Survival, body mass and potential fecundity of the invasive moth *Cameraria ohridella* (Lepidoptera: Gracillariidae) on its original host plant *Aesculus hippocastanum* and *Aesculus glabra*

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**Abstract.** Performance of the invasive horse-chestnut leaf miner, *Cameraria ohridella* Deschka & Dimic, 1986 (Lepidoptera: Gracillariidae), was studied on two host plants: the white-flowering horse-chestnut *Aesculus hippocastanum* L. and the Ohio buckeye *Aesculus glabra* Willd. *C. ohridella* developed successfully on both host plants; however, mine density and survival were much higher on *A. hippocastanum* than on *A. glabra*. The pupal mass and potential fecundity were strongly affected by the host plant on which the larvae fed. On *A. hippocastanum* pupae were significantly heavier and females more fecund than those on *A. glabra*. Furthermore, on both host plants there was a significant positive correlation between the number of oocytes in ovaries and pupal body mass, and as a consequence, heavier females produced more eggs. Our study demonstrates that the mine density, survival, pupal mass and potential fecundity were significantly lower on *A. glabra* than on *A. hippocastanum*. The observed lower performance of *C. ohridella* on the exotic host plant was assumed to be due to its poor food quality (nutritional and chemical composition).

**INTRODUCTION**

Performance of herbivorous insects (e.g. development time, survival, pupal mass, fecundity) is strongly affected by the quality of the host plant upon which they feed (Awmack & Leather, 2002; Wetzel et al., 2016). Phylogenetically related plants are more likely to be similar in structural, chemical and nutritional traits, and therefore novel host plants are suitable for colonization if they are related to the native host (Connor at al., 1980; Odegaard et al., 2005; Pearse & Hipp, 2009; Bertheau et al., 2010; Ness et al., 2011). However, in some cases remarkably distantly related novel host plants may be utilized (Ronquist & Liljeblad, 2001).

The horse-chestnut leaf miner, *Cameraria ohridella* Deschka & Dimic, 1986 (Lepidoptera: Gracillariidae), is a small leaf-mining moth of Balkan origin (Valade et al., 1986; Lees et al., 2011) that has spread rapidly into most European countries (Šefrová & Laštůvka, 2001; Augustin et al., 2009). In 1998 this species was recorded for the first time in Southern Poland (Labanowski & Soika, 1998; Wittenberg, 1998), since when it has spread all over this country. In Poland this species has three generations per year, and the last one overwinters in the pupal stage in dead leaves. The larvae of the first generation appear from the end of May to the end of June, those of the second generation from the middle of July to the middle of August, and those of the third generation from September to the middle of October (Baraniak et al., 2005). Its main host plant is the white-flowering horse-chestnut *Aesculus hippocastanum* L. ( Sapindaceae), which is native to Southeastern Europe and is a popular urban-tolerant tree widely cultivated in parks and along streets throughout the temperate zone. *C. ohridella* can also attack and develop on some other species of *Aesculus* (Grabenweger & Grill, 2000; Freise, 2001) and occasionally on *Acer pseudoplatanus* L. (Pere et al., 2010). Host suitability for the leaf miner is likely associated with the phylogenetic distance between species of the genus *Aesculus* (Straw & Tilbury, 2006; D’Costa et al., 2013). Two species in the section *Aesculus*, *A. hippocastanum* and the Japanese horse-chestnut *A. turbinata* Blume, are the most heavily infested. In contrast, species belonging to the sections *Macrothyrsus* and *Calothyrsus* are highly resistant. North American species belonging to the section *Pavia* show varying degrees of infestation, with a low or intermediate percentage of the larvae reaching the pupal or adult stages (Freise, 2001; D’Costa et al., 2013).
In subsequent studies, D’Costa et al. (2014) investigated the influence of physical and chemical leaf traits on the leaf miner’s preference and performance on its original and four exotic host plants. This study shows that *C. ohridella* laid eggs on all the hosts studied; however, only on *A. hippocastanum* and *A. turbinata*, which have very similar leaf traits, did it complete its development and have a similar performance. This study also indicates that leaf toughness, which is similar in *A. flava* Aiton, *A. chinensis* Bunge and *A. indica* (Wall. ex Camb.), may be one of the factors responsible for the reduced performance on these exotic hosts.

