Arch Biol Sci. 2019;71(1):167-176 https://doi.org/10.2298/ABS180927058N

Physiological responses of pedunculate oak (*Quercus robur* L.) to *Corythucha arcuata* (Say, 1832) attack

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Received: September 27, 2018; **Revised**: November 30, 2018; **Accepted**: December 14, 2018; **Published online**: December 26, 2018

Abstract: The spread and occurrence of the oak lace bug *Corythucha arcuata* out of its natural distribution area across European and Asian countries has been reported during the past decades. The ecological and economic significance of oak stands and the vulnerability of plants to various abiotic and/or biotic factors requires in-depth knowledge of plant-pest interaction. The present study examined the influence of *C. arcuata* feeding on the photosynthetic characteristics and gas-exchange parameters, mineral nutrient concentrations and defense mechanisms (the activities of some antioxidant enzymes) of leaves of pedunculate oak. The rate of photosynthesis, transpiration and stomatal conductance were lowered by 58.84, 21.66 and 35.71%, respectively, in comparison to non-infested plants. The concentrations of photosynthetic pigments and activities of antioxidant enzymes, catalase and ascorbate peroxidase, were affected by the presence of *C. arcuata*. To our knowledge this is the first paper providing a report on the physiological responses of *Quercus robur* plants exposed to *C. arcuata* infestation. Understanding the impact of pests, such as the invasive species *C. arcuata* on physiological processes and vitality of young plants and plant responses, could provide a foundation for efficient preservation of oak forests endangered by the oak lace bug.

Keywords: mineral element concentrations; oak lace bug; oxidative stress; photosynthesis; *Quercus*

INTRODUCTION

Among 42 alien true bugs (Insecta: Hemiptera: Heteroptera) recognized in Europe, 19% of the species are represented by lace bugs that belong to the family Tingidae [1]. The oak lace bug *Corythucha arcuata* (Say, 1832) is an important pest species originating from North America, with a wide natural distribution in the United States and Canada [2]. Since the first occurrence of *C. arcuata* in Europe recorded in Italy in 2000 [3], the presence of the pest has been observed in other countries, including Turkey, Croatia, Serbia, Switzerland, Romania, Bulgaria, Hungary, Russia, Iran, Slovenia, with the most recent report of its spreading in Bosnia and Herzegovina [4-14]. Human activities, probably in combination with other factors, are considered as the driving force that enable the spread of the range of alien insects beyond the borders of their natural distribution area [1,7]. There are implications of possible natural spreading of *C. arcuata* into non-infested regions in neighboring countries [13]. The spread of adults is promoted by wind or other factors in view of the poor flying capability of the oak lace bug [2]. However, a moderate expansive spread of the oak lace bug across Europe usually occurs either via trade and transport of their host plants or other goods (as imperceptible contaminates), or as stowaways within or on the exterior of transport vehicles [1].

C. arcuata adults and nymphs feed on the abaxial leaf surface by piercing and sucking the cell sap [15]. The feeding mode of the lace bugs negatively affects leaf photosynthetic activity, while heavy infestations may result in premature leaf fall and dieback of branch tips [13,16]. The occurrence of chlorotic discoloration at the adaxial leaf surface is considered a typical symptom, while at the abaxial leaf surface, characteristic black spots appear [2].

C. arcuata frequently colonizes woodlands, resulting in a negative impact on native biodiversity and individuals [1]. Numerous oak species are the main hosts of the insect [2-3,11,17]. However, the polyphagous oak lace bug has been reported to infest other plant species belonging to genera *Malus*, *Ulmus, Rubus, Castanea, Pyrus, Acer* and *Rosa* [8,18]. In view of significant defoliation and reduced photosynthetic activities of remaining leaves of infested plants, which can result from the feeding of only one lace bug generation, these herbivorous insects are considered as possible candidates for biological control of invasive plants [16].

Environmental factors, such as water conditions at sites inhabited by potential lace bug host plants, could influence leaf characteristics and thus interfere with the bug's ability to pierce leaves and reach the sap from mesophyll cells [15]. The importance of leaf characteristics in plant resistance to lace bug attack has been investigated recently by comparison of leaf toughness and nutrient composition, surface wax and stomatal characteristics in resistant and susceptible *Pieris* taxa [19]. Reduced reproduction and survival of *Stephanitis takeyai* adults was attributed to leaf toughness (high fiber, lignin and cellulose content), low moisture content and stomatal characters of tolerant taxa. Due to the negative correlation between leaf toughness and its water content, *C. arcuata* prefers *Q. alba* plants that have a higher water content of leaves [15,20]. Considering climate change at a global level, with scarce and irregular rainfall accompanied by global warming, appropriate modification of leaf characteristics might occur in host plant species, leading to a reduction in *C. arcuata* abundance and/or successful spreading. The oak lace bug was added to the European and Mediterranean Plant Protection Organization (EPPO) Alert List in 2001, but it was removed from it in 2007 due to the inefficiency of phytosanitary measures to prevent its natural spreading [21]. According to extensive field and laboratory investigations on host plant distribution and the suitability of climate conditions for insect development, Bernardinelli [17] elucidated the possibility of the oak lace bug spreading all over Europe.

