Spreading-the-risk hypothesis may explain *Cameraria ohridella* oviposition in relation to leaf blotch disease

Radosław Jagiełło¹ · Piotr Łakomy² · Adrian Łukowski^{1,2} · Marian J. Giertych^{1,3}

Received: 24 July 2018 / Accepted: 15 March 2019 / Published online: 16 April 2019
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Abstract

The horse chestnut, *Aesculus hippocastanum* L., is a common tree in urban and rural environments of Europe. Invasion by *Cameraria ohridella* (Deschka & Dimić) started three decades ago, and to date, the whole European range of *A. hippocastanum* has been infected. With the addition of the fungal agent of leaf blotch *Phyllosticta paviae* (Desmazières), a new tripartite interaction arose, and a possible complex disease has been established. In this study, we aimed to answer the following question: do females of *C. ohridella* deposit eggs more frequently on healthy leaflets than on infected ones? In two experiments, leaves were previously infected by leaf blotch, and leaf miners were absent. In the choice test, one fertilised female was put into a box with healthy and infected leaflets. The second experiment was conducted under greenhouse conditions; an unknown number of females were exposed to leaf-blotch-infected and healthy saplings. Eggs were deposited mainly on healthy areas of leaves and always in leaf depressions. A positive relationship between the number of chosen leaflets and the number of deposited eggs was found when a single female was allowed to choose. In both experiments, we found that healthy leaflet area rather than the presence or absence of leaf blotch disease was the factor explaining the number of deposited eggs. We conclude that *C. ohridella* females are spreading the risk by ovipositing on many leaflets rather than avoiding the potential decrease in offspring survival.

Keywords Gracillariidae · Leaf miner · Lepidoptera · Oviposition preference · Tripartite interaction

Introduction

Miners are insects that spend most of ontogenesis concealed inside plant tissues. Usually, such insects are unable to change the location where they live and become space limited as a result of their feeding. Hence, the places where mothers deposit fertilised eggs are crucial for the maintenance of species. This phenomenon is explained by the optimal oviposition behaviour model (Jaenike 1978) or more generally by the unified theory of animal behaviour (Mangel

and Clark 1986). The process leading to the decision of where to oviposit, preceded by host selection, is very complex and takes place in the central nervous system as a result of chemo- and mechanosensory stimulation (Schoonhoven et al. 2012). Moreover, the interaction between a plant and an herbivorous insect may be altered when a third organism (for instance, a phytopathogenic fungus) is present. In a review study, Hatcher (1995) proposed four categories of interactions between herbivorous insects and phytopathogenic fungi: synergistic, additive, inhibitory and equivalent. From an evolutionary point of view, depending on the interaction type and specificity of participants' responses, the interaction may have an impact on the oviposition site selection process if such an insect-phytopathogen-plant system exists for a long enough amount of time. Considering the response of preference behaviours of insect herbivores, Tack and Dicke (2013) indicated that there may be no effect, attraction or avoidance of pathogen-infected plant tissues. The preference and performance of insects are generally negatively affected by fungal infections of host plants (Fernandez-Conradi et al. 2018), but the cited authors indicated

Handling Editor: Livy Williams.

✉ Radosław Jagiełło
rjagiello@man.poznan.pl

¹ Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, Poland

² Faculty of Forestry, Poznań University of Life Sciences, Poznań, Poland

³ Faculty of Biological Sciences, University of Zielona Góra, Zielona Góra, Poland

that oviposition preference and offspring performance could differ depending on the insect guild (chewing or piercing-sucking) and fungus lifestyle (necrotrophic, biotrophic or endophytic).

The horse chestnut leaf miner (*Cameraria ohridella* Deschka & Dimić) has achieved great ecological success. From historical herbarium data (Lees et al. 2011), we know that the miner was causing outbreaks before its taxonomic identification in 1984 (Deschka and Dimić 1986). Since the late 1980s it has spread throughout the European range of its main host plant—*Aesculus hippocastanum* L. (Šefrová and Laštůvka 2001; Rämert et al. 2011). A recent report indicated its invasion in Kazakhstan (Gninenko et al. 2017). Polyphagous parasitoids are ineffective at controlling this herbivore (Kenis et al. 2005; Ferracini and Alma 2007) and studies focused on chemical control methods by systemic application of insecticides have yielded promising results (Ferracini and Alma 2008; Jagiełło et al. 2018). Horse chestnut leaf miner is an oligophagous species and may complete a full generation on few tree species, mainly from the genus *Aesculus*, such as *A. hippocastanum*, *A. turbinata* Blume (D’Costa et al. 2014) and *A. glabra* Willdenow (Walczak et al. 2017), but also on *Acer pseudoplatanus* L. (Péré et al. 2010), which belongs to the same order as *Aesculus* spp. (*Sapindales*). Péré et al. (2010) indicated that females of *C. ohridella* may also oviposit on many species beyond those in *Sapindales* but mostly on trees located near *A. hippocastanum* and with a much lower frequency than on *A. hippocastanum*.

