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The invasive alien leaf miner *Cameraria ohridella* and the native tree *Acer pseudoplatanus*: a fatal attraction?

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Abstract

1 The horse-chestnut leaf miner *Cameraria ohridella* is an invasive moth in Europe and a serious pest of horse-chestnut *Aesculus hippocastanum.* The moth also occasionally attacks sycamore maple *Acer pseudoplatanus*, when situated beside infested horse-chestnuts.
2 The main objective of the present study was to provide an overview of the relationship between *C. ohridella* and *A. pseudoplatanus* and to determine whether *C. ohridella* has the potential to shift to this native tree.
3 In the field, females oviposit on different deciduous tree species. Although less frequently attacked than *A. hippocastanum*, *A. pseudoplatanus* was clearly preferred for oviposition over 12 other woody species investigated.
4 Surveys in Europe demonstrated that the majority of *A. pseudoplatanus* trees found beside infested *A. hippocastanum* had mines of *C. ohridella*, even though more than 70% of the larvae died within the first two instars. Attack rates and development success greatly varied from site to site. Attack levels on *A. pseudoplatanus* were not always correlated with those on *A. hippocastanum*, and mines on *A. pseudoplatanus* were sometimes observed beside weakly-infested *A. hippocastanum.*
5 Field observations, experimental exposure of *A. pseudoplatanus* saplings and rearing trials in a common garden study showed that individual trees may vary in their susceptibility to *C. ohridella*, whereas there was no evidence that *C. ohridella* populations vary in their performance on *A. pseudoplatanus*.
6 To date, there is little evidence that *C. ohridella* represents a major risk for *A. pseudoplatanus*.

Keywords

Acer pseudoplatanus, *Cameraria ohridella*, ecological impact, host shift, invasive alien insect, leaf miner.

Introduction

Host shifts may occur in herbivorous insects when they encounter a novel plant species, either because the insect or the plant is exotic. Leaf miners are particularly prone to host shifts. A noteworthy example is the Californian gracillariid moth *Marmara gulosa*, which expanded its host range from native willows to various introduced plants, such as citrus, avocado, cotton and oleander (Guillén *et al*., 2001). Another gracillariid, the European *Phyllonorycter messaniella*, extended its host range to several new families when introduced into New Zealand (Wise, 1953).

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The horse-chestnut leaf miner *Cameraria ohridella* Deschka & Dimić (Lepidoptera: Gracillariidae) provides an ideal new model to study host shifts in leaf miners. This species, which apparently originated from isolated horse-chestnut (*Aesculus hippocastanum* L.) stands in the Balkan mountains (Valade *et al*., 2009), was first discovered in Macedonia in 1984 and subsequently has invaded most of Europe (Augustin *et al*., 2009). It has two to four generations per year, causing spectacular and unabated defoliation of horse-chestnut trees throughout Europe (Girardoz *et al*., 2007a). Its main host is the European horse-chestnut *A. hippocastanum*. However, it can also attack and develop on other species of the genus *Aesculus*, although not all of them. For example, the leaf miner is able to develop on *Aesculus turbinata*, *Aesculus flava* and *Aesculus glabra* but rarely or never on *Aesculus* x *carnea* (hybrid *A. hippocastanum* x *Aesculus pavia*), *Aesculus indica*, *Aesculus chinensis*, *Aesculus californica*, and *Aesculus parviflora* (Tomiczek & Krehan, 1998; Freise *et al*., 2003, 2004; Dimić *et al*., 2005). In the vicinity of heavily-infested horse-chestnut trees, *C. ohridella* is also occasionally found attacking and developing on sycamore maple *Acer pseudoplatanus* and, very rarely on Norway maple *Acer platanoides* (Pschorn-Walcher, 1997; Hellrigl, 1998, 2001; Freise *et al*., 2003). The same individual maple trees may be attacked year after year (Kenis *et al*., 2005) and, in some rare cases, heavy defoliation may occur, although most larvae generally die in early instars (Pschorn-Walcher, 1997; Hellrigl, 1998, 2001). It is assumed that these attacks occur mainly when neighbouring horse-chestnut trees are saturated with mines (Pschorn-Walcher, 1997).

Surprisingly, very few studies have investigated the attack of *C. ohridella* on maple. Hellrigl (1998) provided some observations on the attack on *A. pseudoplatanus* in South Tyrol (Italy), and an unpublished laboratory and field cage study by Heitland and Schlinsog (in Kenis *et al*., 2005) showed that the moth was able to develop successfully on only a few *Acer* spp. such as *A. pseudoplatanus* and development was particularly favourable on the American maple *Acer circinatum*. There is, for the moment, neither reliable information on the frequency of oviposition, the attack level or the survival rates on maple, nor on how these traits vary among maple species. Furthermore, nothing is known about potential genetic variation among *C. ohridella* populations in their ability to attack and develop on *A. pseudoplatanus.* If such variation exists, it could be expected that the total defoliation of horse-chestnut would produce selective pressure for the utilization of maples as hosts (Kenis *et al*., 2005).