The quality of host plants, being the source of nutritive compounds necessary for their growth, reproduction and development, affects the population dynamics and performance of herbivores (i.e., insect and other animals) [22,23]. Environmental stress factors may enhance the breakdown and mobilization of nitrogen compounds making them more readily available to young invertebrate herbivores, thus affecting their performance [24]. Bearing in mind that the leaves of woody plants represent the main photosynthetic organs, biotic (including herbivore attack) and abiotic environmental factors could influence the physiological processes involved in plant growth i.e., productivity and development. Along with the production of new tissues (i.e. dry matter), the primary products of photosynthesis are also used either for respiration or for the production of other carbohydrates, proteins, fats and secondary compounds, some of which are responsible for resistance to insect attack [25]. Previous investigations have studied and documented various effects of herbivorous insects on photosynthetic characteristics [16,26-30], the foliar nutrient status [31-33] and oxidative status of plant tissues [34-36] in various host plant species. The present study was aimed at examining the influence of oak lace bug (*Corythucha arcuata*) feeding on the photosynthetic characteristics and gas-exchange parameters (net photosynthesis, chlorophyll and carotenoid concentration, transpiration, stomatal conductance, substomatal concentration of CO_2), mineral nutrient concentrations and defense mechanisms (activities of some antioxidant enzymes) of pedunculate oak leaves. Antioxidative enzymes seem to play a considerable role in plant resistance to insect herbivores due to their engagement in the synthesis of defensive compounds, plant tolerance to oxidative stress and signaling pathways [37]. Previous investigations suggested different levels of activities between infested and control (non-infested) plants at different times following infestation [37-38]. Hence, the activities of catalase (CAT), guaiacol and ascorbate peroxidase (GPX and APX, respectively) were evaluated in relation to *C. arcuata* infestation. It seems that catalase and peroxidases play an important role in plant interaction with herbivore insects since their enhanced activity decreases the levels of H_2O_2 and thus its toxic effect [30,35,39-40].

To our knowledge, the obtained data will be the first reported on the changes in the physiological properties of leaves in infested pedunculate oak plants in response to lace bug feeding. Elucidation of the physiological responses should significantly contribute to the cognition and understanding of mechanisms involved in the response of host plants to stress induced by insect herbivory.

MATERIALS AND METHODS

Plant material and cultivation conditions

The experimental location was selected in state forests of PE "Vojvodinašume", Forest Estate Sremska Mitrovica in management unit Vinična-Žeravinac-Puk (coordinates N 44°56′33″; E 19°11′35″). The study area was a shelterwood system, semi-naturally regenerated (natural seed dispersal plus additional acorn sowing), 9-year-old pedunculate oak stand on black meadow soil with the following properties: total clay and sand content – 53.56 and 46.44%, respectively; pH – 5.74; humus content – 6.6%; $CaCO_3$ content – 2.09%. Silvicultural practices of the selected stand were carried out on a regular basis, including weeding in the first years after establishment and selective thinning to promote oak seedlings versus perennial weeds and other naturally occurring codominant competitive tree species, such as narrow-leaved ash and hornbeam. At the beginning of summer, first insect damage became apparent on the upper leaf surface of young oak plants, caused by infestation by overwintered adults, and it reached a climax during August. Therefore, in the selected stand, 7 sample plots of 10 m^2 were marked for observation and data collection. Measurements and sampling in the selected sample plots were performed on leaves developed during the growing season on long terminal shoots of selected infested and healthy plants. Damaged leaves had an even distribution of chlorosis symptoms all over their surfaces, while healthy leaves had no evident signs of damage. Prior to measurement and sampling, insects were removed from the leaves.

Gas-exchange parameters

Rate of photosynthesis (Pn) and transpiration (Tr), stomatal conductance (g_s) and intercellular concentrations of CO_2 (Ci) were measured under ambient levels of CO_2, temperature and humidity on August 14th 2016. Parameters were measured on fully expanded current-year long terminal shoot leaves using a LCpro+ portable photosynthetic system (ADC BioScientific Ltd, UK). Light conditions were set to 1200 μmol m^{-2} s^{-1}. A flow of ambient air to the leaf chamber was set to a constant 300 L min^{-1}, while temperature, CO_2 concentration and humidity were at ambient levels. The gas-exchange parameters of five either infested or non-infested individual plants were determined during a clear sunny day between 10.00 and 12.00 A.M. (n=5).

Following the measurement of gas-exchange parameters, the same leaves were collected and used for assessment of physiological and biochemical parameters. Before sampling, the insects and their remains were gently removed with a fine brush from the selected leaves.

Physiological and biochemical parameters

The concentrations of photosynthetic pigments (chlorophylls and carotenoids) were assayed spectrophotometrically (Beckman DU-65, CA), following extraction in absolute acetone [41], and expressed as mg g^{-1} fresh weight. Levels of chlorophyll were also estimated using a portable chlorophyll meter (Minolta SPAD-502). These measurements were performed at two time points (first on June 27th, 2016; second on July 7th, 2016) during the vegetative period. Five measurements were performed on five either infested or non-infested individual plants during a clear sunny day, between 10.00 and 12.00 A.M. (n=25).

For antioxidative enzyme assays, 500 mg of plant material were ground to a fine powder in liquid nitrogen and homogenized using 5 mL of the extraction buffer (pH 7.8), containing 100 mmol KH_2PO_4/K_2HPO_4, 400 mg insoluble polyvinylpolypyrrolidone, 5 mmol ascorbate and 2% Triton X-100 [42]. Following incubation on ice for 30 min, the homogenate was centrifuged for 30 min at 48400 g at 4°C. For measurement of catalase (EC 1.11.1.6, CAT) and guaiacol peroxidase (EC 1.11.1.7, GPX) activities, the supernatant was eluted by gel-filtration over polyacrylamide columns (Bio-gel P-10, Bio-Rad, CA) using 20 mmol KH_2PO_4/K_2HPO_4 buffer (pH 7.8) with 0.5% Triton. For determination of ascorbate peroxidase (EC 1.11.1.11, APX) activity, the supernatant was eluted by gel-filtration over polyacrylamide columns (Bio-gel P-10, Bio-Rad, CA) using 100 mmol KH_2PO_4/K_2HPO_4 buffer (pH 7.0) containing 1 mmol ascorbate. The activity of CAT was determined in the reaction mixture that consisted of 50 mmol of potassium phosphate buffer with pH 7.0, hydrogen peroxide and 100 μL enzyme extract. H_2O_2 depletion was measured as the decrease of absorbance at 240 nm [43]. APX activ-

