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The effect of the oak powdery mildew, oak lace bug, and other foliofagous insects on the growth of young pedunculate oak trees

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Pedunculate oak (*Quercus robur* L., 1753) is one of the widely distributed oak species in Europe. A large number of organisms develop on its leaves. To determine the extent to which the oak powdery mildew, oak lace bug, and other foliofagous insects affect the growth of young oak trees, three experimental fields were selected in a 10-year-old pedunculate oak stand. In each of them, 50 trees were randomly selected, and their height was measured at the beginning of the vegetative season. The first experimental field was treated with a systemic insecticide, the second with a systemic fungicide, and the third, a comparison area, with water, during the entire vegetative season. At the end of the vegetative season, 25 plants with one apical branch were selected in each experimental field. Their height was measured, and 20 leaves were taken from each plant to determine the extent of the damage on them at the end of the experiment. After processing the obtained data, it was determined that: 1. Both foliofagous insects and oak leaf inhabiting fungi affect the growth of the oak trees significantly; 2. The oak lace bug did not influence the growth of the young trees significantly, as its abundance was low in all of the experimental areas; 3. The greatest damage on the leaves was caused by defoliator insects, which is why they contributed the most to the decrease in growth caused by insects; 4. The influence of the foliofagous insects on the growth of the trees was not significantly different from the influence of fungi; 5. Suppression of oak powdery mildew and foliofagous insects on young trees is useful as it positively influences the vitality and growth of those trees, and contributes to economic and ecological gain.

KEYWORDS

foliofagous insects, chemical control, *Corythucha arcuata*, defoliators, *Erysiphe alphitoides*, *Quercus robur*, height growth

1 Introduction

Pedunculate oak (*Quercus robur* L., 1753) is one of the most widely distributed oak species in Europe (Bobinac et al., 2012; Puchałka et al., 2017). Its range covers most of Europe, excluding its most southern and northern parts (Eaton et al., 2016). This species always had significant meaning for the people, as it provided construction and fuel material, food for livestock, and bark for tanning (Eaton et al., 2016). Because of its impressive appearance and longevity, it has a symbolic role in many cultures in Europe (Askeyev et al., 2005; Mills, 2013; Eaton et al., 2016). Its forests have a significant ecological value as they provide high biodiversity (Mölder et al., 2019). Due to its high quality of wood, it is one of the most important species in managed forests in Europe (Eaton et al., 2016).

A large number of organisms develop on pedunculate oak leaves (Karadžić, 2010; Dobrosavljević et al., 2020; Ermolaev et al., 2021; Mladenović et al., 2021; Marković, 2022). Among them, the oak lace bug - *Corythucha arcuata* (Say, 1832) (Hemiptera: Tingidae) and oak powdery mildew, which is most frequently caused by *Erysiphe alphitoides* (Griffon and Maubl). Braun and Takam (Erysiphales: Erysiphaceae), present the most problematic ones in southeastern Europe (Glavaš, 2011; Pap et al., 2013; Simov et al., 2018; Drekić et al., 2019; Bălăcenoiu et al., 2021a; Franjević et al., 2023). Defoliators such as *Lymantria dispar* Linnaeus, 1758 (Lepidoptera: Erebidae), *Tortrix viridana* Linnaeus, 1758 (Lepidoptera: Tortricidae), *Erannis defoliaria* (Clerck, 1759) and *Operophtera brumata* (Linnaeus, 1758) (Lepidoptera: Geometridae) can also cause significant damage as their outbreaks can spread over large areas (Marović et al., 1998; Harapin and Jurc, 2000; Pernek et al., 2008; Tomescu and Netoiu, 2008).