Until the invasion by *C. ohridella*, the fitness of the *Aesculus* genus was already poor due to leaf blotch disease. The agent of this disease, *Phyllosticta paviae* Desm., was initially found in France on *A. parviflora* Walter (Desmazières 1847). Desmazières (1847) described symptoms of the fungal disease as red-brown necroses with yellow edges (on the adaxial and abaxial leaf surfaces) and the presence of pseudothecia. Stewart (1916) used a synonymic name for this phytopathogen (*Guignardia aesculi* (Peck) Stewart) and stated that it was the most important disease of *A. glabra* and *A. parviflora* in North America. The disease is widely distributed in Europe (Hudson 1987; Phillips and Burdekin 1992) on *A. hippocastanum* and other not specified *Aesculus* species (Pastirčáková et al. 2009). In effect, two species infecting one host plant creates a specific interaction, and few studies report the co-occurrence of *C. ohridella* and *P. paviae* (Kraus 1996; Heitland et al. 1999; Bhatti et al. 2013; Gninenko et al. 2017; Jagiełło et al. 2017). However, it is difficult to unambiguously answer the question of whether the two species had the opportunity to interact before the horse chestnut leaf miner invasion in Europe. *Phyllosticta paviae* has a very similar ecological niche to *C. ohridella*. The fungal pathogen may come into contact with previously mined leaf areas, but feeding larvae have no such opportunity and

cannot spread the necroses because hyphal mycelia utilise the whole leaf. Thus, the choice of a site by a female during egg deposition is crucial for the offspring. In a study by Jagiełło et al. (2017), the authors indicated that defoliation of *A. hippocastanum* saplings was higher when only fungal infection occurred than when saplings were occupied by both pests, and this pattern was most likely related to the higher content of condensed tannins in the leaves of the latter. In turn, Johné et al. (2008) recognised that volatile organic compounds emitted by leaves of *A. hippocastanum* and *A. x carnea* Hayne in response to fungal infections by *P. paviae* and *Erysiphe flexuosa* (Peck) Braun and Takam stimulated *C. ohridella* antennae. In their experiments, females less frequently deposited eggs on leaves with attached filter paper soaked with compounds emitted by leaves of *A. hippocastanum* in response to infection by *P. paviae*. However, the importance of other factors in the females’ decision-making process (to deposit eggs or not), in near-natural conditions (for instance, realistic leaf blotch symptoms and variation in leaflet area), is still unknown. Laboratory and greenhouse experiments were designed to answer one question: do females of *C. ohridella* deposit eggs on healthy leaflets more frequently than on infected ones?

Materials and methods

Plant material

In the spring of 2017, 2-year-old horse chestnut saplings ($n = 40$) growing in 15-L pots containing sieved, composted soil well mixed with neutral (pH of ~ 7.0) peat (7/3, v/v) were put into four greenhouses, with ten plants in each greenhouse (five control and five infected in the manner described hereunder). This design was used to keep leaves free from eggs and mines of *C. ohridella* 1st generation and to allow fungal infection development. These greenhouses, built from polycarbonate, were covered by shading net (75% light reduction, BQM quantum meter, Apogee Instruments Inc.) hung outside for additional prevention of the entrance of leaf miners during the 1st generation. At the beginning of May, when saplings had developed full foliage, leaves of 20 saplings (five in each greenhouse) were inoculated with *P. paviae* by a suspension prepared in the following manner. Leaf litter collected from an experimental plot where infection by *P. paviae* was confirmed by molecular analyses (Jagiełło et al. 2017) was mixed with purified water. The concentration of ascospores in the derived suspension was not measured. Then, leaves were sprayed using this solution, and control saplings were sprayed with only purified water. Suspensions were evenly distributed on all leaves of each sapling with a standard hand sprinkler.

Fig. 1 Copulation of *Cameraria ohridella* in a tube. Photograph taken with a Zeiss (Göttingen, Germany) AxioCam ERc5s attached to a Stemi 2000-C microscope (scale bar 1 mm)

Insect specimens

Development of the 1st generation of the *C. ohridella* was observed on *A. hippocastanum* in an alley located 1.5 km from the location of the experiments. At the end of June, the *C. ohridella* mating flights were observed. When the flights started, approximately 200 leaves with pupae inside leaf mines were collected from randomly chosen trees. The collected leaves were divided into two groups. One group was used for the greenhouse experiment and put into two greenhouses on the same day. The second group of leaves was carried to the laboratory and searched for *C. ohridella* pupae. Each collected pupa was placed in a transparent, polystyrene tube (5 ml volume) and enclosed with a cap and kept under room conditions. The sex of each specimen was determined based on the characteristics described by Freise and Heitland (1999). Tubes with insects were examined every day in the morning for an indication of freshly emerged imaginal stages. When an imago was observed, one male and one female were put together into one tube and observed for indication of mating (Fig. 1). The tubes in which mating was observed were used for the box experiment.