ity was measured using a reaction mixture containing 50 mmol of potassium phosphate (pH 7.0), 1 mmol hydrogen peroxide, 250 μmol Na-ascorbate and 150 μL enzyme extract following a decrease in the absorbance at 290 nm [44]. GPX activity was assayed at 436 nm in a reaction mixture with 50 mmol of potassium phosphate buffer (pH 5.25), 10 mmol hydrogen peroxide, 40 mmol guaiacol and enzyme extract [44]. The activity of nitrate reductase (NR) was determined *in vivo* following the incubation of fresh leaf samples in a buffered medium containing NO_3^-, and spectrophotometric measurement of the concentration of nitrites (Beckman DU-65, CA) at 520 nm [45]. The activities of CAT, GPX and APX were calculated as U/g fresh weight, and of NR as μmol NO_2^- g^{-1} h^{-1}. Changes in enzyme activities in infested plants were expressed as the percentage of deviation with respect to the control value.

Chemical analysis of plant samples

Following oven-drying of leaves at 80°C until a constant weight, the samples were milled and digested with HNO_3 using a closed vessel microwave digestion system (Milestone D series Microwave Digestion System). The concentration of phosphorus was determined using the ammonium molybdate-vanadate method proposed by Gericke and Kurmies [46]. The concentration of potassium and calcium was determined by flame photometry (flame photometer, Jenway model PFP7), and of nitrogen by the Kjeldahl method [47]. Analysis of each sample was performed in three independent replicates.

Statistical analysis

Analyses of gas-exchange parameters and enzyme activities were performed in five replicates (n=5), and of photosynthetic pigments and macroelement concentrations, in three replicates (n=3). Data were calculated and statistically processed using Microsoft Office Excel and STATISTICA for Windows, ver. 13.3. An independent *t*-test was used to compare the mean values of the studied parameters obtained for the control (non-infested) and infested plants. Two-way analysis of variance (ANOVA) was performed to detect significance levels (*P* values) for the effects of infestation, time, and their interaction on chlorophyll levels measured by a Soil-Plant Analyses Development (SPAD) meter.

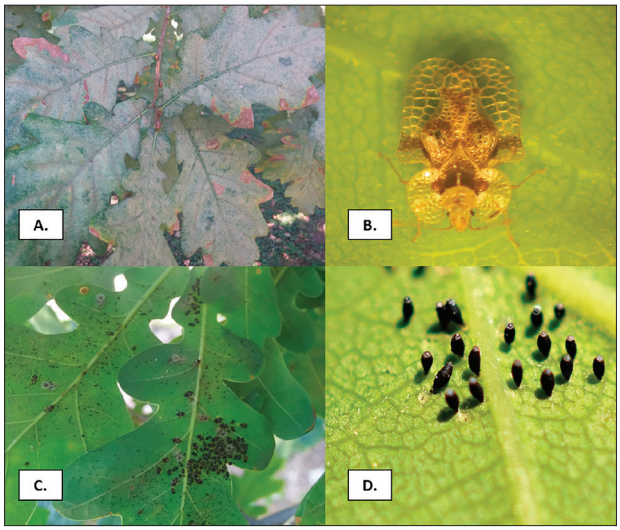

Fig. 1. The adaxial (upper) leaf surface of *C. arcuata*-infested *Q. robur* plants. Chlorotic discoloration (**A**), adults (**B**), nymphs (**C**) and egg clusters (**D**) on the leaves of infested plants.

RESULTS

Chlorosis was evident on the adaxial leaf surface in infested plants (Fig. 1A), while adults, nymphs and egg clusters were observed on the abaxial (lower) leaf surface (Fig. 1B, C and D, respectively).

Photosynthetic characteristics

According to the results presented in Table 1, gas-exchange parameters were considerably changed in infested plants. Rates of photosynthesis and transpiration were lowered by 58.84 and 21.66%, respectively, compared to non-infested plants. A 35.71% decrease in stomatal conductance was recorded in infested plants, and the intercellular CO_2 concentration was increased by 47.44%. The oak lace bug infestation negatively affected the concentration of chlorophyll *a*, while chlorophyll *b* and carotenoids concentrations were not considerably changed (Table 2).

Antioxidant enzymes and NR activity

The activities of antioxidant enzymes, CAT, GPX and APX, of *Q. robur* leaves were not uniformly affected by the oak lace bug infestation (Fig. 2). The activity of CAT was considerably decreased (by 93.9%) in leaves of infested oak plants with respect to the

Table 1. Rate of photosynthesis (Pn) and transpiration (Tr), stomatal conductance (gs), and intercellular concentrations of CO_2 (c_i) of infested and non-infested *Q. robur* leaves.

Parameters	Treatments		ANOVA	
	Control	Infested plants	CV(%)	F
Pn (μmol of CO_2 m^{-2} s^{-1})	12.39±0.61	5.10±0.21***	45.33	128.57
Tr (mmol of H_2O m^{-2} s^{-1})	2.17±0.08	1.70±0.08**	15.54	17.41
g_s (mol H_2O $m^{-2}s^{-1}$)	0.14±0.006	0.09±0.003***	26.13	67.29
c_i (μmol mol^{-1})	196±8.15	289±3.14*	20.89	112.75

Values are the mean±SE. According to Student's t-test, the significance of differences between the means within a row is indicated as: *, ** and *** – significant at p<0.05, p<0.01 and p<0.001, respectively; ns – not significant (n=5); CV (%) – coefficient of variation.

