ECOLOGICAL IMPACT OF THE INVASIVE HORSE-CHESTNUT LEAF MINER, *CAMERARIA OHRIDELLA* DESCHKA & DIMIĆ (LEPIDOPTERA: GRACILLARIIDAE), ON NATIVE SPECIES

Dissertation submitted to the Faculty of Sciences
Institute of Biology, University of Neuchâtel

For the Degree of Doctor of Philosophy ès Sciences

By

Christelle Péré

Defended on February 20, 2009
Accepted by the Thesis Committee Members:

Prof. Ted C. J. Turlings, University of Neuchâtel, thesis director
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Photo by C. Péré (August 2008):

Alignment of horse-chestnut, *Aesculus hippocastanum*, attacked by *C. ohridella*

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Photos by C. Péré (from left to right):

Leaf of maple, *Acer pseudoplatanus*, attacked by *C. ohridella*
Adult of *C. ohridella* on horse-chestnut trunk
Leaf of horse-chestnut, *Aesculus hippocastanum*, attacked by *C. ohridella*
Minotetrastichus frontalis, parasitoid of *C. ohridella*
Leaf of beech, *Fagus sylvatica*, attacked by *Orchestes fagi*

University of Neuchâtel, Switzerland

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IMPRIMATUR POUR LA THESE

Ecological Impact of the Invasive Horse-Chestnut Leaf Miner, *Cameraria ohridella,* DESCHKA & DIMIC (Lepidoptera : Gracillariidae) on Native Species

Christelle Péré

UNIVERSITE DE NEUCHATEL

FACULTE DES SCIENCES

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PREFACE

The present PhD dissertation of Christelle Péré in Biology of the Doctoral Programme "Plants and their Environment", NCCR "Plant survival", of the University of Neuchâtel (http://www2.unine.ch/nccr/page7451.html) was written as a thesis by publications. This work of research was part of ALARM (*Assessing LArge scale Risks for biodiversity with tested Methods*), an integrated Project of the European Union's Sixth Framework Programme (2004-2009) (http://www.alarmproject.net/alarm/). It was also partly funded by the Loterie Romande and the University of Neuchâtel. This PhD project was conducted in the "Forestry and Ornamental Pests Research" laboratory of Dr. Marc Kenis at CABI Europe-Switzerland, at Delémont, in Switzerland (http://www.cabi.org/).

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Abstract / Résumé

"Ecological impact of the invasive horse-chestnut leaf miner, *Cameraria ohridella* Deschka & Dimić (Lepidoptera: Gracillariidae), on native species"

The horse-chestnut leaf miner, *Cameraria ohridella*, is the first leaf miner and first major pest known to attack the white flowering horse-chestnut tree, *Aesculus hippocastanum*, native to the Balkans and introduced in Central Europe as an ornamental urban tree. This moth, which probably originates from the Balkans, was first discovered in Macedonia in 1984 and has since invaded most of Europe (Chapter 1). This thesis is the first attempt to investigate the ecological impact of an invasive alien leaf miner on native fauna and flora.

Cameraria ohridella is attacked by several European parasitoids and predators. The first aim of this thesis was to assess the interactions of *C. ohridella* with native leaf miner species through their shared natural enemies, and in particular, parasitoids. This indirect impact is known as apparent competition. Despite the low parasitism rates observed in *C. ohridella*, populations are so high that an unusual number of polyphagous parasitoids is produced two to four times per year. These parasitoids then have the opportunity to attack other leaf miners and, consequently, affect their density, especially in spring. After overwintering, the parasitoids emerge from dead leaves of horse-chestnut at the same time as, or earlier than, their host, and at least five weeks before suitable *C. ohridella* larvae or pupae are available. Our results showed that species richness and abundance of native leaf miners were lower in the vicinity of horse-chestnut trees infested by *C. ohridella* compared to control sites (Chapter 2). However, no evidence for apparent competition was found between *C. ohridella* and the native beech leaf mining weevil, *Orchestes fagi*, as total mortality, parasitism and predation were not significantly different. Several parasitoid species commonly attacking *C. ohridella* were reared from *O. fagi*, but, in general, their density was not higher in the vicinity of infested horse-chestnut trees. Possible explanations for these unexpected results are provided (Chapter 3).