Box experiment

The box experiment was designed to answer the following question: does the presence of leaf blotch symptoms on leaflets influence the oviposition preference of 2nd generation *C. ohridella* females (number of chosen leaflets and relative number of deposited eggs)? The experiment started on 29 June 2017, when the first pairs of adult insects after mating were obtained (see “[Insect specimens](#)” section for details), and replicates were established

Fig. 2 Design of a replicate for the box experiment. The leaf on the left side was collected from a healthy plant. On the opposite side, a leaf collected from an infected plant with visible chocolate-brown necroses with yellow edges is shown. Inside the box is an opened tube where specimens of *Cameraria ohridella* copulated before being placed in the box

individually as insects became available after mating. One hole (3 mm in diameter) was made on each of the two opposite sides of transparent, flat polystyrene boxes ($L \times W \times H$ of $270 \times 270 \times 100$ mm, ~ 7.3 L). For each box, one leaf was collected from a control sapling and one from a leaf-blotch-infected sapling growing in greenhouses not included in the greenhouse experiment (described in the next paragraph). Infected leaves were selected in such a way that, on as many leaflets as possible, leaf blotch symptoms (chocolate-brown necroses) were present. Then, control leaves were selected based on two criteria: (1) the same number of leaflets as on the infected leaf and (2) a similar leaf area based on preliminary visual assessment. Petioles were inserted through the holes in the box, with the leaf blades inside the box and the petiole sticking out of the box. The space surrounding the petiole in the hole was stuffed with cotton wool to prevent insects from escaping. A portion of the leaf petiole section sticking out of the box was put inside a tube with water to maintain the appropriate turgor pressure in the leaf. The water level in the tubes was controlled, and the tubes were refilled when needed. Finally, the enclosed tube containing insects that had mated was put into a box with leaves prepared in the abovementioned manner (Fig. 2), and the box was closed after ensuring that the insect had not escaped during the procedure. In total, 36 boxes were established for the experiment. Leaves were collected from boxes for inspection after confirmation of the insects' death.

Greenhouse experiment

The greenhouse experiment was designed to address the following question: do females deposit eggs less frequently on leaves infected by leaf blotch if they have to choose between infected and healthy leaves? Two greenhouses were used for the experiment, with five control and five infected plants in each ($n = 20$ plants in total). The experiment started on 26 June 2017. Similar numbers of leaves occupied by *C. ohridella* pupae (detailed description in “Insect specimens” section) were put into the greenhouses without any assessment of the number of insect specimens. Before oviposition started, two leaves on each sapling ($n = 40$ in total) were chosen randomly and marked permanently (small dot on the petiole marked with a felt-tip pen). This marking was performed to avoid confirmation bias during the collection of leaves. There was one criterion for leaves on infected saplings during selection: leaf blotch symptoms (chocolate-brown necroses) had to be present on as many leaflets as possible. Leaves were collected for inspection after 2 weeks.

Data collection

Leaves collected from both experiments were thoroughly inspected under a microscope (Stemi 2000-C, Zeiss, Göttingen, Germany), and eggs deposited on the adaxial leaf surface were counted. For the greenhouse experiment, the number of replicates was standardised: one or two outer leaflets were rejected if a leaf comprised six or seven leaflets, respectively. As a result, 200 leaflets from 40 compound leaves from the greenhouse experiment and 366 leaflets from 72 compound leaves from the box experiment were inspected. After inspection, the leaflets were scanned (Epson Perfection V700 Photo; 200 dpi = 62 pixels mm⁻²). The area of leaflets was measured in WinFOLIA 2013 Pro software (Regent Instruments, Quebec, Canada) with the use of colour analysis based on colour sets determined by the operator for three layers (background, healthy and damaged). Hence, the total leaflet area (TLA), healthy leaflet area (HLA) and leaflet area covered by leaf blotch spots were obtained for each leaflet. If mines were present, they were covered in the digitised material with a colour representing healthy tissue before analyses.

Statistical analyses

The number of leaflets chosen by a female (in other words, the number of leaflets with at least one deposited egg) in each box was explained by two fixed effects: the number of available leaflets (control and infected counted separately for each box) and the experimental variant (nominal variable with two levels: control or infected). For this purpose, a generalised linear mixed model (GLMM) with a Poisson

distribution of the dependent variable and a log link function was used. The box was considered a random effect. The relationship between the number of leaflets chosen by a female and the total number of eggs deposited on all leaflets in each box (realised fecundity, $\ln$ transformed) was modelled with a linear model. The number of eggs deposited on each leaflet was modelled using GLMMs (negative binomial distribution, log link function) with two fixed effects: experimental variant (nominal variable with two levels: control and infected) and leaflet area (continuous variable), and two random effects: box and leaf nested within box. Two analyses were performed: in the first, TLA, and in the second, HLA was considered a covariate (leaflet area). Two additional GLMMs with zero inflation were built, but in both cases, the zero inflation effect was not significant and did not improve the models (Akaike information criterion), and the results from the previously mentioned GLMMs were taken into consideration. The number of eggs deposited on leaflets in the greenhouse experiment was analysed using GLMMs (negative binomial distribution, log link function). Experimental variant (control or infected) and leaflet area were considered fixed effects, and greenhouse, sapling and leaflet nested within sapling were considered random effects. Two analyses similar to those conducted with data from the box experiment were carried out: in the first, TLA, and in the second, HLA was considered a covariate (leaflet area). Statistical analyses were performed in R 3.4.4 (R Development Core Team 2018) using the glmmPQL (MASS) function to build GLMMs (Venables and Ripley 2002), anova.lme (nlme) for F tests of fixed effects (Pinheiro et al. 2018) and zeroinfl (pscl) to build GLMMs with a zero-inflated distribution of the dependent variable (Zeileis et al. 2008).