Corythucha arcuata and *Erysiphe alphitoides* are invasive species' (Marçais and Desprez-Loustau, 2014; Bălăcenoiu et al., 2021a). The first one originates from North America (Csóka et al., 2020), while the origin of second one is most probably from Asia (Desprez-Loustau et al., 2017). The first finding of *C. arcuata* in Europe happened in Italy in 2000 (Bernardinelli and Zandigiacomo, 2000), while *E. alphitoides* was first found in France in 1907 (Hariot, 1907). These two species are now one of the most widely distributed oak leaf-inhabiting pest organisms in Europe (Marçais and Desprez-Loustau, 2014; Csóka et al., 2020). *C. arcuata* causes significant damage during each vegetative season. Severe outbreaks of this species have been reported in many European countries (Paulin et al., 2020). Its larvae and adults damage the leaves by sucking the sap on the underside of the leaf. Necroses which their feeding causes can cover the entire leaf area in the case of high abundance. That is why decolorization, lower photosynthetic activity, transpiration, and stomatal conductance occur on those plants (Nikolic et al., 2019; Paulin et al., 2020; Bălăcenoiu et al., 2021a). All of these effects can consequentially lead to a decrease in growth, premature leaf abscission, and a decrease in the size of the acorn (Tomescu et al., 2018; Drekić et al., 2019; Paulin et al., 2020). *E. alphitoides* is constantly present in oak forests. This obligate parasite creates an epiphyte mycelium on the leaf, which takes nutrients from the host and covers the leaf surface (Karadžić and Milijašević, 2005). All this consequentially causes a reduction in photosynthetic activity and transpiration (Pap et al., 2014b). That causes a decrease in growth and can cause dieback of younger plants (Karadžić and Milijašević, 2005; Bert et al., 2016). The dieback of young oak trees which this fungus causes is a significant problem (Karadžić and Milijašević, 2005; Pap et al., 2012). That is why the control of this pathogen is conducted during forest regeneration (Bobinac and Karadžić, 1994; Glavaš, 2011; Pap et al., 2012). *E. alphitoides* causes problems even in older forests in the cases of defoliation, when it can significantly diminish the vitality of oak trees (Pap et al., 2014b).

As *E. alphitoides* has been present in Europe for more than 100 years, a lot is known about it (Desprez-Loustau et al., 2011; Marçais and Desprez-Loustau, 2014; Lonsdale, 2015; Kebert et al., 2022; Mieslerová et al., 2022). *L. dispar*, *T. viridana*, *E. defoliaria,* and *O. brumata* have also been a topic of many studies (Ivashov et al., 2002; Tikkanen and Julkunen-Tiitto, 2003; Glavendekić, 2010; Milanović et al., 2020a,b, 2022). *C. arcuata* is still a new species for Europe so it is currently intensively studied (Bernardinelli, 2006; Franjević et al., 2018; Drekić et al., 2019; Nikolic et al., 2019; Csóka et al., 2020; Kern et al., 2021; Marković et al., 2021a; Bălăcenoiu et al., 2021b; Paulin et al., 2023; Stancă-Moise et al., 2023; Valdés-Correcher et al., 2023). As pedunculate oak is one of the most significant European oaks (Eaton et al., 2016; Mölder et al., 2019) we conducted a study to determine: how *C. arcuata* and other foliophagous insects affect the growth of young pedunculate oak trees; which type of foliofagous insect damage is dominant on the leaves; how oak powdery mildew affects the growth of young trees; and does the influence of foliofagous insect on the growth of young oak trees differ from the influence of oak powdery mildew.

2 Materials and methods

2.1 Study area

The study was conducted in 2022 in a 10-year-old regenerated pedunculate oak stand[1] (44° 45′ 2.88″ N and 19° 59′ 45.88″ E). It is located in a plane, at an altitude of 74 m. The average tree height was 2.4 m and the average diameter at root collar was 2.8 cm. The studied area is located on an alluvial deposit of clay and sand, where the soil is eutric cambisol. The average annual temperature is about 11°C, while the annual precipitation is 569.6 mm. The climate is characterized as continental with some features of the Pannonian-steppe temperate continental climate. The plants in the investigated stand use only atmospheric water and underground water in spring since the area is not flood-prone.

2.2 Experimental design

Three experimental areas, measuring 20 × 10 m, separated by a distance of 100 m, were selected in the stand. At each of them, 50 randomly selected trees were singled out at the beginning of the study (on April 1st). They were labeled and their heights were measured by a tape measure with a precision of 1 cm. The first study area was treated with the systemic insecticide Tonus (active substance Acetamiprid 200 g/kg) in a concentration of 0.25 g per liter of water to prevent and suppress insect damage. The second area was treated with the systemic fungicide Falcon 460-EC (Tebuconazole 167 g/L + Triadimenol 43 g/L + Spiroxamine 250 g/L, in a concentration of 0.35 mL) per liter of water to prevent and suppress the harmful fungi. The third area was the comparison area which