In Europe, mines of *C. ohridella* have also been recorded on two native maple species, *Acer pseudoplatanus* and *Acer platanoides*, but attacks are highly variable. The second aim of this thesis was to provide an overview of the relationship between *C. ohridella* and *A. pseudoplatanus* and to assess the possibility of a host shift by *C. ohridella* to *A. pseudoplatanus*. So far, our results have shown that high numbers of eggs are laid on *A. pseudoplatanus* when the trees are surrounded by horse-chestnut and that the majority of the larvae died in the first two instars. Our outcomes also showed that there is no clear indication that the level of attack increases with time in Europe. 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*. Thus far, there is little evidence that *C. ohridella* represents a major risk for *A. pseudoplatanus* (Chapter 4).

Overall the present thesis provides, to our knowledge, one of the first examples suggesting that invasive alien insects may indirectly affect native species of insect through apparent competition. Moreover, this thesis is an important contribution to the knowledge of the ecology of *C. ohridella* because it provides the first detailed information on the relationship between *C. ohridella* and *A. pseudoplatanus*.

Keywords: biological invasions, invasive alien species, horse-chestnut leaf miner, *Cameraria ohridella*, indirect impact, apparent competition, natural enemies, parasitoid, native leaf miners, beech leaf mining weevil, *Orchestes fagi*, direct impact, native flora, host-shift, maple sycamore, *Acer pseudoplatanus*.

"Impacts écologiques de la mineuse du marronnier d'Inde, *Cameraria ohridella* Deschka & Dimić (Lepidoptera: Gracillariidae), sur les espèces natives"

La mineuse du marronnier d'Inde, *Cameraria ohridella*, est la première mineuse et le premier ravageur important attaquant le marronnier à fleurs blanches, *Aesculus hippocastanum*, natif des Balkans et introduit en Europe Centrale en tant qu'arbre ornemental. Le papillon, probablement originaire aussi des Balkans, a été découvert pour la première fois en Macédoine, en 1984, et s'est depuis répandu dans toute l'Europe (Chapitre 1). Cette thèse est la première tentative d'évaluation de l'impact écologique d'une mineuse exotique et invasive sur la faune et la flore natives.

Cameraria ohridella est attaquée par plusieurs espèces natives de parasitoïdes et prédateurs. Le premier objectif de cette thèse était d'évaluer les interactions de *C. ohridella* avec les espèces de mineuses natives à travers le partage d'ennemis naturels communs, et en particulier les parasitoïdes. Cet impact indirect est connu sous le nom de "compétition apparente". En dépit du faible taux de parasitisme observé chez *C. ohridella*, les populations du papillon sont tellement importantes qu'un grand nombre de parasitoïdes, considérés comme polyphages, sont produits deux à quatre fois par an. Ces parasitoïdes ont alors la possibilité d'attaquer d'autres espèces de mineuses et, par conséquent, affecter leur densité, tout particulièrement au printemps. En effet, après la diapause, les parasitoïdes émergent des feuilles mortes de marronnier en même temps, voire plus tôt, que leur hôte et, au moins cinq semaines avant que les larves âgées et les chrysalides de *C. ohridella* soient disponibles. Nos résultats ont montré que la richesse spécifique et l'abondance des mineuses natives étaient plus basses à proximité de marronniers infestés par *C. ohridella* comparé aux sites de contrôle (Chapitre 2). Cependant, aucune évidence de compétition apparente n'a été trouvée entre *C. ohridella* et le charançon du hêtre, *Orchestes fagi*, dont la mortalité totale, le parasitisme et la prédation n'étaient pas significativement différents en présence et en absence de marronniers infestés. Plusieurs espèces de parasitoïdes attaquant communément *C. ohridella* ont été élevées sur *O. fagi*, mais en général, leur densité n'était pas plus importante à proximité de marronniers infestés. Des explications plausibles pour expliquer ces résultats inattendus sont fournies (Chapitre 3).