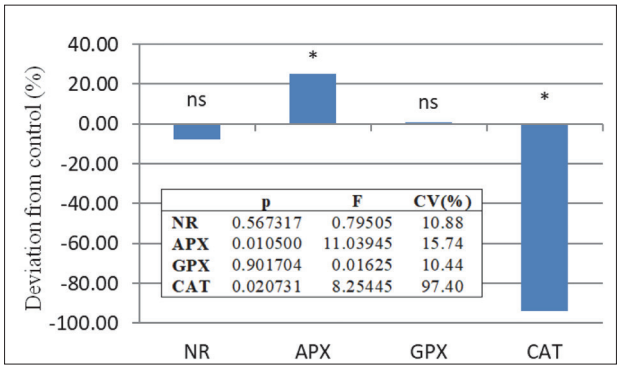

Fig. 2. Changes of nitrate reductase (NR), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and catalase (CAT activity) in *C. arcuata* infested *Q. robur* leaves with respect to the control (%). According to Student's t-test, the significance of differences between the means within a row is indicated as: *, ** and *** – significant at p<0.05, p<0.01 and p<0.001, respectively; ns – not significant (n=5); CV (%) – coefficient of variation.

Table 2. Concentration of photosynthetic pigments and chlorophyll levels (SPAD values) in infested and non-infested *Q. robur* leaves (mean±SE).

Photosynthetic pigments (mg g^{-1} FW)	Treatments		ANOVA	
	Control	Infested plants	CV(%)	F
Chlorophyll *a*	2.71±0.12	2.09±0.16*	16.91	9.068
Chlorophyll *b*	0.82±0.06	0.75±0.07 ns	13.56	0.597
Total chlorophylls (*a*+*b*)	3.53±0.18	2.84±0.23ns	15.53	5.513
Carotenoids	0.77±0.06	0.65±0.05ns	14.65	2.703

SPAD values							
Period of measurement				ANOVA			
June		July					
Control	Infested plants	Control	Infested plants	CV(%)	Infested plants	Period	Interaction
38.93±0.4a	31.28±0.55b	40.21±0.26a	29.35±0.62c	6.95	***	***	ns

According to Student's t-test, the significance of differences between means within a row is indicated as: *, ** and *** – significant at p<0.05, p<0.01 and p<0.001, respectively; ns – not significant (n=5); CV (%) – coefficient of variation.
*Means within a row followed by different letters are significantly different at p≤0.05 (Duncan's test). Two-way ANOVA was used to evaluate the coefficient of variation (CV(%)), the infestation and the effect of the period of measurement (n=25).

Table 3. Concentrations of nitrogen, phosphorus, potassium and calcium (%) in infested and non-infested *Q. robur* leaves (mean ± S.E.).

Macroelements (%)	Treatment		ANOVA	
	Control	Infested plants	CV (%)	F
Nitrogen	2.36±0.02	2.31±0.04ns	2.07	1.271
Phosphorus	0.49±0.04	0.61±0.03ns	16.28	6.368
Potassium	0.76±0.04	0.86±0.01ns	8.85	5.488
Calcium	0.16±0.003	0.17±0.008ns	5.95	0.358

According to Student's t-test, the significance of differences between means within a row is indicated as: *, ** and *** – significant at p<0.05, p<0.01 and p<0.001, respectively; ns – not significant (n=5);
CV (%) – coefficient of variation.

control, while the activity of APX was considerably increased. However, the activity of NR and GPX remained unchanged due to insect attack (Fig. 2).

Concentration of mineral elements in leaves

The *C. arcuata* attack did not considerably affect the concentrations of N, P, K and Ca in infested oak leaves when compared to control plants (Table 3). The order of macroelement concentrations in dry matter for both infested and non-infested pedunculate oak leaves was as follows: N>K>P>Ca.

DISCUSSION

In the present study we investigated the physiological responses of *Q. robur* plants with respect to *C. arcuata* infestation. The obtained results related to gas-exchange parameters corroborate previous findings on photosynthetic activity in plants infested by mesophyll-feeding insects [26-27,29]. The observed decreases in stomatal conductance for gasses (CO_2, O_2) and water vapor in our experiment could be ascribed to stomatal closure, i.e. the decrease of stomatal aperture dimensions in infested plants. Stomatal closure accompanied with restriction of gas exchange due to lace bug feeding was previously observed in azalea plants [27]. A reduction of CO_2 entrance into destructed mesophyll cells was followed by decreased stomatal conductance that occurred because of herbivore feeding, as reported for spider mite-injured soybean [29]. Analysis of gas-exchange parameters in infested oaks pointed to the possible mechanism responsible for the significant reduction of photosynthetic activity in relation to non-infested plants. Regarding the increase of intercellular CO_2 concentration, it appears that decreased stomatal conductance did not affect the availability of this gas necessary for the carboxylation reaction of the Calvin cycle. Therefore, mesophyll-related limitations are likely responsible for the decreased photosynthetic activity of pedunculate oak leaves attacked by *C. arcuata*. However, maintenance of optimal rates of carbon-related dark reactions through preserved normal levels of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity and ribulose-1,5-bisphosphate regeneration has been related to a plant's resistance to insect infestation [30]. A significant reduction in the Rubisco carboxylase activity has been reported in plants under lace bug attack [16]. Analysis of transcriptome data originating from microarray experiments in various plant species experiencing biotic stress (including herbivore attack) revealed the downregulation of photosynthesis-related genes as a part of a defensive reaction [48]. The authors reported silencing of genes coding for photosynthetic pigments, proteins involved in electron transport, as well as for other components of the photosynthetic process (i.e. light and dark reactions). Enhancement of photosynthesis in infested plants is rarely observed despite the increased investment of resources for the synthesis of secondary metabolites, due to the low cost of the process with respect to the plant's energy and carbon budget [49]. Photosynthetic tissue in infested plants is directly influenced (damaged) by cell-content-feeding insects, which makes the reduction in photosynthetic activity expected. However, Zangerl et al. [28] described a negative indirect effect on photosynthetic activity in remote leaf tissue impacted by folivory, which was positively correlated with the synthesis of defensive compounds. Such a response enables the use of nitrogen-rich resources, such as leaf proteins, in secondary defense pathways, such as the regulation of transcription of related genes [48,50]. Therefore, the decreased photosynthetic activity of oaks exposed to *C. arcuata* attack might be, at least in part, considered as a defensive response.