1 All the data except the plant dimensions were gathered from the Public company "Vojvodinašume" which manages the forests in which the experiment was conducted.

was treated only with water. These pesticides were selected because they have a broad spectrum of effect and were already successfully used or they gave satisfactory results in similar experiments (Pap et al., 2015; Drekić et al., 2021). The pesticides were applied by spraying from the ground with a backpack sprayer. Each experimental area was treated with 10 liters of the listed formulation prepared with water (500 L/ha). All of the areas were treated simultaneously, every 15 days starting from April 1 to October 1 (entire vegetation). After that, 25 plants with a single apex and similar initial heights (± 25 cm) were selected in each experimental field to isolate extreme values, as some plants were broken while some formed multiple apical branches. Their heights were measured on the 15th of October when the experiment was finished. Twenty leaves were then randomly selected from each of the plants to assess the amount of damage caused by the analyzed organisms. They were packed in plastic bags and brought to the laboratory of the University of Belgrade Faculty of Forestry, where they were kept in the refrigerator for 2 days until the analyses were done. Damages were divided into the following groups: defoliators, miners, gallers, sucking insects, and oak powdery mildew (Figure 1). The assessment of the damage by category was done visually by the naked eye. The damaged area was estimated as the share of leaf area covered by mycelia, mines, galls, discoloration or simply eaten (missing) in relation to the total surface area of the leaf. The damaged area was measured in percentages as a relative measure because the leaf size differed significantly between and within each tree.

2.3 Leaf damaging organisms

Before each treatment and at the end of the experiment, leaves from randomly selected plants in the comparison area were analyzed to identify the foliofagous insect fauna on them. The noted species were identified on the site as they are common for the area in which the study was conducted. As oak powdery mildew can be caused by multiple fungi (Karadžić and Milijašević, 2005) of which *E. alphitoides* is listed as the most important one in the studied area, the damage on the leaves was labeled only as oak powdery mildew.

