.e. distinct reduction in leaf gas exchange and pigment content) than it is case in older leaves. This is probably related to leaf ontogenic development, because during the development, older leaves store larger amounts of photosynthetic products, so physiological processes are more intensive. Shirke (2001) stated that, during leaf development, stomatal conductance, net photosynthe-



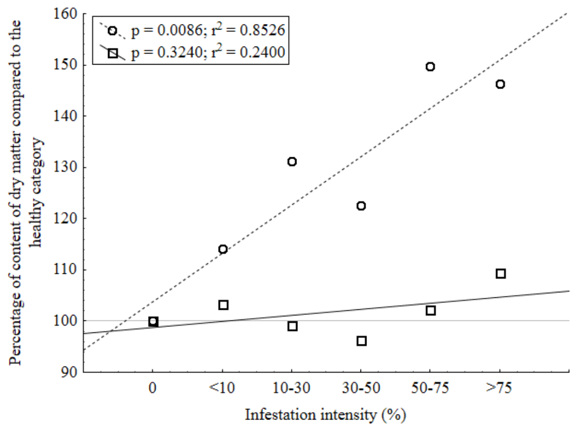


Figure 1. Impact of infestation intensity (%) (x axis) on investigated leaf parameters expressed in percentage compared to the healthy category (y axis), in the leaves aged 7–10 days (open square, dotted lines) and the leaves aged >20 days (closed squares, solid line)

sis and pigment content increase with leaf expansion and are related to efficiencies of radiation energy utilisation, electron transport chain and photophosphorylation. Also, it can be noted that the content of dry matter in older leaves was on average higher, probably as a result of the higher percentage of mechanical tissues in older leaves. Pap (2012) stated that the seedlings are under the highest risk of powdery mildew during the onset of new shoots with young leaves, but it is very rarely, only in extremely unfavourable conditions, that the fungus can destroy the new leaves on secondary shoots. Even in the case of complete coverage of the assimilation apparatus with the mycelium, the leaves are not killed, and over time, the presence of powdery mildew infection is significantly eliminated (Pap 2012, Pap et al. 2012). Presumably, this is the case of compensation effects reported by Hewitt and Ayres (1976). Previous studies also indicate that, in addition to good light conditions, normal physiological processes also depend on sufficient moisture and nutrients for the roots in the soil. Intensive physiological processes generate a faster ontogenetic development, and in this way reduce the powdery mildew pressure and fungus-induced negative consequences (Pap 2012, Pap et al. 2012).

Our results showed that net photosynthesis decreased for about 56% in younger and 50% in older leaves under the moderately severe infestation (30-50%). Net photosynthesis was almost interrupted in young leaves infected over 75%, while in the older leaves it declined for approximately 70%. Study conducted by Liović (2011) showed that net photosynthesis in non-infected oak leaves was on average 40% higher compared to infected leaves. Similar results were reported by Hewitt and Ayres (1975), Brüggemann and Schnitzler (2001) and Hajji et al. (2009), who found that presence of oak powdery mildew on leaves caused a milder impact on net CO_2 assimilation under moderately severe infestation (50%), while the higher infection levels (85-100%) reduced photosynthesis by 40-50%. The differences in the values of net photosynthesis in our experiments were primarily the consequence of different leaf ages, i.e. different responses of young and old leaves to the presence of pathogens. The same authors did not succeed in proving that the decrease in photosynthesis in infected leaves of one-year-old pedunculate oak seedlings was compensated by an increase in photosynthesis in healthy leaves on the same plant, as detected by Mayr et al. (2001) on spruce needles infested by *Chrysomyxa rhododendri*. Hewitt and Ayres (1976) reported that infected leaves translocated a lower amount of photosynthetic products than did healthy leaves, but infected leaves took up a greater part of photosynthetic products from healthy leaves on the same plant, than did healthy leaves on healthy plants. As a result, in mildewed leaves, the import of photosynthetic products increases, and the export of photosynthetic products decreases. According to the above mentioned authors, healthy leaves provide a compensation effect because they create and translocate greater amounts of photosynthetic products to the infected leaves.