Results

Symptoms of fungal activity (red-brown necrotic lesions) became apparent in early June on the sprayed leaves (infected variant), and no symptoms were observed on the control leaves. The presence of *P. paviae* was confirmed by the presence of pycnidia (Fig. 3a, b), which produced ovoid and hyaline conidia, 11–15 $\mu\text{m} \times 7$ –9 μm in size (Fig. 3b). Females of *C. ohridella* always oviposited in leaf depressions, most often on 2nd- and 3rd-order veins (Fig. 3c, d) and seldom on/near the midrib. Generally, only a few eggs were observed on leaf areas covered by necrosis, and it is possible that some of those eggs were deposited on healthy areas later damaged by the fungus, because all of those eggs were located on the margins of necroses.

The box experiment consisted of 36 replicates (boxes). Females deposited eggs on leaflets in 21 of the replicates and no eggs were observed on 62 of the 215 total leaflets from the boxes. Within those replicates, necrosis on infected

Fig. 3 *Phyllosticta paviae* and *Cameraria ohridella* on the adaxial side of leaflets of *Aesculus hippocastanum* saplings from the experiment: **a** surface with necrosis and visible pycnidia (scale bar 0.4 mm), **b** pycnidium of the *P. paviae* anamorph (scale bar 0.05 mm), **c** eggs of *C. ohridella* deposited on 2nd- and 3rd-order leaf veins, with the egg on the latter already hatched and causing a visible leaf mine (scale bar 1 mm), and **d** developing larvae of *C. ohridella* in leaf mines from eggs deposited very close to necroses caused by *P. paviae* (scale bar 5 mm). Photographs were taken with a Zeiss (Göttingen, Germany) AxioCam ERc5s attached to a Stemi 2000-C or Primo Star (only **b**) microscope

leaflets ($n=90$) covered, on average, 31% (95% confidence interval (CI) 26.3–35.8%) of the total leaflet area. Realised fecundity estimated as the number of observed eggs on all leaflets in each box ranged from 2 to 104 (on average, 37 ± 6 SE, $n=21$). Regardless of the presence of leaf blotch symptoms, this feature was strongly correlated with the number of leaflets in the box (Fig. 4). The number of chosen leaflets, where at least one egg was deposited by a female, was not related to the presence of leaf blotch symptoms ($F_{(1,19)}=2.67$, $p=0.12$) but was related to the number of exposed leaflets ($F_{(1,19)}=4.93$, $p=0.04$). In both GLMMs we built (first: TLA, second: HLA), the covariate consisting of an area parameter but not the presence of leaf blotch symptoms strongly explained the number of deposited eggs (TLA: $F_{(1,171)}=68.0$, $p<0.001$; HLA: $F_{(1,171)}=64.3$, $p<0.001$). The variable representing the experimental variant was statistically significant in the model with covariate values for TLA ($F_{(1,171)}=4.80$, $p=0.03$), but when it was substituted by HLA, the variability explained by the experimental variant decreased and became nonsignificant ($F_{(1,171)}=0.03$, $p=0.87$). In the greenhouse experiment, necrosis of infected leaflets ($n=100$) covered on average

22.5% (95% CI 19.0–26.1%) of the TLA. In this experiment, where the number of deposited eggs resulted from the oviposition of an unknown number of females, the results were similar to those from the box experiment. The model including TLA (Fig. 5a) yielded $p=0.06$ for the factor “treatment”. The second model, including the truly available area at the time of leaf collection (HLA) instead of TLA, did not reject the null hypothesis about the lack of a treatment influence (Fig. 5b).

Discussion

Based on our results, we have no reason to reject the statistical null hypotheses that there is no oviposition preference for healthy or leaf-blotch-infected leaflets. Females of *C. ohridella* chose spots for egg deposition independently of the presence of visible leaf blotch symptoms (red-brown necroses). We have indicated that (1) leaflet area is a factor explaining the number of deposited eggs, and (2) realised fecundity is a factor explaining the number of chosen leaflets. However, the location of oviposition on a leaflet

Fig. 4 Relationship between the number of deposited eggs and the number of chosen leaflets in the box experiment ($n=21$). Data points are transparent to indicate where they overlap. The grey quadrangle represents the region predicted by the model. The pink area represents an area where the possible relationship is not covered by the data points and model. The results for significance (t test) of model parameters are as follows: intercept ($t=-0.65$, $p=0.52$); $\ln(\text{DE})$ (95% CI 2.11–2.78, $t=15.3$, $p<0.001$)

is specific. Females deposit eggs directly on leaf veins and avoid areas with necroses. Because we did not inspect the abaxial leaf side, we cannot unambiguously say that females oviposit on only the adaxial side under such experimental conditions. However, oviposition on the latter side is biologically plausible, because the 1st instar behaves as a sap feeder (Kosibowicz 2005). Based on our results, we cannot

explain the causes of avoidance of egg deposition on damaged leaf areas; however, this result does lead to a question: does a female recognise damaged leaf areas by vision, smell or touch?