En Europe, des mines de *C. ohridella* ont également été trouvées sur deux espèces d'érables natifs, *Acer pseudoplatanus* (érable sycomore) et *Acer platanoides* (érable plane), mais les taux d'attaques sont très variables. Le second objectif de cette thèse était de fournir une synthèse de la relation trophique entre *C. ohridella* et *A. pseudoplatanus* et, d'évaluer la possibilité d'un changement d'hôte de *C. ohridella* vers *A. pseudoplatanus.* Nos résultats ont montré qu'un grand nombre d'œufs sont pondus sur *A. pseudoplatanus* situés à proximité de marronniers infestés et, que la majorité des larves meurt aux jeunes stades. Par contre, il n'y a pas d'évidence que le niveau d'attaque augmente avec le temps, en Europe. Des observations sur le terrain, des études expérimentales d'exposition de jeunes arbres d'*A. pseudoplatanus* et, des essais d'élevage dans un jardin commun ont montré que les érables sycomores pouvaient varier individuellement dans leur sensibilité face à *C. ohridella*, tandis qu'il n'y avait pas d'évidence que les populations de *C. ohridella* varient dans leur performance sur *A. pseudoplatanus*. Par conséquent, les populations de *C. ohridella* ne représentent pas, à court terme, un risque majeur pour *A. pseudoplatanus* (Chapitre 4).

De manière générale, cette thèse fournit un des premiers exemples suggérant que les insectes invasifs peuvent affecter indirectement les espèces d'insectes natifs via la compétition apparente. De plus, cette thèse est une importante contribution à la connaissance de l'écologie de *C. ohridella* car elle fournit les premières informations détaillées de la relation trophique entre *C. ohridella* et *A. pseudoplatanus.*

Mots clés: Invasions biologiques, espèce exotique envahissante, mineuse du marronnier, *Cameraria ohridella*, impact indirect, compétition apparente, ennemis naturels, parasitoïde, mineuses natives, charançon du hêtre, *Orchestes fagi*, impact direct, flore native, changement d'hôte, érable sycomore, *Acer pseudoplatanus*.

Chapter 1

General introduction

Invasive alien species

Under the Convention on Biological Diversity, invasive alien species are those that are introduced deliberately or unintentionally, establish, naturalize, and spread outside of their home range and whose impacts involve significant harm. Biological invasions by alien species are large-scale phenomena of widespread importance and represent one of the major current threats to natural ecosystems and biodiversity. Important economic impacts have arisen from non-indigenous species to agriculture, horticulture, aquaculture, stored produced, and forestry (Pimentel 2002). However invasive alien species may also have profound environmental consequences and are taxonomically diverse, though certain groups (e.g., mammals, plants, and insects) have produced particularly large numbers of damaging invaders.

The ecological impact caused by invasive insects on the environment can be observed at various organizational levels - genetic, individual, population, community and ecosystem (Kenis & Péré 2007). It may occur at different spatial scales, from microhabitat to landscape, and through various mechanisms, such as predation, competition, herbivory, hybridization etc.

Leaf miners

Leaf miners are insects whose immature stages feed creating channels (mines) inside the parenchyma or epidermis tissues of leaves (Hering 1951). These mines provide both living and feeding quarters for the larva. Leaf miners are found in four insect Orders: Lepidoptera and Diptera comprise the greatest number of species, while Hymenoptera and Coleoptera contain less species. The majority of leaf miners are monophagous, in other words confined to one definite plant genus or species, which greatly facilitates determination. For oligophagous and polyphagous species the determination is more difficult. Among them are a number of very closely related species, which produce very similar mines, and which cannot be identified by their mines alone. However, in Europe, most species can be determined by identification of the host-plant, position of the mine on the leaf (lowerside, upperside, edge…), shape of the mine (e.g., linear mine, blotch mine, serpentine mine) (Fig. 1) and the presence, location, way of deposition and colour of faeces (Fig. 2) (Hering 1951; Askew & Shaw 1974).

Many generalist predators are known to prey on leaf mining larvae (wasps, bugs, ants, insectivorous birds). Parasitoid insects play the most important role as natural enemies of leaf miners. They penetrate the mines with their ovipositors and lay their eggs in or on the bodies of the mining larvae. They also destroy many larvae merely by feeding on their body fluids (Askew & Shaw 1974). Parasitoids of leaf miners are found almost exclusively among the Hymenoptera. Most leaf miner parasitoids are Chalcidoidea, but species belonging to Ichneumonoidea (Ichneumonidae and Braconidae) also parasitise leaf miners (Hespenheide 1991).