For the moment, the impact of *C. ohridella* on *A. hippocastanum* is considered to be primarily aesthetic because no sign of decline or dieback on mature trees has been observed (Salleo *et al*., 2003). However, Thalmann *et al*. (2003) showed that severe defoliation of *A. hippocastanum* affects seed quality, which, in forests, may have an impact on natural regeneration and seedling survival. Through most of its range in Europe, *A. hippocastanum* is planted as an ornamental tree. Furthermore, *A. hippocastanum* does not host any other leaf miner and generally is utilized by few herbivores, limiting the potential for competition with native fauna. However, should similar damage occur on *A. pseudoplatanus*, the impact of the moth may increase significantly. *Acer pseudoplatanus* is a very common component of several European forest ecosystems and there is a risk that heavy defoliation may impact tree growth and survival, in particular young seedlings. Furthermore, maples host many herbivores, including leaf miners with which *C. ohridella* could compete, either directly or by apparent competition through natural enemies (Kenis *et al*., 2009; Péré *et al*., 2009).

The objective of the present study is to provide the first general overview of the relationship between *C. ohridella* and *A. pseudoplatanus*. In particular we will attempt to answer or provide preliminary responses to the several questions: (i) How frequent are mines of *C. ohridella* on *A. pseudoplatanus* in the field? Are attacks restricted to the direct vicinity of infested *A. hippocastanum*, particularly in situations where horse-chestnut foliage has become depleted through defoliation? (ii) Does *C. ohridella* oviposit 'at random' on *A. pseudoplatanus* or is it specifically attracted by this species compared with other non-*Aesculus* hosts? (iii) Do *C. ohridella* populations vary in their ability to attack and develop on *A. pseudoplatanus*? (iv) Is there variation among individual *A. pseudoplatanus* in their susceptibility to *C. ohridella*? (v) Do attacks on *A. pseudoplatanus* increase with time and is there evidence suggesting that *C. ohridella* will become a serious pest of this valuable European tree species?

Materials and methods

Incidence of oviposition and larval development success on various host plants

Cameraria ohridella is difficult to rear in the laboratory and oviposition success is highly variable (Girardoz *et al*., 2007a). Therefore, the assessment of host plant preference and suitability was carried out through direct observations on oviposition frequency and larval development under natural conditions in Switzerland. Natural oviposition rates and larval development of *C. ohridella* were measured on *A. hippocastanum* and 13 indigenous tree and shrub species that are commonly found with horse-chestnut in Central Europe (Fig. 1). Observations were made at five sites, twice per year (early July and September) in 2006 and 2007. Only plants that were situated less than 3 m from the trunk of heavily- and continuously-infested horse-chestnut trees (i.e. a minimum mean of 20 mines per leaf) were considered. At each site and for each plant species, 20 leaves from the lower half of the canopy were randomly collected and the number of eggs, mines and larval stages per leaf were counted in the laboratory. Because the size of leaves varies considerably among plant species, we calculated an average leaf area for all plant species considered in the analysis, based on ten randomly selected leaves at the collection sites. These estimates were then used to transform counts of life stages into densities expressed as numbers of eggs or larvae per square decimeter of leaf area. These leaf areas were measured using the Scion Image program (Scion Image, Alpha 4.0.3.2.; Scion Corporation, Frederick, Maryland). The number of eggs and larvae found per leaf was compared among host plant species. Because not all plant species could be found at all sites in both years, we compared potential host plants pairwise using Wilcoxon rank tests.

Assessment of the attack level of *C. ohridella* on *A. pseudoplatanus*

Twenty random leaf samples were collected from the lower half of the canopy of *A. pseudoplatanus*. The leaves were sampled at distances of 0–3, 10–20 and 50–100 m from infested horse-chestnut trees during surveys in Upper Austria (region of Linz), North-western Switzerland (Cantons Aargau, Basel-Landschaft, Basel-Stadt, Jura, Neuchâtel, Solothurn) and various regions in France (Alsace, Aquitaine, Bourgogne, Centre, Champagne-Ardenne, Ile de France, Rhône-Alpes, Lorraine, Midi-Pyrénées, Pays de la Loire, Provence-Alpes-Côte d'Azur), in September 2005 and 2006. These countries differ considerably in their