The current study focuses on the performance of *C. ohridella* on two host plants: *A. hippocastanum* and *A. glabra* Willd. An earlier study (Freise, 2001) has shown that *A. glabra* is the most suitable host among the species in the section *Pavia*, and on which the leaf miner can develop to the adult stage. However, no previous study has compared survival, pupal mass and fecundity on these hosts. Because these host plants belong to different evolutionary lineages within the same genus (Harris & Xiang, 2009; Harris et al., 2009, 2016) we hypothesized that they are presumably more different in their chemical and nutritional traits, and therefore the leaves of *A. glabra* are likely to be of a lower quality for the leaf miner. Consequently, we predict that the performance of *C. ohridella* on the exotic will be poorer than on its original host plant.

**MATERIALS AND METHODS**

**Study site**

The study was carried out at the Kórnik Arboretum of the Institute of Dendrology of the Polish Academy of Sciences (western Poland, 52°14´30˝ N, 17°05´44˝ E), which is the oldest arboretum in Poland. The collection contains about 3,000 species and varieties of trees and shrubs originating from temperate climate zones in the Northern Hemisphere. In this arboretum, we recorded *C. ohridella* for the first time in autumn 1999.

**Host plants**

We chose two *Aesculus* species for this study, both are not native to Poland. The white-flowering horse-chestnut, *A. hippocastanum* (section *Aescules*), is indigenous to Greece and the central Balkan Peninsula and has been introduced throughout Europe and North America. It was one of the first ornamental trees of foreign origin introduced into Poland, where it has been planted since the 17th century. The Ohio buckeye, *A. glabra* (section *Pavia*), is native to Southeastern and Central North America and is occasionally planted as an ornamental in Europe (Seneta & Dolatowski, 2004).

**Assessment of performance**

Only two out of 8 specimens of *A. glabra*, present in the collection of Kórnik Arboretum, were infested by horse chestnut leaf miner in 2003. All the specimens are of similar age and grow a short distance from each other. One tree was moderately infested, while on another we observed only a few mines. Therefore, in May 2003 only one specimen of *A. glabra*, and one nearby specimen of *A. hippocastanum* were selected for study. In 2008, still only two specimens of *A. glabra* were infested, and both were moderately infested. Consequently, two *A. glabra* trees and the three nearest *A. hippocastanum* trees were chosen for study. On 27 May 2003 and on 25–27 May 2008 from each tree we chose the first 5 encountered compound leaves (25 leaflets per tree) with at least a single mine of first generation larvae of *C. ohridella*. Leaves were marked with tape for later identification, and the position of the mines on the leaves was noted. All the marked first generation larvae were followed until death or the emergence of an adult. Then leaves were removed and each mine was assigned to one of the following categories (Connor, 1991; slightly modified): (1) successful emergence (presence of emergence hole or pupal evisceration), (2) preyed upon (predation by birds or arthropods), (3) parasitized (presence of emergence hole, larva, pupa or pupal evisceration of parasite) or host fed (the dead, flattened larva of the leaf miner adhering to the inner surface of the mine), or (4) dead of other causes (i.e., chemical unsuitability of the host plant, bacterial and fungal death, or intra-specific competition). The surface area of each mine was estimated using a transparent millimeter grid. In the next step the area of the leaflets of each monitored leaf was measured using ImageJ software (http://imagej.nih.gov/ij/), and mine density was estimated (number of mines per square centimeter of leaf area).

On 30 June 2015 from each tree (3 *A. hippocastanum* and 2 *A. glabra*) we randomly collected samples of 100 leaves infested with *C. ohridella* (first generation). In the laboratory the leaves were dissected and pupae were isolated and sexed under the stereomicroscope according to Freise & Heitland (1999). A maximum of 5 pupae were dissected per leaflet. Female pupae were weighed on a Sartorius semi-micro balance (CPA225D-OCE) to an accuracy of 0.00001 g. Then the pupae were individually transferred to glass vials that were closed with small pieces of sponge and stored at room temperature. Females were frozen within a day of emergence, pinned in a Petri dish coated with a Sylgard silicone elastomer and then dissected under Ringer’s solution. Ovaries were stained with a 0.5% solution of Evans blue in Ringer’s solution, and the number of oocytes in the ovaries counted.

**Data processing and analysis**

Several parameters of the two host plant species were compared: (a) density of mines on leaflets; (b) survival of the moth from larva to adult stage; (c) body mass of female pupae of the first generation, and (d) potential fecundity of females of the first generation in terms of the number of oocytes.