Cameraria ohridella is diurnally active while mating (Skuhřavý 1999; Kalinová et al. 2003). An analysis of *C. ohridella* eye ultrastructure indicated that they are intermediate to apposition and superposition eyes (Fischer et al. 2012), which calls into question the importance of the sense of sight. For instance, colour and shape are factors strongly affecting the landing preference of *Apis cerana* F. (Hymenoptera: Apidae) (Guez et al. 2017). However, it is difficult to answer the question of whether sight rather than the other senses helps during leaflet penetration and acceptance of a location for egg deposition. Johné et al. (2008) convincingly indicated a repellent role of synthetic volatile organic compounds (VOCs), which were found to be emitted by leaves of *A. hippocastanum* in response to fungal infections (*P. paviae* and/or *E. flexuosa*). In their laboratory tests, females deposited fewer eggs when such substances (1-octen-3-ol, benzyl alcohol and dodecane) were present. On the other hand, there were no differences in methyl salicylate and tri-decane, which were also emitted by leaves in response to *P. paviae* infection. The results of our experiments in which the fungal activity of *P. paviae* manifested as visible necroses are in contrast to the results from Johné et al. (2008). Wist and Evenden (2016) investigated the preference and performance of *Caloptilia fraxinella* Ely (Lepidoptera: Gracilariidae), a species taxonomically related to *C. ohridella*. The authors studied the preference and performance of *C. fraxinella* on two related host plants (*Fraxinus nigra* Marshall and *F. pennsylvanica* Marshall) and found an inconsistency between laboratory (wind tunnel) and field preference tests. This finding suggests that results from laboratory tests should not be exclusively taken into account when drawing

Fig. 5 Relationship between the number of deposited eggs on leaflets from different experimental variants (“treatment” fixed effect with two levels: control and infected) and total leaflet area (a) or healthy leaflet area (b). Each point represents one leaflet. The shaded area covers 90% of observations for the respective treatments. The results of F tests for fixed effects of generalised linear mixed models are presented in each chart (n.s. $p>0.05$, $*p<0.05$, $***p<0.001$)

conclusions about the preference of insect species. Considering the results published in the study by Johné et al. (2008), we should conclude that other cues are also, if not more, important for the decision made by *C. ohridella* females during oviposition. Roessingh and Städler (1990) conducted several tests with a set of simulated physical stimulants (i.e. shape, size, colour, surface cover or 3D folding) using leaf models made of paper and found that they had an impact on the oviposition behaviour of *Delia radicum* (Diptera: Anthomyiidae) L. The authors found that this insect preferred to oviposit on leaf models with bright green and yellow colours rather than red or blue (1), laid more eggs on models with a greater area (2) and preferred models with vertical folds rather than those with folds set horizontally (3). The first two results from the Roessingh and Städler (1990) study are consistent with our results. The density and length of trichomes are other features affecting oviposition preference. Although these features were not taken into account in our study, it is worth mentioning results published in a study by Coapio et al. (2018). The results indicated that *Trichoplusia ni* (Lepidoptera: Noctuidae) Hübner females preferred leaf surfaces with lower densities of trichomes. The other issue is why females avoid leaf surfaces covered by necrosis. A leaf blotch agent utilising leaf tissue changes the leaf surface, and as a result, spots encompassed by necrosis may be unacceptable for this reason alone. In a study, Fernandez-Conradi et al. (2018) concluded that insects (chewing and piercing-sucking feeding guilds) preferred healthy hosts rather than those infected by necrotrophic pathogens, which is the opposite of our result. However, in the abovementioned review, the leaf miner guild was defined very narrowly.

Leaflet area was the variable explaining the number of deposited eggs in both experiments, with a positive correlation. From a clear, logical point of view, it is possible that the probability of finding a leaflet and a suitable place for offspring increases with an increase in leaflet area. An experiment conducted by Faeth (1991) indicated that *Cameraria agrifoliella* (Braun) females most often deposit eggs singularly and seldom deposit eggs on leaves with smaller areas. Such a relationship was also observed by Preszler and Price (1995), who investigated *Phyllonorycter* sp., belonging to the same family as *C. ohridella* and *C. agrifoliella* (Lepidoptera: Gracillariidae). Taking into account the values of realised fecundity from our experiment (on average, 37 ± 6 SE, $n=21$), values reported by other authors (Girardoz et al. 2007; 34 and 82 eggs on average, depending on the survey year), and the abovementioned examples (Faeth 1991; Preszler and Price 1995), we can assume that *C. ohridella* females are spreading the risk by such behaviour. Although unanticipated, in our opinion, this convincing result about the nature of the relationship between the number of deposited eggs and the number of leaflets chosen by *C. ohridella* females (Fig. 4) supports such a conclusion.