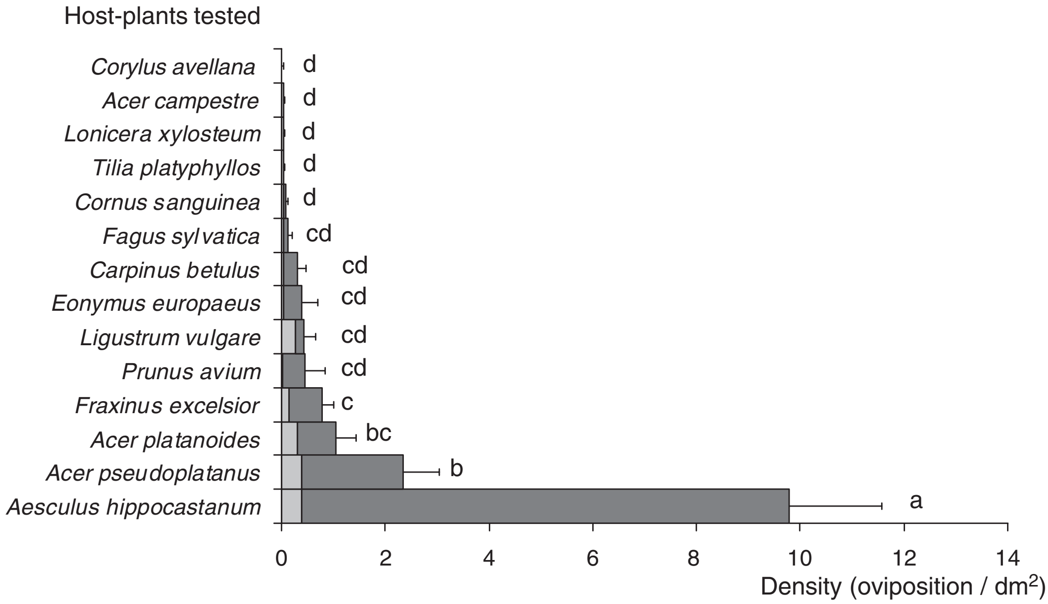

Figure 1 Mean number of eggs (light grey) and larvae (dark grey) of *Cameraria ohridella* per square decimeter of leaf on various potential host plants found within 0–3 m of an infested *Aesculus hippocastanum*. Error bars represent standard errors of the mean number of oviposition (eggs + larvae). Different letters indicate significant difference ($P < 0.05$, Wilcoxon rank tests).

history of invasion by *C. ohridella*, which appeared in Upper Austria in 1989, in North-western Switzerland in 1998, and in France from 2000 to 2003.

Approximately 40 samples (30–46) per distance and country were collected. At several sites, only one or two of the distances could be sampled. When possible, the same trees were sampled in both years. Leaves were returned to the laboratory in a cool box to avoid development during transport and were inspected under a stereomicroscope. The information collected for each sample comprised: (i) the number of eggs and mines of *C. ohridella* per leaf and per site; (ii) the proportion of respective developmental stages; (iii) mortality rate; and (iv) the level of attack by *C. ohridella* on the nearest *A. hippocastanum* using the damage code of Gilbert & Grégoire (2003), reflecting the relative infested area on each leaf. Mean values of these parameters were compared among countries using Kruskal–Wallis and Bonferroni post-hoc tests and variation was related to the history of infestation. Proportions of sites with mines were compared among countries using chi squared. The correlation between the level of attack on *A. pseudoplatanus* and the nearest infested horse-chestnut tree was calculated.

Intraspecific variation in *C. ohridella* and *A. pseudoplatanus*

In 2007 and 2008, five times ten *A. pseudoplatanus* saplings (approximate height 120 ± 10 cm) originating from the provenance in the same nursery were planted close to infested horse-chestnut trees at five sites in Switzerland: two sites (Munchenstein: 47°31′09″N, 7°37′14″E; Lignières: 47°04′08″N, 7°03′40″E) where full development of *C. ohridella* on *A. pseudoplatanus* had been commonly observed in previous years and three sites (Delémont-CABI: 47°22′21″N, 7°19′30″E; Delémont-Birse: 47°22′17″N, 7°21′39″E; Berlincourt: 47°19′37″N, 7°13′23″E) where no development beyond the second instar had been observed on *A. pseudoplatanus* (i.e. in total 50 *A. pseudoplatanus* saplings and five populations of *C. ohridella*). The saplings were left on site from April (before the emergence of overwintering pupae) to mid-August (at the end of the second generation oviposition period). Variation in oviposition rates and development success were monitored. The first set of observations was carried out in June, directly in the field using a magnifying glass. The second, more precise set of observations was carried out in September by collecting all leaves and counting the number of *C. ohridella* mines and eggs per leaf and per sapling with a stereomicroscope. The relative frequency of various developmental stages and mortality rates on *A. pseudoplatanus* saplings were recorded, as was the level of attack by *C. ohridella* on the nearest *A. hippocastanum*. Because most larvae died during the first two instars, the proportion of larvae reaching the third instar was used as a measure of development success. The effect of moth populations and saplings on oviposition rates and development success were analysed using linear mixed models, with moth population as a fixed factor and sapling as a random factor. Oviposition and development success data were log transformed prior to statistical analyses.

In 2008, a rearing experiment was carried out in a common garden in Delémont, Switzerland. The same five populations of *C. ohridella* were tested on 40 *A. pseudoplatanus* saplings (approximate height 120 cm ± 10 cm) originating from the same nursery. Dead leaves of horse-chestnut infested by *C. ohridella* were collected at the five previously mentioned sites in December 2007 and stored in plastic bags in a cold room at 2°C, then moved to 10°C a week before incubation in April. Next, pupae were collected from the dead leaves, sexed using the method of Freise & Heitland (1999) and left at room temperature in plastic containers (200 mL). At emergence (i.e. in mid May), ten adults (five males and five females) were put in gauze cages (200 × 60 × 60 cm) containing one sapling and placed outdoors under natural conditions but protected from direct rain and sunlight. Eight replicates per population of *C. ohridella* were made. Two months later, the number of eggs, mines and respective developmental stages were noted for each leaf of each replicate. The fraction of larvae reaching the third instar was used as a measure of development success. The effect of moth populations and saplings on development success was analysed using linear mixed models with moth population as a fixed factor and sapling as a random factor. Development success measurements were log transformed prior to statistical analysis.

All statistical analyses were performed using SPSS software (SPSS Inc., version 16.0, Chicago, Illinois).