The density of mines was calculated for each leaflet, consisting of each leaf studied and expressed as the number of mines per square centimeter. To test the differences between the host species, a generalized linear mixed model (GLMM; Bolker et al., 2009) with Gamma distribution and log link function was used. Because the data from the individual leaflets are not independent, the hierarchical structure of the data (tree → leaflet → leaflet) was included in the model, with the tree species and year as fixed factors and the tree ID and leaf ID as random effects included in the model to account for the spatial dependence of the data collected. The survival of the moth from larva to adult was also compared between the host species by using a GLMM but with a binomial distribution and logit link function. Similar to the density analysis, the hierarchical structure of the data was included in the model, with the tree species, year and density of mines per leaflet as fixed factors and the tree ID and leaf ID as random effects.

The comparisons between the different species of host plant in terms of pupal body mass and potential fecundity, expressed as the number of oocytes, were performed using Student’s *t* test for independent samples and Bonferroni correction was applied to adjust alpha values for the increased probability of obtaining statistical significance from multiple testing. Furthermore, the Fisher-Freeman-Halton Exact test (Freeman & Halton, 1951) was used to check the differences in the distribution of the different causes of death in the mines on the two host plants. All calcula-
ifications were performed using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp. Released 2016). Throughout the text, mean values are presented with 95% confidence limits (CL).

RESULTS

Cameraria ohridella developed successfully on both host plants, for which 664 mines were recorded on A. hippocastanum and 45 on A. glabra. The mine density calculated for leaves with at least 1 mine was much higher on A. hippocastanum than on A. glabra (GLMM; whole model: $F_{2, 202} = 42.03, p < 0.001$, species: $F_{1, 202} = 84.03, p < 0.001$, Fig. 1a) and no effect of year was found ($F_{1, 202} = 1.18, p = 0.279$). Furthermore, the survival of the moth was higher on A. hippocastanum than on A. glabra (GLMM; whole model: $F_{3, 705} = 7.35, p < 0.001$, species: $F_{1, 705} = 21.02, p < 0.001$, Fig. 1b), was not affected by the mine density (fixed coefficient = $–3.52$, 95% CL: $–4.22–1.72$; $t = –3.34, p = 0.739$) and did not depend on year ($F_{1, 705} = 0.08, p = 0.782$).

A total of 396 (59.6%, CL: 55.8–63.4, $n = 664$) larvae on A. hippocastanum and 7 (15.6%, CL: 6.5–29.5, $n = 45$) on A. glabra survived to the adult stage.

The distribution of causes of mortality differed between host plants (Fisher-Freeman-Halton Exact test, $\chi^2 = 21.52$, df = 2, $P < 0.001$). In the case of A. hippocastanum, ($n = 268$) 13.1% (CL: 9.3–17.7, $n = 35$) of the larvae and pupae died from parasitism and host feeding, 42.2% (CL: 36.2–48.3, $n = 113$) from predation and 44.8 % (CL: 38.7–50.9, $n = 120$) from other causes, whereas in the case of A. glabra ($n = 38$), 10.5% (CL: 2.9–24.8, $n = 4$) died from parasitism and host feeding. 7.9% (CL: 1.7–21.4, $n = 3$) from predation and 81.6% (CL: 65.7–92.3, $n = 31$) from other causes.

Pupae of the first generation collected from A. hippocastanum were significantly heavier than those from A. glabra (Student’s t test, $t = –14.41$, df = 180, $p < 0.001$, Fig 1c). The same difference was recorded for potential fecundity, as the females reared on A. hippocastanum were more fecund than those reared on A. glabra (Student’s t test, $t = –12.07$, df = 134, $p < 0.001$, Fig. 1d). Furthermore, on both host plants a significant positive relationship was found between the number of oocytes in ovaries and pupal body mass (simple linear regression; A. glabra: $F_{1, 48} = 27.70, p < 0.001$, $R^2 = 0.36$, $y = 8.43 + 17005.81 \times x$; A. hippocastanum: $F_{1, 84} = 152.81, p < 0.001$, $R^2 = 0.64$, $y = –6.82 + 36066.91 \times x$).

DISCUSSION

According to optimal oviposition theory (Jaenike, 1978; Thompson, 1988; Gripenberg et al., 2010), female insects prefer to oviposit on plants that maximize the performance of their offspring. This could be especially important for endophagous insects, such as leaf miners and gall makers, because of their sedentary nature. Pere et al. (2010) and D’Costa et al. (2013) show that C. ohridella lay eggs on suboptimal hosts or even on hosts that do not support larval development. However, in both studies, a significantly greater density of eggs was recorded on A. hippocastanum than on other hosts, which suggests that females preferentially select the most suitable host. Although we did not
study preference, the lower mine density on *A. glabra* recorded in our study, presumably also reflects a lower tendency to oviposit on this exotic plant. Moreover, the actual density of mines on *A. glabra* could be even lower as our results refer to the densities on the leaves tested (with ≥ 1 mine).