Although most leaf miners are usually not considered as major agricultural or forestry pests, in cases of outbreaks some species can cause significant damage (Hespenheide 1991). For example, agromyzid flies can damage a wide variety of agricultural crops (bean, wheat, sugar beet, onion, maize, etc.). Many of the most damaging leaf miners are exotic. The best example in Europe is *Cameraria ohridella* Deschka & Dimić (Lepidoptera: Gracillariidae), the horse-chestnut leaf miner, which is the focus of this thesis. In less than two decades, this moth has invaded most of Europe, seriously defoliating, year after year, one of the most popular urban trees, without any sign of population decline (Freise et al. 2002).

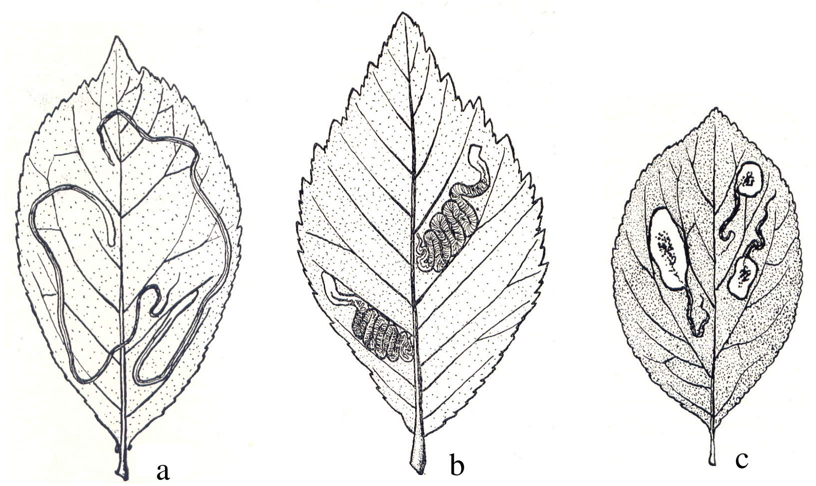

Fig. 1: Examples of different shapes of mines. a, linear mine of *Lyonetia clerkella* on *Prunus cerasus*; b, serpentine mine of *Nepticula viscerella* on *Ulmus*; c, linear and blotch mine of *Nepticula plagicolella* on *Prunus domestica*. Source: Hering (1951).

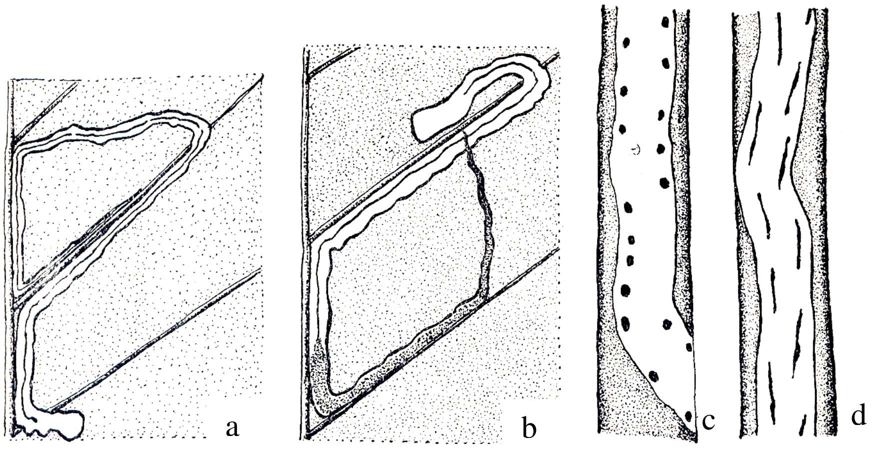

Fig. 2: Disposal of faeces in mines. a, faeces throughout as a thin, central line; b, to begin with the faeces fills the whole width of the mine; c, faeces in isolated grains; d, faeces in threads. Source: Hering (1951).