Results

Incidence of oviposition and larval development success on various host plants

Eggs or larvae were found on all the 14 tree and shrub species investigated, although their numbers varied greatly among species (Fig. 1). *Aesculus hippocastanum* was more heavily attacked than any indigenous species. Among the latter, we found significantly more eggs and larvae on *A. pseudoplatanus* than on any other plant species, except Norway maple *A. platanoides*. This latter and European ash *Fraxinus excelsior* were significantly more frequently attacked than several other species, including field maple *Acer campestre* on which very few dead eggs and no larvae were found. Successful development to the pupal stage was observed only on *A. hippocastanum* and, very rarely, on a few *A. pseudoplatanus*. In all other cases, *C. ohridella* eggs did not hatch or larvae died in the first larval stage, shortly after they had started feeding.

Assessment of the attack level of *C. ohridella* on *A. pseudoplatanus*

The proportion of sites in Austria, Switzerland and France where mines of *C. ohridella* were found on *A. pseudoplatanus* varied in the range 53–87% over the 2 years, with most of the mines being found within 0–3 m of *A. hippocastanum* trees (Fig. 2). Few mines were found at 10–20 m and almost none at 50–100 m. The mean number of mines per *A. pseudoplatanus* leaf varied in the range 2–14 over the 2 years and the three countries during which sampling was conducted (Fig. 3). In both years, we found more sites with mines (Fig. 2) and more mines per leaf (Fig. 3) in Austria, although significant differences were only found in 2006 in the number of sites with mines between Austria and France and in the number of mines per leaf between Austria and Switzerland (χ^2 test, Fig. 2A, 0–3 m: $\chi^2 = 0.081$, d.f. = 2, $P = 0.960$; 10–20 m: $\chi^2 = 1.83$, d.f. = 2, $P = 0.400$; 50–100 m: $\chi^2 = 1.72$, d.f. = 2,

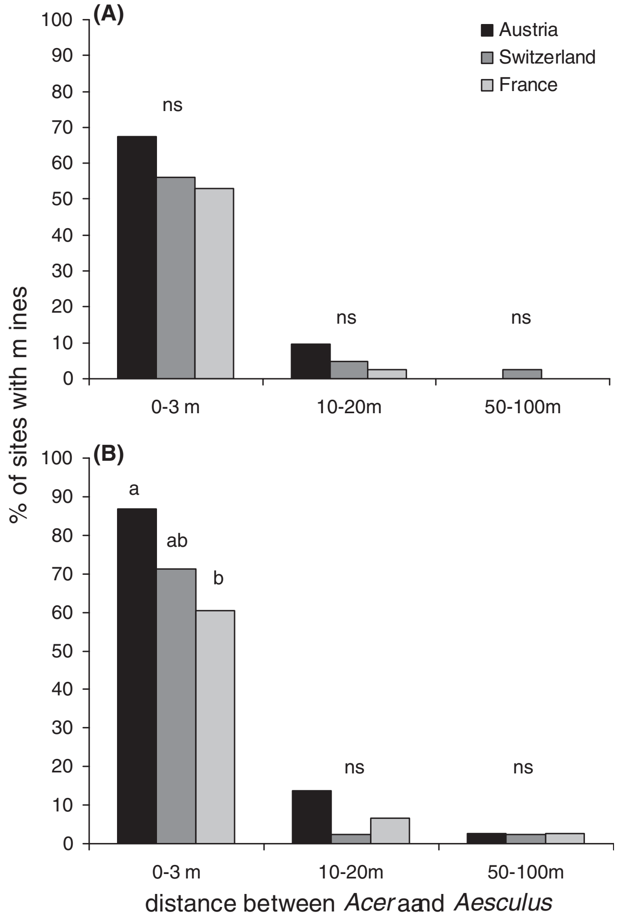

Figure 2 Percentage of sites with mines on *Acer pseudoplatanus* in the three countries at three distances from an infested *Aesculus hippocastanum* in (A) 2005 and (B) 2006. Different letters above bars indicate significant differences between countries ($P < 0.05$, χ^2). ns, not significant.

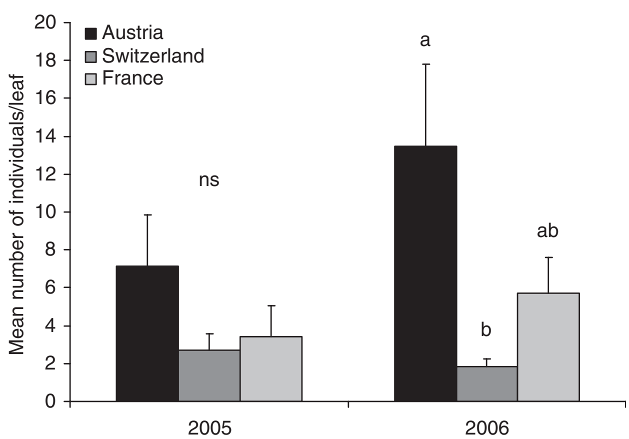

Figure 3 Mean number of individuals (eggs + larvae) of *Cameraria ohridella* on *Acer pseudoplatanus* situated within at 0–3 m of an infested *Aesculus hippocastanum*, in 2005 and 2006, in the three countries. Error bars represent standard errors. Different letters above bars indicate significant differences among countries ($P < 0.05$, Kruskal–Wallis and Bonferroni post-hoc tests). ns, not significant.