Another reason for decreased photosynthetic activity might be ascribed to the loss of photosynthetic pigments. A positive correlation between net photosynthesis and chlorophyll content has been reported in plants experiencing lace bug feeding [27]. In our study, the concentration of photosynthetic pigments was differentially affected by the presence of *C. arcuata*. The concentration of chlorophyll *a* was considerably reduced in infested plants, while a tendency for a non-significant decrease was observed for chlorophyll *b* and carotenoids levels. Reduction in chlorophyll content was reported in plants following insect herbivory [27]. Reduction in the chlorophyll content in host-plant leaves is positively correlated with the density of adult lace bugs and their offspring [16]. SPAD values indicated a considerable decrease in chlorophyll levels in infested oak leaves at both periods of measurement. According to our results, both infestation and the period of measurement affected the obtained SPAD values (i.e. chlorophyll levels), indicating a possible use of this technique for fast and early evaluation of photosynthetic pigment disorder caused by *C. arcuata* feeding in *Q. robur* plants.

The production of reactive oxygen species (ROS) such as hydrogen peroxide, the superoxide radical, or hydroxyl radicals, regularly occurs in different plant cell organelles as products of aerobic metabolism under both optimal and stressful conditions [36]. Because of their cytotoxicity, the production and removal of different ROS types is rigorously coordinated by nonenzymatic and enzymatic components of defense mechanisms [40]. These molecules play an important role in plant defense against biotic stress factors, includ-

ing herbivory [36]. The rapid accumulation of H_2O_2 in soybean and poplar leaves as an early signaling event following herbivory damage has been reported by Bi and Felton [34] and Hu et al. [39], respectively. In order to prevent the toxic effects of high H_2O_2 levels, the activities of antioxidant enzymes involved in detoxification is enhanced [35]. Various genes, as well as those coding for ROS-scavenging enzymes, are regulated by herbivore attack [51]. The antioxidant enzymes studied in the present work were affected in oak leaves by *C. arcuata* feeding. The activity of APX was increased by 25% in the leaves of infested oak plants compared to the control, while the activity of GPX remained unchanged. A 1.5-fold increase in APX activity was also found in soybean leaves following herbivory [34]. Peroxidases are multifunctional enzymes known to be upregulated in plants coping with different abiotic and biotic stresses, they regulate H_2O_2 levels and they are involved in the generation of reactive oxygen species (ROS) and cell-wall hardening due to polymerization of cell wall compounds [52]. These enzymes oxidize various substrates in the presence of hydrogen peroxide: ascorbate peroxidase catalyzes the reduction of H_2O_2 to water by using the reducing power of ascorbate [53], whereas guaiacol peroxidase oxidizes guaiacol. Upregulation of peroxidase activity has been reported in wheat cultivars resistant to *Diuraphis noxia* (Mordvilko) feeding [38], *Vigna mungo* plants experiencing *Bemisia tabaci* (Gennadius) attack [37], in poplar (*Populus simonii* × *P. pyramidalis* 'Opera 8277') leaves infested by *Clostera anachoreta* [39] as well as in tobacco induced by whiteflies feeding [40]. Also, profiling of gene expression in a tolerant barley (*Hordeum vulgare* L.) cultivar in response to herbivory insects revealed upregulation of peroxidase genes, indicating the significance of the enzyme in the attacked plant's tolerance process [30]. Among numerous functions of peroxidases, these enzymes are responsible for providing a physical barrier to biological attack by the hardening of cell walls in a passive defense mechanism [52]. However, a considerable decrease in CAT activity was observed in leaves of infested oak plants compared to the control. This enzyme catalyzes the decomposition of hydrogen peroxide into water and oxygen [53]. The results obtained in the present work are in agreement with the decreased catalase activity observed in various plant species experiencing insect attack [34,37]. The activity of CAT can vary in infested plants, with leaf position and insect feeding time (it was significantly increased in distant (non-infested) leaves whereas it was suppressed in local (attacked) leaves of tobacco plants 15 days following infestation), with respect to the control [40]. Changes in the foliar oxidative status probably contributed to plant defense against herbivores [34,37].

Regarding the nutritional quality of plants used for insect herbivores' consumption, the main constituents necessary for insect metabolism include appropriately balanced carbohydrates, amino acids, vitamins, mineral elements, sterols, lipids, as well as water [32,54]. Considering the role of mineral elements in insect physiological processes during development (such as enzyme activation, structure formation, control mechanisms, etc.), their requirements for K, P and Mg are higher than for Ca, Na and chlorides [32]. The concentrations of macroelements P, K and Ca in infested oak leaves were not considerably changed with respect to control plants. However, the tendency of increase by 24.49, 13.16 and 6.25% was observed for P, K and Ca concentration, respectively. Leaf herbivore feeding may differentially alter the mineral composition in both above-ground and below-ground host-plant tissues. Significant decreases of leaf N, S, Ca and P concentrations were observed in *Hordeum vulgare* leaves, while significant increases in root N, S, Ca and K levels were reported [33]. Mineral elements are necessary for the metabolic processes of both plants and animals, and changes in a host plant's mineral status consequently affect herbivorous insects feeding on them [32].