The horse-chestnut leaf miner

The horse-chestnut leaf miner, *C. ohridella*, is the first leaf miner and first major pest known to attack the white flowering horse-chestnut tree, *Aesculus hippocastanum* L. (Sapindale: Sapindaceae) in Europe. Until the appearance of the moth, this tree was largely free of pests and diseases, with the notable exception of another alien species, *Guignardia aesculi* (Peck), a fungus of the family Ascomycetes.

The horse-chestnut is native to the Balkans. It has been widely planted in Europe as an ornamental tree in streets, urban parks, recreation areas and public gardens since the second half of 17th Century.

Cameraria ohridella, probably originates from some remote horse-chestnut stands in the Balkan mountains (R. Valade, unpublished data), was first discovered near lake Ohrid (hence its name) in Macedonia in 1984 (Deschka & Dimić 1986) and since then has invaded most of Europe (Augustin et al. 2009) (Fig.3). *Cameraria ohridella* is the only species of its genus present in Europe, other species being found in North America and Asia. Only five years after its first appearance, in 1989, *C. ohridella* was found in Austria in the region of Linz, 800 km north of Macedonia (Tomiczek & Krehan 1998). Since then, it has spread very rapidly to other parts of Europe (Gilbert et al. 2005). It appears to have spread from its first infestation point in Macedonia to Albania, Serbia, Greece, Bulgaria, Romania, Croatia and from its second infestation in Austria to Italy (1992), Germany and Czech Republic (1993), Slovakia (1994), Slovenia (1995), Holland and Belgium (1999), France (2000), and England (2002). It was first noticed in Switzerland in 1998 (Kenis & Forster 1998).