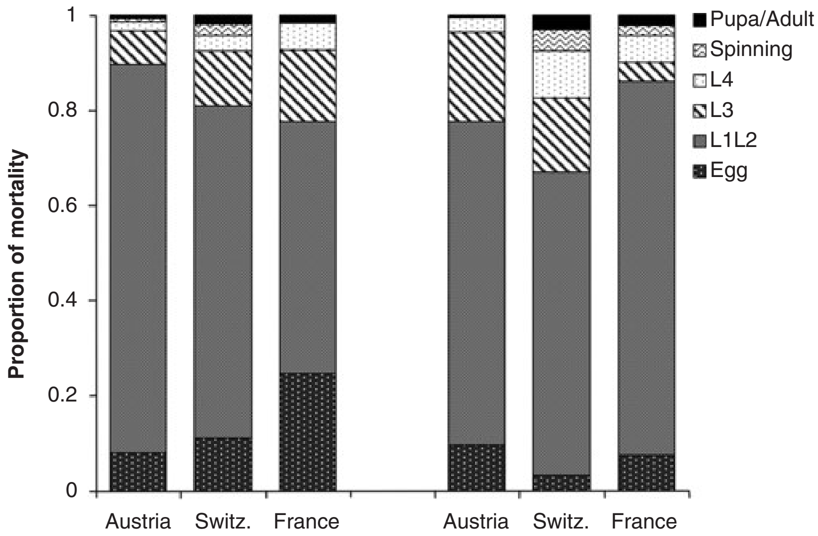

Figure 4 Proportional mortality of *Cameraria ohridella* at different development stages on *Acer pseudoplatanus* in Austria, Switzerland and France (0–3 m from an infested horse-chestnut), in 2005 (left) and 2006 (right).

$P = 0.423$; Fig. 2B, 0–3 m: $\chi^2 = 7.04$, d.f. $= 2$, $P = 0.030$; 10–20 m: $\chi^2 = 3.78$, d.f. $= 2$, $P = 0.150$; 50–100 m: $\chi^2 = 0.01$, d.f. $= 2$, $P = 0.994$; Kruskal–Wallis test, Fig. 3; 2005: $\chi^2 = 4.14$, d.f. $= 2$, $P = 0.110$; 2006: $\chi^2 = 12.74$, d.f. $= 2$, $P = 0.002$). Over 70% of the larvae died in the first two instars (Fig. 4). Nevertheless, some larger mines (L3 and older) were found at half of the sites and pupae and emergence holes were observed in all countries. There was no significant difference among countries in the proportion of individuals reaching the third instar ($\chi^2 = 3.18$, d.f. $= 2$, $P = 0.204$ and $\chi^2 = 0.54$, d.f. $= 2$, $P = 0.765$ in 2005 and 2006, respectively, Kruskal–Wallis test).

The level of attack by *C. ohridella* on *A. pseudoplatanus* in the three countries investigated was positively correlated with the attack level on *A. hippocastanum* in 2005 ($r = 0.314$, $n = 113$, $P = 0.001$) but not in 2006 ($r = 0.071$, $n = 122$, $P = 0.439$) (Fig. 5). However, there was considerable variation in this among the different sites. Some of the highest densities on *A. pseudoplatanus* were observed at sites where the level of attack on *A. hippocastanum* was low, whereas, in several cases, no mines were found on *A. pseudoplatanus* at sites where *A. hippocastanum* was heavily attacked (Fig. 5).

The numbers of *C. ohridella* mines on *A. pseudoplatanus* in 2005 were positively correlated with numbers in 2006, both when the three countries were analysed together ($r = 0.684$, $n = 102$, $P < 0.001$) and separately (Austria: $r = 0.607$, $n = 33$, $P < 0.001$; Switzerland: $r = 0.736$, $n = 38$, $P < 0.001$; France: $r = 0.720$, $n = 31$, $P < 0.001$). This correlation was also significantly positive when only large mines (L3 and older) were included ($r = 0.642$, $n = 59$, $P < 0.001$).

Intraspecific variation in *C. ohridella* and *A. pseudoplatanus*

Eggs and larvae of *C. ohridella* were found on *A. pseudoplatanus* saplings exposed at the five sites (Table 1). Although many more eggs and mines were found in September, *C. ohridella* laid a sizeable number of eggs in spring, despite the availability of fresh *A. hippocastanum* leaves. There was considerable variation in the mean number of ovipositions (Fig. 6), which was significantly different among the saplings (linear mixed model: $\sigma^2 = 0.09 \pm 0.02$, Wald's $Z = 4.50$, $P \leq 0.001$ and $\sigma^2 = 0.04 \pm 0.01$, Wald's $Z = 3.39$, $P = 0.001$ in 2007 and 2008, respectively) and among the five sites in 2007 (linear mixed model: $F_{4,44} = 5.66$, $P = 0.001$) but not among four sites in 2008 ($F_{3,34} = 2.40$, $P = 0.085$; the saplings in Berlincourt were accidentally destroyed). As in the previous experiment, most hatched larvae died in the first two larval stages. The development success (i.e. % of larvae surviving to the third instar) was not significantly different among the sites ($F_{4,44} = 0.54$, $P = 0.704$ and $F_{3,34} = 1.89$, $P = 0.148$ in 2007 and 2008, respectively). In particular, we did not find more successfully developed mines at the two sites where successful development was observed in the previous years on local *A. pseudoplatanus* compared with the three other sites where full development had never been observed. By contrast, the development success significantly differed among saplings ($\sigma^2 = 0.008 \pm 0.002$, Wald's $Z = 4.29$, $P \leq 0.001$ and $\sigma^2 = 0.02 \pm 0.004$, Wald's $Z = 3.64$, $P \leq 0.001$ in 2007 and 2008, respectively).