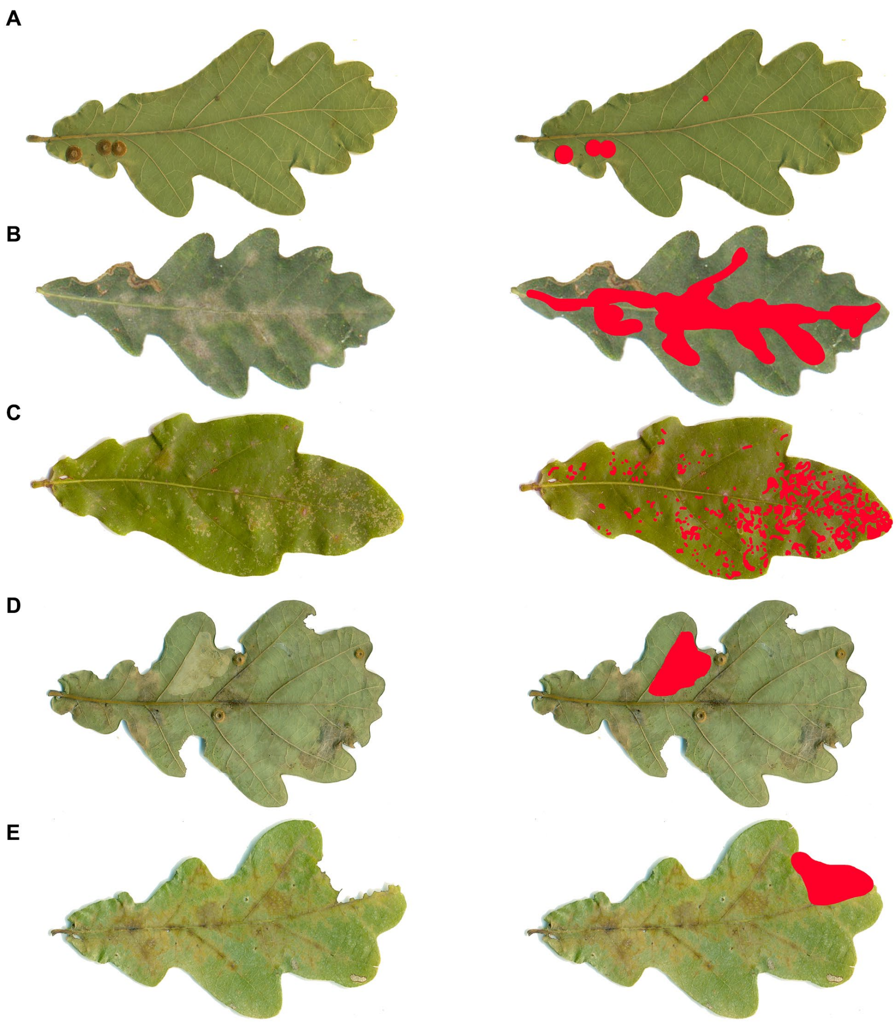

FIGURE 1
Illustration of how the different damage types were identified and estimated: **(A)** galls (Cynipidae sp.); **(B)** oak powdery mildew; **(C)** oak lace bug (*Corythucha arcuata*); **(D)** mines (*Phyllonorycter* sp.); **(E)** defoliation.

2.4 Statistical analysis

As the distribution of the analyzed parameters did not fit any of the standardized distributions (Kolmogorov–Smirnov test), nonparametric tests were used for further analysis. Kruskal-Wallis ANOVA by Ranks was used to determine the influence of the treatments on the growth of the analyzed trees and the influence of the treatments on the damage caused by different insect groups. Mann–Whitney U test was used as a post-hoc test, to determine the differences between individual treatments where the Kruskal-Wallis ANOVA showed significant differences. Mann–Whitney U test was also used to determine the differences between the leaf areas damaged by different insect groups in each treatment. All the data were analyzed at the tree level, at the level of significance 0.05. All of the statistical analyses were performed using Statistica 8.0 (StatSoft, Inc., Tulsa, OK, United States).

3 Results

Among the insects observed on the experimental areas, the most significant sucking species was *C. arcuata*; defoliators *L. dispar*, *E. defoliaria*, *O. brumata T. viridana* and *Periclista* sp. (Hymenoptera, Tenthredinidae); gallers *Andricus curvator* Hartig, 1840, *Neuroterus numismalis* (Fourcroy, 1785) and *N. quercusbaccarum* (Linnaeus, 1758) (Hymenoptera, Cynipidae); miners *Profenusa pygmaea* (Klug, 1816) (Hymenoptera, Tenthredinidae), *Phyllonorycter harrisella* (Linnaeus, 1761), *Ph. roboris* (Lepidoptera, Gracillariidae), and *Tischeria ekebladella* (Bjerkander, 1795) (Lepidoptera, Tischeriidae). The dominant fungal damage on the leaves was caused by oak powdery mildew.

Statistically significant differences were identified between the treated and the comparison area in the intensity of the damage caused by oak powdery mildew, defoliator insects, sucking insects, and leaf miners (Figure 2; Table 1). No significant differences were observed in the damage caused by gallicolous insects. In the area treated with fungicide, the intensity of the damage caused by leaf miners and sucking insects was significantly higher compared to the comparison area.

Statistically significant differences in growth were identified between each of the treated and the comparison area (Insecticide - Zadj = 4.590, $p = 0.000$; Fungicide - Zadj = 4.910, $p = 0.000$). There were no significant differences between the treated areas (Zadj = −1.136, $p = 0.256$). The greatest increase in growth was detected in the area treated with fungicide, less with insecticide and the lowest was in the comparison area (Figure 3). The growth on the area treated with fungicide was on average 62.3% higher, and on the surface treated with insecticide 50.5% higher in respect to the comparison area.

4 Discussion

Pedunculate oak hosts a large number of insects and fungi (Županić et al., 2009; Marković and Stojanović, 2011; Wrzesińska, 2017; Demeter et al., 2021; Ermolaev et al., 2021; Milanović et al., 2021; Jankowiak et al., 2022; Pilipović et al., 2022; Marković and Dobrosavljević, 2023). Many of them can be effectively suppressed by using insecticides and fungicides (Mihajlović and Glavendekić, 2006; Margaletić et al., 2007; Glavaš, 2011; Pap et al., 2012, 2014a; Pajnik et al., 2017; Drekić et al., 2021). The results of our study showed that the insecticide applied in the experiment can be successfully used for the control of sucking insects, defoliators, and leaf-mining insects, as the leaf area damaged by these groups was significantly lower in the insecticide-treated area. It should not be used to control gallicolous insects because it is not very effective. The applied fungicide can be used to efficiently control the oak powdery mildew as the damage caused by it was significantly lower in the fungicide-treated area.