Leaf mining has many advantages: a lower risk of predation, better hygrothermal conditions and the avoidance of plant defences (Connor and Taverner 1997), but low mobility causes female choice of oviposition location to be highly important for offspring performance. We put forward the assumption that the interaction between *C. ohridella* and *P. paviae* will lead to competition for similar resources, and fungal infection will have the ability to occupy previously mined areas of a leaf and *vice versa* (R. Jagiełło personal observation). To date, no data have been published on this particular interaction and with a focus on *C. ohridella* performance. In the previously cited study (Fernandez-Conradi et al. 2018), the authors concluded that the performance of insects interacting with necrotrophic phytopathogens was generally less affected than their preference. Hence, we have no arguments to support or reject the preference–performance hypothesis (PPH), which is actually the leading theory (Gripenberg et al. 2010) accounting for the oviposition preferences of insects. There are instead premises about the negative effects of *C. ohridella* on the fungal activity of *P. paviae* (Jagiełło et al. 2017). However, host-plant quality may be less important when females are distributing eggs on more than one leaflet and the risk-spreading strategy is key. On the other hand, PPH is well suited to *C. ohridella* when the host-plant species is taken into consideration because the most preferred trees, i.e. *A. hippocastanum* and *A. turbinata*, are also the most suitable (Rämert et al. 2011; D’Costa et al. 2013, 2014), and other species are oviposited on relatively less frequently.

Conclusions

In this study, we put forward a question: do females of *Cameraria ohridella* deposit eggs on healthy leaflets more frequently than on infected ones? Based on the results of two experiments, we can confidently say that: no, they do not. We found two cues that are important at least during the oviposition process (availability of leaf depressions and healthy leaf area) and we have no reason to add the presence of leaf blotch symptoms to this list. In view of our results, the presence of leaf blotch symptoms may decrease the oviposition preference only by reducing the abovementioned leaflet area. We found inconsistency between our results and those from a previously published study, where only VOCs were under experimental control. This example leads to a conclusion about designing such experiments. We recommend conducting experiments in conditions similar to those met in nature, because synergistic action of more than one factor may be responsible for the observed behaviour of insects. From the plant point of view, the oviposition pattern of *C. ohridella* creates a risk of complex *Aesculus hippocastanum* disease origination because the insect is utilising previously

unexploited leaf areas. Thus, in *C. ohridella*, females are spreading the risk by ovipositing on many leaflets rather than avoiding a potential decrease in offspring survival.

Acknowledgements The research was financially supported by the National Science Centre, Poland (Grant No. 2012/07/B/NZ9/01315). We thank the anonymous reviewers for their insightful comments and suggestions.