A similar observation was made in the gauze cage experiment in the common garden. The development success was not significantly different among the five populations of *C. ohridella* ($F_{4,24} = 0.217$, $P = 0.927$) but it was significantly different among the different saplings ($\sigma^2 = 0.05 \pm 0.02$, Wald's $Z = 2.39$, $P = 0.017$).

Discussion

The present study provides the first comprehensive information on *A. pseudoplatanus* as a host of *C. ohridella* and allows us to

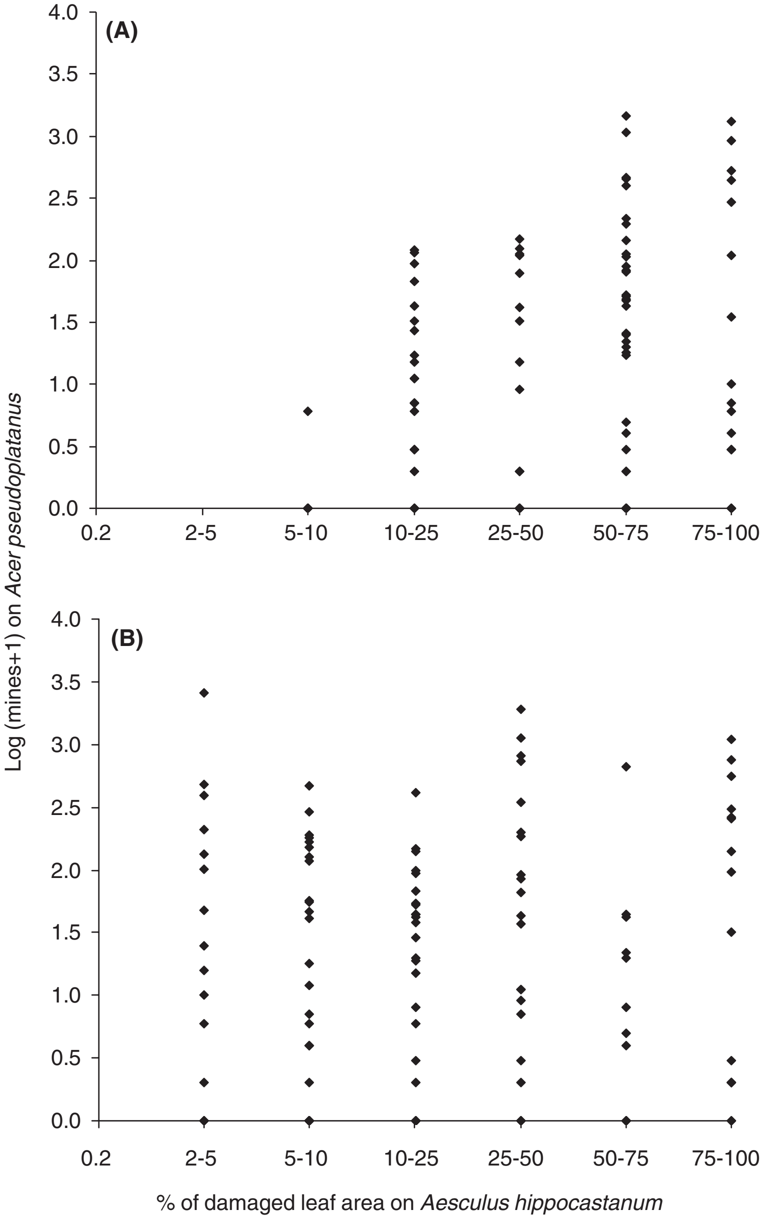

Figure 5 Relationship between log-transformed mine numbers [log10(number of mines + 1)] on *Acer pseudoplatanus* and the percentage of damaged leaf area on *Aesculus hippocastanum*, at 0–3 m in Austria, Switzerland and France in (A) 2005 and (B) 2006. Data on mine counts were log-transformed to eliminate heteroscedasticity observed at sites.

answer some questions as well as provide preliminary responses to others.

How frequent are mines of *C. ohridella* on *A. pseudoplatanus* in the field?