Among the fungi, oak powdery mildew caused the greatest damage on the observed leaves on the area treated with insecticide, and the comparison area. This was expected because oak powdery mildew is one of the biggest problems on the leaves of young pedunculate oak trees in Southeastern Europe (Karadžić and Milijašević, 2005; Glavaš, 2011; Pap et al., 2013). The plants treated with fungicide showed lower oak mildew damage in comparison to other plots, and also the greatest height increment. This height increment is most likely connected to the lower share of damaged leaf area and subsequentially more available leaf area for photosynthesis

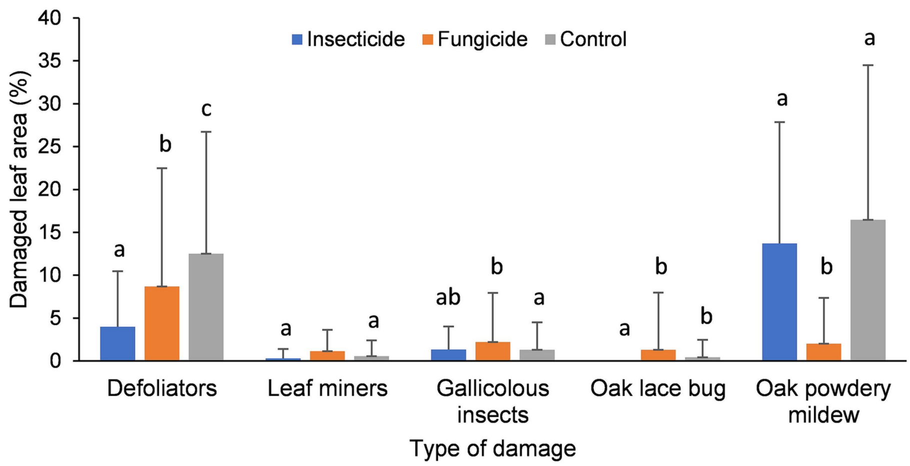

FIGURE 2
Damaged leaf area per type of damage (%) + SD, per each treatment, with letters above indicating the significant differences.

TABLE 1 Results of the Mann–Whitney U test between the damaged area of the leaf per category.

	Insecticide - control		Fungicide - control		Insecticide - fungicide	
	Z_{adj}	p	Z_{adj}	p	Z_{adj}	p
Defoliators	−12.52	0.00	−6.40	0.00	−6.14	0
Leaf miners	−1.37	0.17	5.29	0.00	−6.74	0
Gallicolous insects	1.24	0.21	2.21	0.03	−1.02	0.31
Sucking insects	−5.75	0.00	0.99	0.32	−6.37	0
Oak powdery mildew	−1.58	0.11	−19.03	0.00	18.13	0

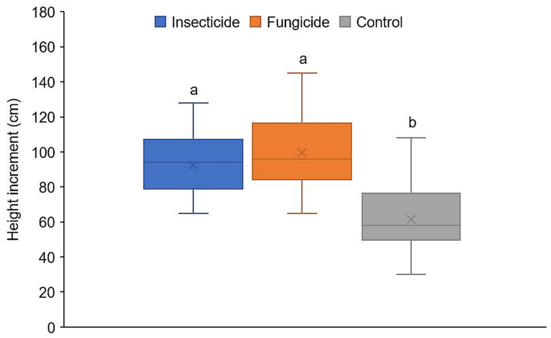

FIGURE 3
Influence of pesticide treatment on the height growth of the analyzed trees (cm) ± SD, with letters above indicating the significant differences.