Previous studies reported on variations of macroelement concentrations (N, P, K, Ca, Na) in leaves of different pedunculate oak genotypes as a result of the interaction of a certain genotype and environmental factors [55]. Furthermore, foliar nutritional quality changes greatly during leaf maturation, as well as insect assimilation efficiency of compounds such as carbohydrates and proteins [56]. Along with these differences, the variability in nutrient amounts between plant species and even within a single plant (leaf maturity, for example) suggests that most insect herbivores facing heterogeneous nutritional sources regulate their nutrient intake [23]. For example, recent experiments conducted by Cease et al. [57] showed that limitation of P intake could be a common feature of

terrestrial insect herbivores, which could exhibit various mechanisms to attain sufficient but not excessive amounts of P since excessive P intake reduces their growth and survival.

The N and water contents in host-plant leaves are important nutritional components for herbivorous insects, positively affecting their growth rate [58]. Along with N concentration, we measured the activity of nitrate reductase (NR) to estimate the effect of *C. arcuata* feeding on N assimilation in infested pedunculate oak leaves, due to its rate-limiting role in this process in plants. In the present study, neither the concentration of N in leaves nor the assimilation of nitrates (i.e. activity of NR) were considerably changed in *C. arcuata*-infested oak plants. Although plant tissue usually contains N levels below the threshold considered optimal for herbivorous insects [22], previous investigations revealed a positive correlation between the N content in leaves and herbivore abundance [59]. In another investigation, the opposite was estimated for aphid density and N concentration, and was attributed to a possible relationship between host-plant alkaloids and aphid performance [32]. Phenols, alkaloids and other allelochemicals are negative host-plant constituents that insect herbivores regularly encounter, and their effect on insect metabolism depends on the nutrient composition of food [23,54]. A recent investigation showed the inhibitory effect of sesquiterpenes alantolactone and isoalantolactone isolated from *Inula racemosa* roots on *Spodoptera litura* (Lepidoptera: Noctuidae) growth, including an extended development period, reduced pupation and adult emergence [60]. However, some plant species may lower their leaf N concentration in response to a high rate of leaf consumption [31]. In the mountain birch (*Betula pubescens* ssp. *tortuosa*), the low nutritional quality of leaves was considered as a protective function that constitutes a potential active defense against herbivorous insects [54]. Concurrent analysis of both herbivore and plant performance, including the nutritional quality of plant tissues consumed, would enable comprehensive clarification of the relations in plant-insect interaction [22]. The results obtained in our study indicate unaffected leaf nutrient concentrations in oak plants experiencing herbivory. We propose that other mechanisms are involved in the interaction of these plants with *C. arcuata,* thus requiring additional investigations.

To the best of our knowledge, this is the first paper to report on the physiological responses of *Q. robur* plants exposed to *C. arcuata* infestation. The ecological and economic significance of oak stands and the vulnerability of plants to various abiotic and/or biotic factors require the understanding of plant-pest interactions. Understanding the impact of pests such as the invasive species *C. arcuata* on the physiological processes and vitality of young plants, as well as on plant responses, may provide a foundation for efficient preservation of oak forests endangered by the oak lace bug.

Funding: This publication was supported by the project: "Dispersal, harmfulness and suppression possibilities of invasive species *Corythucha arcuata* in common oak forests", funded by the Provincial Secretariat for Higher Education and Scientific Research, Autonomous Province of Vojvodina, Republic of Serbia.

Acknowledgments: The authors are grateful to Dr. Ivana Maksimović, Faculty of Agriculture, University of Novi Sad, Republic of Serbia and her associates for the assistance with the chemical analyses. We sincerely appreciate Mr. Ed Bauer from USDA Forest Service Northern Research Station, Rhinelander, WI, for editing the manuscript for English language and style. We are also grateful to the anonymous reviewers for their constructive and invaluable comments that have improved our manuscript. Part of the results shown in this paper were presented at the International Conference of Kaszo-Life Project, "Water in Forests", 29-30 May 2018.

Author contributions: Andrej Pilipović, Milan Drekić and Leopold Poljaković-Pajnik designed the research and were responsible for conducting the experiments and data collection in the field. Nataša Nikolić, Danijela Kojić and Danijela Arsenov were responsible for the sample collection and laboratory analyses. Nataša Nikolić processed all of the data and wrote the entire manuscript. Danijela Arsenov performed the technical preparation of the manuscript.

Conflict of interest disclosure: The authors declare that there are no conflicts of interest.

REFERENCES

1. Rabitsch W. Alien True Bugs of Europe (Insecta: Hemiptera: Heteroptera). Zootaxa. 2008;1827:1-44.
2. Mutun S, Ceyhan Z, Sözen C. Invasion by the oak lace bug, *Corythucha arcuata* (Say) (Heteroptera: Tingidae), in Turkey. Turk J Zool. 2009;33:263-8.
3. Bernardinelli I, Zandigiacomo P. Prima segnalozione di *Corythucha arcuata* (Say) (Heteroptera, Tingidae) in Europe. Inf Fitopatol. 2000;12:47-9.
4. Mutun S. First report of the oak lace bug, *Corythucha arcuata* (Say, 1832) (Heteroptera: Tingidae) from Bolu, Turkey. Israel J Zool. 2003;49(4):323-4.
5. Forster B, Giacalone I, Moretti M, Dioli P, Wermelinger B. Die amerikanische Eichennetzwanze *Corythucha arcuata*