Unexpectedly, surveys in Austria, Switzerland and France showed that most *A. pseudoplatanus* in the close vicinity of infested horse-chestnut had numerous mines of *C. ohridella*. This observation had never previously been made, probably because the majority of the larvae feeding on *A. pseudoplatanus* die in the first two instars, leaving only very small mines that are not easily visible to the naked eye. Larger mines were also found at more than half of the sites investigated. The number of mines on *A. pseudoplatanus* quickly decreased with distance from an infested horse-chestnut, although some mines were found at 50–100 m. Thus, in contrast to previous conclusions made in previous studies (Pschorn-Walcher, 1997; Avtzis & Avtzis, 2003), the utilization of *A. pseudoplatanus* by *C. ohridella* is not limited to situations when horse-chestnut foliage becomes unavailable as a result of defoliation. Attacks on *A. pseudoplatanus* were frequently observed in the first generation when the level of attack on horse-chestnut was still

Table 1 Total number of eggs and larvae of *Cameraria ohridella* on *Acer pseudoplatanus* saplings placed at five sites, counted in June and September 2007 and 2008

	2007		2008	
Sites	June	September	June	September
Berlincourt	482	10371	198	0[a]
Birse	149	2981	266	1882
Cabi	17	715	56	1899
Munchenstein	122	2642	77	2027
Lingières	17	4131	0	264

[a]At the Berlincourt site, saplings were accidentally destroyed during the summer 2008.

low. Later in the year, the attack rate on *A. pseudoplatanus* was not clearly correlated with that on horse-chestnut.

Does *C. ohridella* oviposit 'at random' on *A. pseudoplatanus* or is it specifically attracted by this species compared with other non-*Aesculus* hosts?

Under natural conditions, females of *C. ohridella* oviposited on various plant species that were nearby horse-chestnut trees with very high densities of leaf mines. Eggs and mines were found on all 14 deciduous tree and shrub species investigated. Although horse-chestnut was the preferred host plant, with the highest number of oviposition per square decimeter, the mean number of oviposition events was significantly higher on *A. pseudoplatanus* than on all other native tree and shrub species, except Norway maple *A. platanoides*. On all trees except *A. hippocastanum*, the vast majority of individuals died at the egg and young larval stages. Successful development to the pupal and adult stages was observed only on *A. hippocastanum* and *A. pseudoplatanus*, although full development is also rarely observed in the field on *A. platanoides* (Tomiczek & Krehan, 1998; Straw & Tilbury, 2006; C. Péré & S. Augustin, unpublished data). *Acer* and *Aesculus* are in the same family, Sapindaceae, and thus it is not surprising that these two genera may share common pest herbivores (Stevens, 2009). Lopez-Vaamonde *et al*. (2003) found that host shifts in gracillariid leaf miners of the genus *Phyllonorycter* have usually occurred between closely-related plant species. Nevertheless, we found that another maple, *A. campestre*, was among the least attacked tree and none of the eggs found on its leaves hatched. Previous laboratory and field cage tests showed that development is possible on some *Aesculus* and *Acer* species but not on all species in these genera (Freise *et al*., 2003, 2004; Kenis *et al*., 2005).

The results obtained in the present study also suggest that females of *C. ohridella* preferentially select the host plant where offspring survival is greater, a phenomenon commonly described as the preference–performance relationship (Singer & Thomas, 1988; Craig *et al*., 2000). Choosing the right host plant is particularly important for endophytic herbivores such as gall makers and leaf miners, whose larval survival entirely depends on the choice made by the adult insect (Faeth *et al*., 1981; Faeth, 1990). In *C. ohridella*, the host selection is far from being perfect, considering the high attack rate and level of mortality on *A. pseudoplatanus.* This 'waste' in oviposition may suggest that *C. ohridella* may not have co-evolved with *A. pseudoplatanus*, although Avtzis & Avtzis (2003) found

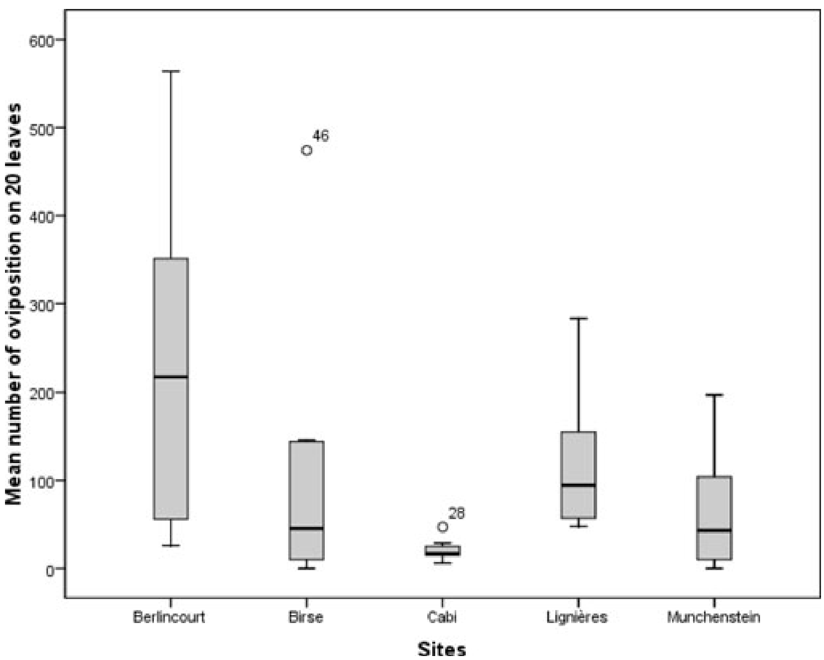

Figure 6 Box plots of the mean number of ovipositions of *Cameraria ohridella* on 20 leaves of *Acer pseudoplatanus* saplings planted in the field at five sites in Switzerland (September, 2007). Boxes depict 25th, 50th (median) and 75th percentiles, whiskers depict 10th and 90th percentiles, and points depict outliers.