*Cameraria ohridella* was able to develop on both host plants, *A. hippocastanum* and *A. glabra*, however, not all specimens of *A. glabra* were infested. These findings are consistent with previous studies of Freise (2001) and D’Costa et al. (2013). A possible explanation for the variation in the susceptibility of *A. glabra* to *C. ohridella* could be genetic diversity in *A. glabra*, which has a patchy distribution in North America (Lim et al., 2002). Such variation in host suitability could presumably occur even among introduced populations. Differences in susceptibility to the leaf miner at the level of individual trees and between cultivars are documented for *A. hippocastanum* (Straw & Tilbury, 2006; Baćovský et al., 2017).

Our study demonstrates that the survival, pupal mass and potential fecundity of *C. ohridella* were significantly lower on *A. glabra* than on *A. hippocastanum* (Figs. 1b, c, d). The lower performance of *C. ohridella* on the exotic host was assumed to be due its poor quality in terms of food (nutritional and chemical composition). Most forest insects suffer a reduction in fitness when feeding on a novel host tree (Bertheau et al., 2010) because of differences in plant traits between original and novel hosts (Leffler et al., 2014). However, there are also examples of an increase in fitness on exotic plants, mainly in polyphagous insects (Bertheau et al., 2010) because they are probably capable of detoxifying various allelochemicals (Bernays & Minkenberg, 1997).

In most phytophagous insects survival and the effect of different mortality factors vary among host plants (e.g., Connor, 1991; Berdegue et al., 1996; Heard et al., 2006). In the case of *C. ohridella*, the causes of mortality are recorded for its original host (e.g., Grabenweger et al., 2005; Girardoz et al., 2007a, b). Predation by birds and arthropods are reported as the main mortality factor of the leaf miner on *A. hippocastanum* (Grabenweger et al., 2005), which is in accordance with the results of our study. Girardoz et al. (2007a) believe that predation on leaf miners may be density-dependent; however, results of research on other species, *Cameraria hamadryadella*, do not confirm this (Connor et al., 1999). Our study showed that on *A. hippocastanum* high mortality of *C. ohridella* from natural enemies was accompanied by mortality from other causes, mostly intra-specific competition. Resource competition among larvae of the same species is often seen in leaf miners at high densities (Bultman & Faeth, 1986; Connor & Beck, 1993). On the contrary, very high mortality due to other causes observed on *A. glabra* was mostly a result of host plant chemistry and not intra-specific competition, because mine density was low. Secondary metabolites play an important role in plant defense against herbivores (Wink, 1988; Bennett & Wallsgrove, 1994) and are probably also responsible for the resistance of some *Aesculus* species to *C. ohridella* (Baćovský et al., 2017). For example, increased level of polyphenolic compounds, especially polymeric proacyanidins, found in *A. glabra*, *A. parviflora* Walt. and *A. ×carnea* H., may explain their lower susceptibility to *C. ohridella* (Oszmiański et al., 2014, 2015).

We also found that host plants on which larvae fed strongly affected pupal mass and female potential fecundity. The performance traits recorded on *A. glabra* were lower than on *A. hippocastanum*. In addition, female pupal weight and potential fecundity of *C. ohridella* on both host plants were positively correlated, and as a consequence, heavier females produced more eggs. In phytophagous insects the chemical and physical quality of the host plant can affect fecundity (Awmack & Leather, 2002), and potential fecundity has been shown to be correlated with female body mass in Lepidoptera (Honek, 1993). However, not only the number of eggs but also egg size and quality may be influenced by plant quality (Awmack & Leather, 2002; Wetzel et al., 2016). On low-quality host plants, females may lay a smaller number of larger eggs, as has been shown for Lepidoptera (Wickman & Karlsson, 1989; Karlsson & Wickman, 1990; Moreau et al., 2006). Large eggs have higher hatchability, and offspring hatching from them have higher survivorship and are often more resistant to environmental stresses (Fox & Czesak, 2000). Therefore, on suboptimal host plants, such as *A. glabra*, *C. ohridella* may possibly balance a decrease in fecundity by producing larger eggs, but this theory requires further investigation.

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