Data Availability The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

- Bhatti I, Ozanne C, Shaw P (2013) Parasitoids and parasitism rates of the horse chestnut leaf miner *Cameraria ohridella* Deschka and Dimić [Lepidoptera: Gracillariidae] across four sites in south-west London. *Arboric J* 35:147–159. <https://doi.org/10.1080/03071375.2013.813706>
- Coapio GG, Cruz-López L, Guerenstein P, Malo EA, Rojas JC (2018) Oviposition preference and larval performance and behavior of *Trichoplusia ni* (Lepidoptera: Noctuidae) on host and nonhost plants. *Arthropod Plant Interact* 12:267–276. <https://doi.org/10.1007/s11829-017-9566-9>
- Connor EF, Taverner MP (1997) The evolution and adaptive significance of the leaf-mining habit. *Oikos* 79:6–25. <https://doi.org/10.2307/3546085>
- D'Costa L, Koricheva J, Straw N, Simmonds MSJJ (2013) Oviposition patterns and larval damage by the invasive horse-chestnut leaf miner *Cameraria ohridella* on different species of *Aesculus*. *Ecol Entomol* 38:456–462. <https://doi.org/10.1111/een.12037>
- D'Costa L, Simmonds MSJJ, Straw N, Castagneyrol B, Koricheva J (2014) Leaf traits influencing oviposition preference and larval performance of *Cameraria ohridella* on native and novel host plants. *Entomol Exp Appl* 152:157–164. <https://doi.org/10.1111/eea.12211>
- Deschka G, Dimić N (1986) *Cameraria ohridella* sp. n. (Lep., Lithocolletidae) aus Mazedonien, Jugoslawien. *Acta Entomol Jugoslavica* 22:11–23
- Desmazières J (1847) Sur les plantes cryptogames récemment découvertes en France. In: Edwards M, Brongniart MA, Decaisne J (eds) *Annales des sciences naturelles. Botanique*. Victor Masson, Paris, pp 9–37
- Faeth SH (1991) Effect of oak leaf size on abundance, dispersion, and survival of the leafminer *Cameraria* sp. (Lepidoptera: Gracillariidae). *Environ Entomol* 20:196–204. <https://doi.org/10.1093/ee/20.1.196>
- Fernandez-Conradi P, Jactel H, Robin C, Tack AJM, Castagneyrol B (2018) Fungi reduce preference and performance of insect herbivores on challenged plants. *Ecology* 99:300–311. <https://doi.org/10.1002/ecy.2044>
- Ferracini C, Alma A (2007) Evaluation of the community of native eulophid parasitoids on *Cameraria ohridella* Deschka and Dimic in urban areas. *Environ Entomol* 36:1147–1153
- Ferracini C, Alma A (2008) How to preserve horse chestnut trees from *Cameraria ohridella* in the urban environment. *Crop Prot* 27:1251–1255. <https://doi.org/10.1016/j.cropro.2008.03.009>
- Fischer S, Müller CHG, Meyer-Rochow VB (2012) Neither apposition nor superposition: the compound eyes of the chestnut leafminer *Cameraria ohridella*. *Zoomorphology* 131:37–55. <https://doi.org/10.1007/s00435-011-0141-0>
- Freise J, Heitland W (1999) A brief note on sexual differences in pupae of the horse-chestnut leaf miner, *Cameraria ohridella* Deschka and Dimic (1986) (Lep., Gracillariidae), a new pest in Central Europe on *Aesculus hippocastanum*. *J Appl Entomol* 123:191–192. <https://doi.org/10.1046/j.1439-0418.1999.00343.x>
- Girardoz S, Quicke DLJ, Kenis M (2007) Factors favouring the development and maintenance of outbreaks in an invasive leaf miner *Cameraria ohridella* (Lepidoptera: Gracillariidae): a life table study. *Agric For Entomol* 9:141–158. <https://doi.org/10.1111/j.1461-9563.2007.00327.x>
- Gninenko YI, Muhamadiev NS, Ashikbaev NZ (2017) *Cameraria ohridella*: the first record in Central Asia. *Russ J Biol Invasions* 8:10–13. <https://doi.org/10.1134/S2075111717010027>
- Gripenberg S, Mayhew PJ, Parnell M, Roslin T (2010) A meta-analysis of preference-performance relationships in phytophagous insects. *Ecol Lett* 13:383–393. <https://doi.org/10.1111/j.1461-0248.2009.01433.x>
- Guez D, Subias L, Griffin AS (2017) Colour and shape preferences of *Apis cerana* (Java genotype) in Australia. *Bull Insectology* 70:267–272
- Hatcher PE (1995) Three-way interactions between plant pathogenic fungi, herbivorous insects and their host plants. *Biol Rev* 70:639–694. <https://doi.org/10.1111/j.1469-185X.1995.tb01655.x>
- Heitland W, Kopelke J-P, Freise J, Metzger J (1999) Ein Kleinschmetterling erobert Europa die Roßkastanien-Miniermotte *Cameraria ohridella*. *Nat Mus* 129:186–195
- Hudson HJ (1987) *Guignardia* leaf blotch of horsechestnut. *Trans Br Mycol Soc* 89:400–401
- Jaenike J (1978) On optimal oviposition behavior in phytophagous insects. *Theor Popul Biol* 14:350–356. [https://doi.org/10.1016/0040-5809\(78\)90012-6](https://doi.org/10.1016/0040-5809(78)90012-6)
- Jagiełło R, Baraniak E, Karolewski P, Łakomy P, Behnke-Borowczyk J, Walczak U, Giertych MJ (2017) Ecophysiological aspects of the interaction between *Cameraria ohridella* and *Guignardia aesculi* on *Aesculus hippocastanum*. *Dendrobiology* 78:146–156. <https://doi.org/10.12657/denbio.078.014>
- Jagiełło R, Walczak U, Iszkuło G, Karolewski P, Baraniak E, Giertych MJ (2018) Impact of *Cameraria ohridella* on *Aesculus hippocastanum* growth and long-term effects of trunk injection with pesticides. *Int J Pest Manag* 65:33–43. <https://doi.org/10.1080/09670874.2018.1454630>
- Johne AB, Weissbecker B, Schütz S (2008) Approaching risk assessment of complex disease development in horse chestnut trees: a chemical ecologist's perspective. *J Appl Entomol* 132:349–359. <https://doi.org/10.1111/j.1439-0418.2008.01283.x>
- Kalinová B, Svatoš A, Kindl J, Hovorka O, Hrdý I, Kuldová J, Hoskovec M (2003) Sex pheromone of horse-chestnut leafminer *Cameraria ohridella* and its use in a pheromone-based monitoring system. *J Chem Ecol* 29:387–404. <https://doi.org/10.1023/A:1022686029051>
- Kenis M, Tomov R, Svatos A et al (2005) The horse-chestnut leaf miner in Europe—prospects and constraints for biological control.