A. pseudoplatanus and other maple species but no mines in the potential region of origin of the moth in Greece. More investigations should be carried out in the original isolated distribution of *C. ohridella* in the Balkan Mountains to verify the extent to which *A. pseudoplatanus* occurs sympatrically with *C. ohridella* and whether the moth attacks and develops on the local maple varieties.

Do *C. ohridella* populations vary in their ability to attack and develop on *A. pseudoplatanus*?

Some sites show high levels of attack and successful development on *A. pseudoplatanus* repeatedly every year (present study; C. Péré and M. Kenis, unpublished data). This suggests either that *C. ohridella* populations vary in their ability to attack and develop on *A. pseudoplatanus* or that individual *A. pseudoplatanus* trees vary in their attractiveness and susceptibility to the moth. Comparisons in a common garden among *C. ohridella* populations collected at sites with a different success of attack on *A. pseudoplatanus* failed to show any significant differences in their performance. However, more tests should be made (e.g. using *C. ohridella* populations emerging directly from *A. pseudoplatanus*) before excluding variations among *C. ohridella* populations or individuals.

Is there variation among individual *A. pseudoplatanus* in their susceptibility to *C. ohridella*?

The results obtained in the present study suggest that variation among individual *A. pseudoplatanus* trees is more likely than among individual *C. ohridella*. It is not yet clear whether some *A. pseudoplatanus* attract more *C. ohridella* than others, although there is little doubt that the moth develops more successfully on some trees than on others, as observed in the present study. We observed a positive year-to-year correlation in the number of large mines at our sample sites in the three countries. Furthermore, there are also empirical observations that the same *A. pseudoplatanus* trees have many large mines every year, whereas, on other trees at the same site, all larvae die in the first two instars (Kenis *et al*., 2005; C. Péré and M. Kenis, unpublished data). Mortality factors affecting young larvae on *A. pseudoplatanus* have not been studied in detail, although the most important factor is probably host defense reaction or chemical or physical unsuitability. Host resistance also occurs on *A. hippocastanum*. Girardoz *et al*. (2007b) observed that approximately 5% of the *C. ohridella* larvae die as a result of host resistance in the first two instars but that this rate may vary among individual trees. Extensive variation in developmental success also occurs among varieties of *A. hippocastanum* and various *Aesculus* and *Acer* species (Freise *et al*., 2003, 2004; Kenis *et al*., 2005). The difference in larval mortality between *A. pseudoplatanus* and *A. hippocastanum* is surely not a result of parasitism and predation. During our investigations, we found hardly any sign of parasitism and predation on young larvae in *A. pseudoplatanus*. Previous studies also demonstrated that parasitism and predation is negligible in the first two instars (Girardoz *et al*., 2007a, b) and, on older larvae and pupae, parasitism is not higher on *A. pseudoplatanus* than on *A. hippocastanum* (Girardoz *et al*., 2007c).

Do attacks on *A. pseudoplatanus* increase with time and is there evidence suggesting that *C. ohridella* has the potential to become a serious pest of this valuable European tree species?

A successful host shift is characterized by three important mechanisms. First, adult females must be able to locate the host plant (e.g. through volatiles, colours, leaf or tree shape). Second, they must find the plant suitable for oviposition. Finally, once eggs are laid, the larvae must develop the ability to survive on a new diet where they may encounter physical or chemical barriers (Bernays & Chapman, 1994). The present study showed that the first two barriers have already been overcome. *Cameraria ohridella* locates *A. pseudoplatanus* leaves up to at least 100 m from horse-chestnut trees and massive numbers of eggs and young larvae were found on several *A. pseudoplatanus* trees in all investigated regions. The only factor that has prevented outbreaks of *C. ohridella* on *A. pseudoplatanus* is the high larval mortality, particularly in the young instars. Although some individuals are able to complete their development on *A. pseudoplatanus*, so far, the survival rate is not sufficient to support self-maintaining populations on this new host, and we have not found any indication that this rate may increase in the near future. Surveys in Austria, Switzerland and France showed that more eggs and mines per leaf and a higher proportion of sites with mines were found in Austria compared with the two other countries. Because *C. ohridella* arrived in Austria some 10–12 years earlier, it may suggest that damage on *A. pseudoplatanus* is increasing. However, because individual *A. pseudoplatanus* trees vary in their attack rates, it may also be that, for various reasons, trees in Austria are more attractive than in the two other countries. Observations in the Balkans, where the moth has been present for at least 25 years, did not reveal particularly high levels of attack on *A. pseudoplatanus* (Avtzis & Avtzis, 2003, 2006; R. Tomov, personal communication). The results obtained in the present study show that high variation in the level of attack arises from variability among trees rather than among moth populations. Nevertheless, at some sites, *C. ohridella* outbreaks on *A. hippocastanum* continue unabated and total defoliation occurs every year; it cannot be ruled out that, under such a strong selection pressure to find alternative hosts, a *C. ohridella* population may at some time in the future overcome the natural defences of *A. pseudoplatanus.*

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