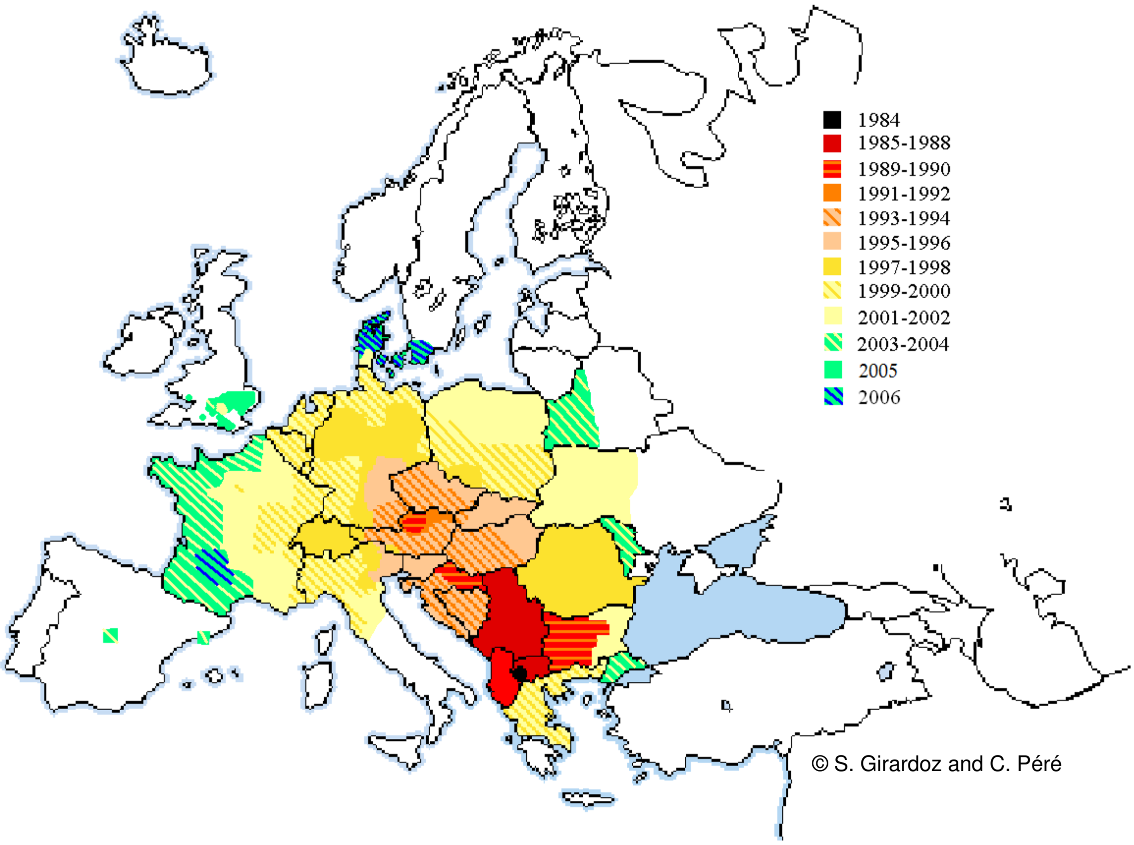

Fig. 3: Map of Europe showing the spreading of *Cameraria ohridella* until 2006.

Cameraria ohridella has two to four generations per year, depending on the climate (Pschorn-Walcher 1994). In cool areas, such as the Jura, only two generations occur. However in southern Europe, four generations are observed. In each generation, a small percentage of pupae enter into diapause. In Delémont (Switzerland), the first flight of the moths takes place at the beginning of May (Fig. 4). As soon as they emerge from their overwintering pupa, adult moths fly to the bark of the horse-chestnut tree. Mating takes place on the bark and the females fly away to lay their eggs. The females then deposit individually about 75 eggs on the upper surface of the leaves, often on the lateral nerves or between them. The development of the eggs takes three to four weeks, depending on weather conditions (Pschorn-Walcher 1994). The first two larval stages are sap-feeders and mine along the margins for a few millimeters. The next two stages are tissue-feeders and extend the mine first in a circular manner, then to a more irregular shape. The mines can grow up to 8 or 9 cm and fuse when the infestation is high. The first spinning stage starts spinning the cocoon, but stops to moult and most of the cocoon will be done by the second spinning stage. The mouthparts of the spinning stage are modified to enable the larvae to spin a cocoon, in which pupation takes place (Pschorn-Walcher 1994). The pupae stay in the mine and complete their development in two to three weeks in the summer. Pupae overwinter in the dead leaves.