- In: Proceedings of the second international symposium on biological control of arthropods. pp 77–90
- Kosibowicz M (2005) Szrotówek kasztanowcowiaczek *Cameraria ohridella* Deschka & Dimić (Lepidoptera, Gracillariidae), nowy inwazyjny szkodnik kasztanowca białego *Aesculus hippocastanum* L. w Polsce—biologia i metody zwalczania. Leśne Pr Badaw 66:121–132 (in Polish with English abstract)
- Kraus M (1996) Erste Nachweise der eingeschleppten Kastanien-Miniermotte *Cameraria ohridella* Deschka et Dimic (Lep., Gracillariidae) in Mittelfranken, Bayern. Berichte Kreises Nürn Entomol 12:82–84
- Lees DC, Lack HW, Rougerie R, Hernandez-Lopez A, Raus T, Avtzis ND, Augustin S, Lopez-Vaamonde C (2011) Tracking origins of invasive herbivores through herbaria and archival DNA: the case of the horse-chestnut leaf miner. Front Ecol Environ 9:322–328. <https://doi.org/10.1890/100098>
- Mangel M, Clark CW (1986) Towards a unified foraging theory. Ecology 67:1127–1138. <https://doi.org/10.2307/1938669>
- Pastirčáková K, Pastirčák M, Celar F, Shin H-D (2009) *Guignardia aesculi* on species of *Aesculus*: new records from Europe and Asia. Mycotaxon 108:287–296. <https://doi.org/10.5248/108.287>
- Péré C, Augustin S, Turlings TCJ, Kenis M (2010) The invasive alien leaf miner *Cameraria ohridella* and the native tree *Acer pseudoplatanus*: a fatal attraction? Agric For Entomol 12:151–159. <https://doi.org/10.1111/j.1461-9563.2009.00462.x>
- Phillips DH, Burdekin DA (1992) Diseases of other broadleaved trees. In: Phillips DH, Burdekin DA (eds) Diseases of forest and ornamental trees, 2nd edn. Palgrave Macmillan, London, pp 396–431
- Pinheiro J, Bates D, DebRoy S, Sarkar D (2018) {nlme}: linear and nonlinear mixed effects models. <https://cran.r-project.org/package=nlme>
- Preszler RW, Price PW (1995) A test of plant-vigor, plant-stress, and plant-genotype effects on leaf-miner oviposition and performance. Oikos 74:485–492. <https://doi.org/10.2307/3545994>
- R Development Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Rämert B, Kenis M, Kärnestam E, Nyström M, Rännbäck L-M (2011) Host plant suitability, population dynamics and parasitoids of the horse chestnut leafminer *Cameraria ohridella* (Lepidoptera: Gracillariidae) in southern Sweden. Acta Agric Scand Sect B 61:480–486. <https://doi.org/10.1080/09064710.2010.501341>
- Roessingh P, Städler E (1990) Foliar form, colour and surface characteristics influence oviposition behaviour in the cabbage root fly *Delia radicum*. Entomol Exp Appl 57:93–100. <https://doi.org/10.1111/j.1570-7458.1990.tb01419.x>
- Schoonhoven LM, van Loon JJA, Dicke M (2012) Host-plant selection: when to accept a plant. In: Schoonhoven LM, Van Loon JJA, Dicke M (eds) Insect-plant biology, 2nd edn. Oxford University Press, New York, pp 169–208
- Šefrová H, Laštůvka Z (2001) Dispersal of the horse-chestnut leafminer, *Cameraria ohridella* Deschka & Dimić, 1986, in Europe: its course, ways and causes (Lepidoptera: Gracillariidae). Entomol Zeitschrift 111:194–198
- Skuhravý V (1999) Zusammenfassende Betrachtung der Kenntnisse über die Rosskastanienminiermotte, *Cameraria ohridella* Desch. (Lep., Gracillariidae). J Pest Sci 72:95–99. <https://doi.org/10.1007/BF02768917>
- Stewart VB (1916) The leaf blotch of horsechestnut. Phytopathology 6:5–19
- Tack AJM, Dicke M (2013) Plant pathogens structure arthropod communities across multiple spatial and temporal scales. Funct Ecol 27:633–645. <https://doi.org/10.1111/1365-2435.12087>
- Venables WN, Ripley BD (2002) Modern applied statistics with S, Fourth. Springer, New York
- Walczak U, Baraniak E, Zduniak P (2017) Survival, body mass and potential fecundity of the invasive moth *Cameraria ohridella* (Lepidoptera: Gracillariidae) on its original host plant *Aesculus hippocastanum* and *Aesculus glabra*. Eur J Entomol 114:295–300. <https://doi.org/10.14411/eje.2017.036>
- Wist TJ, Evenden ML (2016) Host plant preference and offspring performance of a leaf-mining moth, *Caloptilia fraxinella*, on two *Fraxinus* species. Entomol Exp Appl 159:311–326. <https://doi.org/10.1111/eea.12442>
- Zeileis A, Kleiber C, Jackman S (2008) Regression models for count data in R. J Stat Softw 27:1–25. <https://doi.org/10.18637/jss.v027.i08>

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