The effect of oak powdery mildew on transpiration rate has been studied by few authors worldwide. Contrary to our results, Hewitt and Ayres (1975) found that transpiration rate increased from the second day after inoculation to the end of the seven-day experiment when the differences between healthy and infected leaves were the highest, both in the light period, and in the dark period. Hajji et al. (2009) showed that there was no difference in the total transpiration rate on severely infected seedlings compared to that in healthy plants. Our findings are similar, to some extent, to the previously mentioned results. In the leaves of both ages, transpiration rate slightly increased with the enhancement of infestation intensity, but that increase was not statistically significant. Mild reduction in transpiration rate was observed in young leaves infected over 50% and old leaves infected over 30%, respectively. However, in the case of leaves aged 7–10 days, statistically significant decrease was observed only in the most infected leaves (above 75%), compared to the completely healthy category.

Stomatal conductance is a parameter on which the fungus had the lowest effect, both in our research and according to other authors. Our results showed that stomatal conductance was significantly affected by infection only in the case of older leaves, even though decrease in stomatal conductance between completely healthy leaves and the most infected leaves occurred in the leaves of both ages. Hewitt and Ayres (1975) reported that fungus did not have any effect on stomatal conductance of young leaves until the sixth day after inoculation, when stomatal opening decreased in the light. However, according to Hajji et al. (2009), infection had a significant impact on stomatal conductance reducing it on average by 15–30% compared to that in healthy leaves. Similar to findings by Hajji et al. (2009), our results showed that the decrease in the rate of stomatal conductance in the leaves ranged between 12% (leaves 7–10 days old) and 20% (leaves older than 20 days).

Concentration of photosynthetic pigments in leaves was highly influenced by intensity of infestation, owing to the result that the expansion of leaf infection led to decreasing in pigment concentration. The results obtained by our study showed that infection by fungi led to the decrease in total chlorophyll concentration (chlorophyll a + b) in the leaves in between 46% (leaves older than 20 days) and 73% (leaves 7–10 days old). Also, linear regression showed statistically significant relationship between the concentration of photosynthetic pigments in the leaves and the incidence of leaf infection. Kuznetsova (1988) reported that chlorophyll content decreased linearly with an increase in the degree of leaf infection, while Brüggemann and Schnitzler (2001) found that a severe leaf infection was accompanied by a 50% decrease in assimilation and total chlorophyll content.

The leaf dry matter content seems to be an important indicator of plant productivity, due to its relationship with plant metabolism (Wilson et al. 1999, Domínguez et al. 2012). Along with dry matter content, parameters SLA (specific leaf area, fresh leaf area: dry mass ratio), or its reciprocal, LMA (leaf dry mass per area) are frequently used predictors of plant growth under certain environmental conditions. LMA is correlated with photosynthetic rate, since it indicates the investment of dry mass into light-intercepting leaf area (Hassiotou et al. 2010). Therefore, these parameters should be included in future studies of effects of the fungus infection on oak plant physiological processes, to define reliably the performance of plants under the fungus attack.

In conclusion, our results point to the occurrence of disturbances in leaf gas exchange, concentration in photosynthetic pigments and leaf dry matter in *Quercus robur* saplings, depending on leaf age and *E. alphitoides* infestation intensity. Also, the most harmful effect of powdery mildew occurred in the physiological processes of young leaves. In this respect, the results obtained by our study combined with previously acquired knowledge, could be applied as some of the criteria in the decisions on oak protection measures.

Acknowledgement

This paper was realized as a part of the project "Studying climate change and its influence on the environment: impacts, adaptation and mitigation" (43007) financed by the Ministry of Education and Science of the Republic of Serbia within the framework of integrated and interdisciplinary research for the period 2011–2014.

References

Aldea, M., Hamilton, J.G., Resti, J.P., Zangerl, A.R., Berenbaum, M.R., Frank, T.D. and DeLucia, E.H. 2006. Comparison of photosynthetic damage from arthropod herbivory and pathogen infection in understory hardwood saplings. *Oecologia* 149: 221-232.

Bassanezi, R.B., Amorim, L., Bergamin Filho, A. and Berger, R.D. 2002. Gas exchange and emission of chlorophyll fluorescence during the monocycle of rust, angular leaf spot and anthracnose on bean leaves as a function of their trophic characteristics. *Journal of Phytopathology* 150: 37-47.

Bobinac, M. 1999. Research of pedunculate oak (*Quercus robur* L.) natural regeneration and the selection of regeneration methods depending on site and stand conditions. PhD, University of Belgrade, Faculty of Forestry, Serbia, 262 pp.