(Say) (Heteroptera, Tingidae) hat die Südschweiz erreicht. Mitt Schweiz Entomol Ges. 2005;78:317-23.
6. Samin N, Linnavuori RE. A contribution to the Tingidae (Heteroptera) from north and northwestern Iran. Entomofauna. 2011;32(25):373-80.
7. Dobreva M, Simov N, Georgiev G, Mirchev P, Georgieva M. First record of *Corythucha arcuata* (Say) (Heteroptera: Tingidae) on the Balkan Peninsula. Acta Zool Bulgar. 2013;65(3):409-12.
8. Hrašovec B, Posarić D, Lukić I, Pernek M. First record of oak lace bag (*Corythucha arcuata*) in Croatia. Šumarski list. 2013;9-10:499-503.
9. Csóka G, Hirka A, Somlyai M. First record of oak lace bag (*Corythucha arcuata* Say, 1832) in Hungary. Növényvédelem. 2013;49(7):293-6.
10. Pap P, Drekić M, Poljaković-Pajnik L, Marković M, Vasić V. Forest health monitoring in Vojvodina in 2015. Topola. 2015;195/196:117-33.
11. Don I, Don CD, Sasu LR, Vidrean D, Brad ML. Insect pests on the trees and shrubs from the Macea Botanical garden. Studia Universitatis 'Vasile Goldiş' Arad Seria Ştiinţe Inginereşti şi Agro-Turism. 2016;11(2): 23-8.
12. Neimorovets VV, Shchurov VI, Bondarenko AS, Skvortsov MM, Konstantinov FV. First documented outbreak and new data on the distribution of *Corythucha arcuata* (Say, 1832) (Hemiptera: Tingidae) in Russia. Acta Zool Bulg. 2017;9:139-42.
13. Jurc M, Jurc D. The first record and the beginning of the spread of oak lace bug, *Corythucha arcuata* (Say, 1832) (Heteroptera: Tingidae), in Slovenia. Šumarski list. 2017;9-10:485-8.
14. Dautbašić M, Zahirović K, Mujezinović O, Margaletić J. First record of oak lace bug (*Corythucha arcuata*) in Bosnia and Herzegovina. Šumarski list. 2018;3-4:179-81.
15. Connor EF. Plant water deficits and insect responses: the preference of *Corythucha arcuata* (Heteroptera: Tingidae) for the foliage of white oak, *Quercus alba*. Ecol Entomol. 1988;13:375-81.
16. Zhang Y, Hanula JL, O'Brien J, Horn S, Braman K, Sun J. Evaluation of the impacts of herbivory by lace bugs on Chinese privet (*Ligustrum sinense*) survival and physiology. Biol Control. 2013;64:299-304.
17. Bernardinelli I. European host plants and potential distribution of *Corythucha arcuata* (Say) (Heteroptera: Tingidae). In: Csóka Gy, Hirka A, Koltay A, editors. Biotic damage in forests. Methodology of forest pest and disease survey in Central Europe; 2006 Sep 12-16; Mátrafüred, Hungary. Mátrafüred, Hungary: Hungarian Forest Research Institute Agroinform Publishing House; 2006. p. 10-7.
18. Drew WA, Arnold DC. Tingoidea of Oklahoma (Hemiptera). Proc Okla Acad Sci. 1977;57:29-31.
19. Nair S, Braman SK, Knauft DA. Resistance mechanisms in Pieris taxa (Ericaceae) to *Stephanitis takeyai* (Hemiptera: Tingidae). Environ Entomol. 2012;41(5):1153-62.
20. Barber NA. Light environment and leaf characteristics affect distribution of *Corythucha arcuata* (Hemiptera: Tingidae). Environ Entomol. 2010;39(2):492-7.
21. European and Mediterranean Plant Protection Organization (EPPO) [Internet]. Paris: Mini data sheet on *Corythucha arcuata*; 2003 [modified 2007 Mar; cited 2018 Aug 15]. Available from: https://gd.eppo.int/download/doc/1059_minids_CRTHAR.pdf.
22. De Bruyn L, Scheirs J, Verhagen R. Nutrient stress, host plant quality and herbivore performance of a leaf-mining fly on grass. Oecologia. 2002;130:594-9.
23. Behmer ST. Insect herbivore nutrient regulation. Annu Rev Entomol. 2009;54:165-87.
24. Cockfield SD. Relative availability of nitrogen in host plants of invertebrate herbivores: three possible nutritional and physiological definitions. Oecologia. 1988;77:91-4.
25. Kozlowski TT, Kramer PJ, Pallardy SG. The Physiological Ecology of Woody Plants. New York, Tokyo, London, Toronto, Sydney: Academic Press; 1991. 277-9 p.
26. Welter SC. Arthropod impact on plant gas exchange. In: Bernays EA, editor. Insect–plant interactions. Boca Raton, FL: CRC Press; 1989. p. 135-51.
27. Buntin GD, Braman SK, Gilbertz DA, Phillips DV. Chlorosis, photosynthesis, and transpiration of azalea leaves after azalea lace bug (Heteroptera: Tingidae) feeding injury. J Econ Entomol. 1996;89:990-5.
28. Zangerl AR, Hamilton JG, Miller TJ, Crofts AR, Oxborough K, Berenbaum MR, DeLucia EH. Impact of folivory on photosynthesis is greater than the sum of its holes. Proc Natl Acad Sci. 2002;99:1088-91.
29. Haile FJ, Higley LG. Changes in soybean gas-exchange after moisture stress and spider mite injury. Environ Entomol. 2003;32(3):433-40.
30. Gutsche AR, Heng-Moss TM, Higley LG, Sarath G, Mornhinweg DW. Physiological responses of resistant and susceptible barley, *Hordeum vulgare* to the Russian wheat aphid, *Diurpahis noxia* (Mordvilko). Arthropod Plant Interact. 2009;3:233-40.
31. Casotti G, Bradley JS. Leaf nitrogen and its effects on the rate of herbivory on selected eucalypts in the jarrah forest. Forest Ecol Manag. 1991;41:167-7.
32. Silva AA, Varanda EM, Primavesi AC. Effect of the inherent variation in the mineral concentration of alfalfa cultivars on aphid populations. Bragantia. 2005;64:233-9.
33. Johnson SN, Hawes C, Karley AJ. Reappraising the role of plant nutrients as mediators of interactions between root- and foliar-feeding insects. Funct Ecol. 2009;23:699-706.
34. Bi JL, Felton GW. Foliar oxidative stress and insect herbivory: primary compounds, secondary metabolites and reactive oxygen species as components of induced resistance. J Chem Ecol. 1995;21(10):1511-30.
35. Łukasik I, Goławska S, Wójcicka A. Effect of cereal aphid infestation on ascorbate content and ascorbate peroxidase activity in triticale. Pol J Environ Stud. 2012;21(6):1937-41.
36. Bittner N, Trauer-Kizilelma U, Hilker M. Early plant defense against insect attack: involvement of reactive oxygen species in plant responses to insect egg deposition. Planta. 2017;245(5):993-1007.
37. Taggar GK, Gill RS, Gupta AK, Sadhu JS. Fluctuations in peroxidase and catalase activities of resistant and susceptible black gram (Vigna mungo (L.) Hepper) genotypes elicited