(Nikolic et al., 2019; Paulin et al., 2020; Bălăcenoiu et al., 2021a). Of the insect groups, defoliators damaged the greatest leaf area. Damage from sucking insects, leaf miners, and gallicolous insects was negligible on all three plots. The fact that the damage from the sucking insects was small is a real surprise because, among the foliophagous insects in the old pedunculate oak forest near the location where the study was carried out, significant discoloration in the leaves caused by *C. arcuata* was noticed. Since the discoloration of the leaves in the old forest was higher, *C. arcuata* may prefer older trees, as it is already known that insect community and abundance change with the forest ages (Nagy et al., 2016; Marković et al., 2021b). The reason for this may be the fact that the allocation of defense chemicals is highest in young trees. On the other side, mature trees require resources for flower and seed production, they are frequently water deficient and have unfavorable photosynthesis/respiration, and saplings need the energy for the production of more aboveground biomass and increase of photosynthetic area, so they have a significantly lower amount of defense chemicals (Boege and Marquis, 2005; Barton and Hanley, 2013). In the forest where the research was carried out, areas with young trees of pedunculate oak, Turkey oak (*Q. cerris* L.), and Hungarian oak (*Q. frainetto* Ten.) of similar age were observed. The damage caused by *C. arcuata* was significantly greater on Turkey and Hungarian oak than on pedunculate oak. The pedunculate oak may be a less favorable host for *C. arcuata* compared to other oak species (Marković et al., 2021a).

There were no significant differences in tree growth between the treated areas. This shows that insects and fungi have a similar effect on their growth. This result is a novelty since the literature only mentions the effect of the oak powdery mildew (Bobinac and Karadžić, 1994; Karadžić and Milijašević, 2005; Glavaš, 2011; Pap et al., 2013; Rađević et al., 2020). In the area treated with the fungicide, the damage from sucking insects and leaf miners was higher than in the comparison area. Since oak powdery mildew was suppressed on it, this higher abundance indicates that there are competitive relationships between them. Such a relationship between the oak powdery mildew and insects is already known (Zargaran et al., 2012; Marković et al., 2021a).

The results of this study show that during the growing season, under the influence of fungi, the height growth of 10-year-old pedunculate oak trees decreases by 62.3%, and under the influence of insects by 50.5%. The real growth decrease is greater since the pesticides used did not achieve complete protection of the leaves from insects and fungi. The influence caused by these organisms is significant, which is why the need to suppress them arises. In plants up to 2 years of age, the control of oak powdery mildew should be carried out, as it is one of the limiting factors of the plants' development (Glavaš, 2011; Pap et al., 2012). Pesticide treatment of young trees older than 2 years is also useful as it positively influences their height growth and vitality. However, the treatment of older trees is complicated because of the characteristics of those stands (high density and height of trees), so the question of cost to benefit arises. The benefits of increased growth contribute both to the ecological functions such as sequestration of carbon dioxide, and economic functions such as the production of more wood. On the other side, any pesticide treatment affects other, non-targeted organisms, so the balance between these two needs to be found. The only place where the suppression of pest organisms on older trees should be carried out is in parks, gardens, and other areas where it does not require the use of expensive techniques and does not cause serious non-target effects.

Pedunculate oak is one of the dominant forest-forming species throughout Europe (Eaton et al., 2016). The restoration of its forests encounters many problems (Rumiantsev et al., 2018; Axer et al., 2023). To assist it, it is important to have a broader knowledge of the factors that can negatively affect those forests. When talking about the influence of insects and fungi on the growth of its young trees, based on the results of this study, it can be concluded that: 1. Both foliofagous insects and fungi significantly affect the height growth of the pedunculate oak, as the trees treated with pesticides had less damaged leaf area and grew significantly higher than trees in the comparison area; 2. *C. arcuata* did not influence the growth of the young trees significantly, as its abundance was low in all of the experimental areas; 3. The greatest damage on the leaves was caused by defoliator insects, which is why they contributed the most to the decrease in growth caused by insects; 4. The influence of the foliofagous insects on the growth of the trees was not significantly different from the influence of the fungi; 5. Suppression of oak powdery mildew and foliofagous insects on young trees is useful as it positively influences the vitality and growth of those trees, and contributes to economic and ecological gain; 6. As pedunculate oak is a less favorable host for *C. arcuata* compared to other oak species, it would be useful to determine whether there are differences between it and other oak species in terms of the influence of oak lace bug on the growth of young trees.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by the Ethics board of Faculty of Forestry, University of Belgrade. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

ČM: Conceptualization, Investigation, Resources, Supervision, Writing – original draft, Writing – review & editing. BK: Data curation, Investigation, Resources, Writing – review & editing. UP: Investigation, Writing – review & editing. JD: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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