Bobinac, M. 2000. Stand structure and natural regeneration of common oak in the nature reserves "Vratična" and "Smogva" near Morović. OAK 2000 – Improvement of wood quality and genetic diversity of oaks, *Glasnik za šumske pokuse* 37: 295-309.

Bowden, R.L. and Rouse, D.I. 1991. Effects of *Verticillium dahliae* on gas exchange of potato. *Phytopathology* 81: 293-301.

Brüggemann, N. and Schnitzler, J.P. 2001. Influence of powdery mildew (*Microsphaera alphitoides)* on isoprene biosynthesis and emission of pedunculate oak (*Quercus robur* L.) leaves. *Journal of Applied Botany* 75: 91-96.

Desprez-Loustau, M.L., Marçais, B., Nageleisen, L., Piou, D. and Vannini, A. 2006. Interactive effects of drought and pathogens in forest trees. *Annals of Forest Science* 63: 597-612.

Domínguez, M.T., Aponte, C., Pérez-Ramos, I.M., García, L.V., Villar, R. and Marańón, T. 2012. Relationships between leaf morphological traits, nutrient concentrations and isotopic signatures for Mediterranean woody plant species and communities. *Plant Soil* 357: 407-424.

Glavaš, M. 1999. Fungal Diseases of Forest Trees. University of Zagreb, Faculty of Forestry, p. 45-57.

Hajji, M., Dreyer, E. and Marçais, B. 2009. Impact of *Erysiphe alphitoides* on transpiration and photosynthesis in *Quercus robur* leaves. *European Journal of Plant Pathology* 125: 63.

Hassiotou, F., Renton, M., Ludwig, M., Evans, J.R. and Veneklaas, E.J. 2010. Photosynthesis at an extreme end of the leaf trait spectrum: how does it relate to high leaf dry mass per area and associated structural parameters? *Journal of Experimental Botany* 61: 3015-3028.

Hewitt, H.G. and Ayres, P.G. 1975. Changes in CO_2 and water vapour exchange rates in leaves of *Quercus robur* infected by *Microsphaera alphitoides* (powdery mildew). *Physiological Plant Pathology* 7: 127-137.

Hewitt, H.G. and Ayres, P.G. 1976. Effect of infection by *Microsphaera alphitoides* (powdery mildew) on carbohydrate levels and translocation in seedlings of *Quercus robur. New Phytologist* 77: 379-390.

Ierusalimov, E.N. 1979. The disruption of physiological processes in trees infested by defoliating insects. *Lesovedenie* 2: 62-71.

Kuznetsova, I.S. 1988. Effect of powdery mildew on the chlorophyll content and reflective ability of *Quercus robur* leaves. *Lesovedenie* 5: 63-67.

Liović, B and Županić, M. 2006. Testing of the effectiveness of fungicides for the control of fungus *Microsphaera alphitoides* (Griff. et Maubl.) on oak seedling plants. *Radovi Šumarskog Instituta* 9: 181-188.

Liović, B. 2011. The influence of powdery mildew (*Microsphaera alphitoides* Griff. et Maubl.) on growth and survival rate of oak seedlings. *Šumarski list* – Special issue, 122-129.

Lopes, D.B. and Berger, R.D. 2001. The effects of rust and anthracnose on the photosynthetic competence of diseased bean leaves. *Phytopathology* 91: 212-220.

Marçais, B. and Bréda, N. 2006. Role of an opportunistic pathogen in the decline of stressed oak trees. *Journal Ecology* 94: 1214-1223.

Marçais, B. and Desprez-Loustau, M.L. 2012. European oak powdery mildew: impact on trees, effects of environmental factors, and potential effects of climate change. *Annals of Forest Science.* DOI 10.1007/s13595-012-0252-x.

Mayr, S., Siller, C., Kriss, M., Oberhuber, W. and Bauer, H. 2001. Photosynthesis in rust-infected adult Norway spruce in the field. *New Phytologist* 151: 683-689.

Moriondo, M., Orlandini, S., Giuntoli, A. and Bindi, M. 2005. The effect of downy and powdery mildew on grapevine (*Vitis vinifera* L.) leaf gas exchange. *Journal of Phytopathology* 153: 350-357.

Niederleitner, S. and Knoppik, D. 1997. Effects of the cherry leaf spot pathogen *Blumeriella jaapii* on gas exchange before and after expression of symptoms on cherry leaves. *Physiological and Molecular Plant Pathology* 51: 145-153.