by Bemisia tabaci (Gennadius) feeding. Plant Signal Behav. 2012;7(10):1321-9.
38. Franzen LD, Gutsche AR, Heng-Moss TM, Higley LG, Sarath G, John D. Burd JD. Physiological and biochemical responses of resistant and susceptible wheat to injury by Russian wheat aphid. J Econ Entomol. 2007;100(5):1692-703.
39. Hu ZH, Shen YB, Shen FY, Su XH. Effects of feeding *Clostera anachoreta* on hydrogen peroxide accumulation and activities of peroxidase, catalase, and ascorbate peroxidase in *Populus simonii* × *P. pyramidalis* 'Opera 8277' leaves. Acta Physiol Plant. 2009;31:995-1002.
40. Zhao H, Sun X, Xue M, Zhang X, Li Q. Antioxidant enzyme responses induced by whiteflies in tobacco plants in defense against aphids: catalase may play a dominant role. PLoS One. 2016;11(10):e0165454.
41. Von Wettstein D. Chlorophyll-letale und der submikroskopische formwechsel der plastiden. Exp Cell Res. 1957;12:427-506.
42. Schwanz P, Polle A. Differential stress responses of antioxidative systems to drought in pedunculate oak (*Quercus robur*) and maritime pine (*Pinus pinaster*) grown under high CO_2 concentrations. J Exp Bot. 2001;52:133-43.
43. Aebi H. Catalase in vitro. Method Enzymol. 1984;105:121-6.
44. Polle A, Chakrabarti K, Schürmann W, Rennenberg H. Composition and properties of hydrogen peroxide decomposing systems in extracellular and total extracts from needles of norway spruce (*Picea abies* L., Karst.). Plant Physiol. 1990;94:312-9.
45. Hageman RH, Reed AJ. Nitrate reductase from higher plants. Methods Enzymol. 1980;69:270-9.
46. Gericke S, Kurmies B. Die kolorimetrische phosphorsäurebestimmung mit ammonium-vanadat-molybdat und ihre anwendung in der pflanzenanalyse. Z Pflanz Bodenkunde. 1952;59:235-47.
47. Nelson DW, Sommers LE. Determination of total nitrogen in plant material. Agron J. 1973;65:109-12.
48. Bilgin DD, Zavala JA, Zhu J, Clough SJ, Ort DR, DeLucia, EH. Biotic stress globally downregulates photosynthesis genes. Plant Cell Environ. 2010;33:1597-613.
49. .Kerchev PI, Fenton B, Foyer CH, Hancock RD. Plant responses to insect herbivory: interactions between photosynthesis, reactive oxygen species and hormonal signalling pathways. Plant Cell Environ. 2012;35:441-53.
50. Schwachtje J, Baldwin IT. Why does herbivore attack reconfigure primary metabolism? Plant Physiol. 2008;146:845-51.
51. Mohanta TK, Occhipinti A, Zebelo SA, Foti M, Fliegmann J, Bossi S, Maffei ME, Bertea CM. *Ginkgo biloba* responds to herbivory by activating early signaling and direct defenses. PLoS One. 2012;7(3):e32822.
52. Passardi F, Cosio C, Penel C, Dunand C. Peroxidases have more functions than a Swiss army knife. Plant Cell Rep. 2005;24:255-65.
53. Apel K, Hirt H. Reactive oxygen species: Metabolism, Oxidative Stress and Signal Transduction. Annu Rev Plant Biol. 2004;55:373-99.
54. Haukioja E, Ruohomäki K, Suomela J, Vuorisalo T. Nutritional quality as a defense against herbivores. Forest Ecol Manag. 1991;39:237-45.
55. Nikolić N, Krstić B, Pajević S, Orlović S. Variability of leaf characteristics in different pedunculate oak genotypes (*Quercus robur* L.). Proc Nat Sci Matica Srpska. 2006;110:95-105.
56. Barbehenn RV, Kapila M, Kileen S, Nusbaum CP. Acquiring nutrients from tree leaves: effects of leaf maturity and development type on a generalist caterpillar. Oecologia. 2017;184:59-73.
57. Cease AJ, Fay M, Elser JJ, Harrison JF. Dietary phosphate affects food selection, post-ingestive phosphorus fate, and performance of a polyphagous herbivore. J Exp Biol. 2016;219:64-72.
58. Coley PD, Bateman ML, Kursar TA. The effects of plant quality on caterpillar growth and defense against natural enemies. Oikos. 2006;115:219-28.
59. Basset Y. Influence of leaf traits on the spatial distribution of insect herbivores associated with an overstorey rainforest trees. Oecologia. 1991;87:388-93.
60. Kaur M, Kumar R, Upendrabhai DP, Singh IP, Kaur S. Impact of sesquiterpenes from *Inula racemosa* (Asteraceae) on growth, development and nutrition of *Spodoptera litura* (Lepidoptera: Noctuidae). Pest Manag Sci. 2017;73:1031–38.