Novak-Agbaba, S. and Liović, B. 1998. Protection of oak new growth from fungi *Microsphaera alphitoides* (Griff. et Maubl.). *Radovi Šumarskog Instituta* 33: 83-93.

Pap, P., Ranković, B. and Maširević, S. 2012. Significance and need of powdery mildew control (*Microsphaera alphitoides* Griff. et Maubl.) in the process of regeneration of the pedunculate oak (*Quercus robur* L.) stands in the Ravni Srem area. *Periodicum Biologorum* 114: 91-102.

Pap, P. 2012. Oak powdery mildew (*Microsphaera alphitoides* Griff. et Maubl.) and its importance in the regeneration of common oak stands. PhD, University of Novi Sad, Faculty of Agriculture, Serbia, 70 pp.

Ploetz, R.C. and Schaffer, B. 1989. Effects of flooding and Phytophthora root rot on net gas exchange and growth of avocado. *Phytopathology* 79: 204-208.

Shirke, P.A. 2001. Leaf Photosynthesis, Dark Respiration and Fluorescence as Influenced by Leaf Age in an Evergreen Tree, *Prosopis Juliflora. Photosynthetica* 39: 305-311.

Soutrenon, A. 1998. Une expérimentation pluriannuelle confirme l'impact de l'oïdium du chêne sur de jeunes sujets [A pluriannual experiment confirms the impact of powdery mildew on young seedlings]. Les cahiers du DSF, 1-2000 (la santé des forêts [France] en 1997). Min. Agri. Pêche (DERF), Paris, 93-94 (in French)

Soutrenon, A. 2003. Évaluation de l'intérêt d'un traitement chimique contre l'oïdium du chêneen régénération naturelle. [Evaluation of the interest of a chemical treatment against oak powdery mildew in natural regeneration]. Document CEMAGREF, 19p (in French)

StatSoft Inc. STATISTICA 2011. (Data analysis software system), version 10. www.statsoft.com.

Thomas, F.M., Blank, R. and Hartmann, G. 2002. Abiotic and biotic factors and their interactions as causes of oak decline in Central Europe. *Forest Pathology* 32: 277-307.

Wettstein, D. 1957. Chlorophyll-letale und submikroskopische formwechsel der plastiden. *Experimental Cell Research* 12: 427-433.

Wilson P.J., Thompson, K. and Hodgson, J. G. 1999. Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. *New Phytologist* 143: 155-162.

Received 03 September 2013
Accepted 15 May 2014

ВЛИЯНИЕ МУЧНИСТОЙ РОСЫ ДУБА (*MICROSPHAERA ALPHITOIDES* GRIFF. ET MAUBL.) НА ФИЗИОЛОГИЧЕСКИЕ ПАРАМЕТРЫ ЛИСТЬЕВ МОЛОДОГО ДУБА

П. Пап, С. Стойнич, Н. Николич, С. Орлович, М. Маркович, В. Васич и М. Стеванов

Резюме

Влияние мучнистой росы дуба – (возбудитель сумчатый гриб *Microsphaera alphitoides* Griff. et Maubl), на главные физиологические процессы в растениях (фотосинтез, транспирация, биосинтез органических веществ), изучается на молодом дубе в зависимости од возраста листьев и интензитета нападения грибы. Интензитет нетто-фотосинтеза прогрессивно уменьшивался развитием мицелии на поверхности листьев, независимо от их возраста. Молодые листья более интенсивно отреагировали на присуствие патогенов. При самом сильном уровне заболевании, процесс фотосинтеза практически полностью прекращается (0.02 mmol m^{-2} s^{-1}). Мучнистая роса умеренно повлияла на транспирацию, и небольшая тенденция ее уменьшения замечена при большем заболевании листьев. Не замечено значительное влияние мучнистой росы на биосинтез органических веществ, и показатели данного параметра в среднем оказались большим у старших листьев. Содержание пигментов хлоропластов линейно снижался с увеличением зараженности листьев, независимо от их возраста. Одновременно, содержание сухого вещества увеличивалось сразмерно са ростом зараженности листьев, и в среднем оно было больше у старших листьев. Результаты показывают на отрицательное влияние мучнистой росы дуба на нетто фотосинтез и остальные физиологические процессы в листьях дуба и могут быть использованы в качестве важного показателя жизнеспособности и угрозы растений.

Ключевые слова: мучнистая роса дуба, молодой дуб, нетто фотосинтез, транспирация, биосинтез органических веществ