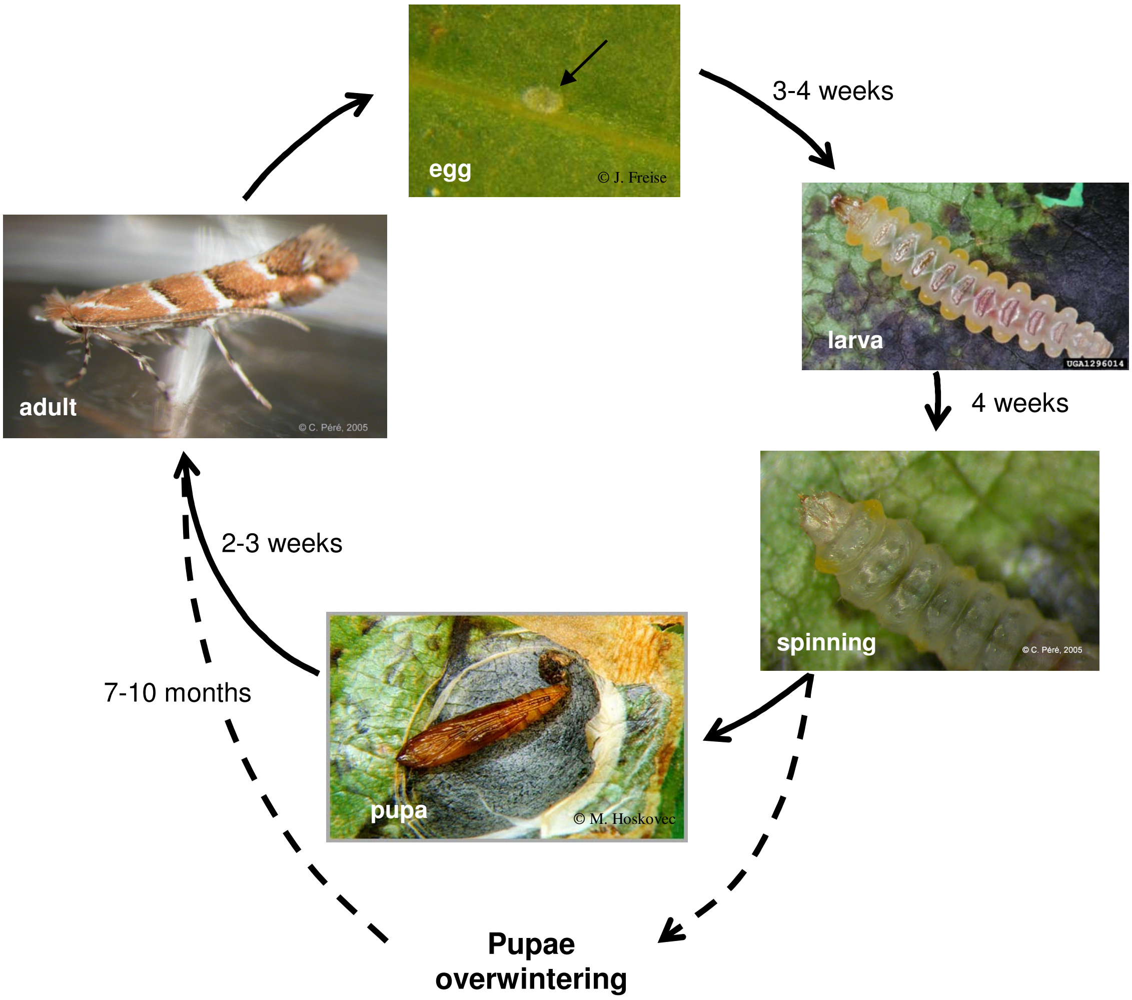

Fig. 4: Life cycle of *Cameraria ohridella.*

The larvae of *C. ohridella* mine the leaves of the tree, creating brown lesions that eventually cover the whole leaf (Fig. 5). This can lead, at high infestation levels, to a total defoliation of the trees as early as in the first generation of the moth, which occurs in June. The defoliation caused by *C. ohridella* is highly damaging from an aesthetic point of view, and it causes concern for the long-term survival of the trees, although studies in Italy showed that mature trees are not seriously affected (Salleo et al. 2003). However, in the few remaining endemic horse-chestnut forest in the Balkans, there is concern for the survival of the species because unpublished studies showed that defoliation is likely to affect regeneration processes.

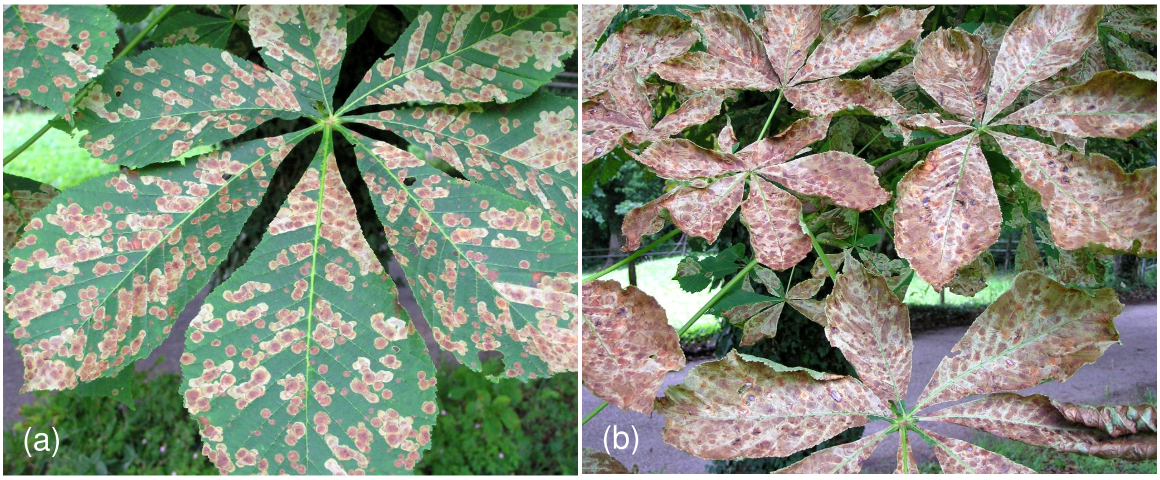

Fig. 5: Leaves of horse-chestnut attacked by *Cameraria ohridella* in mid-June (a) and mid-August (b).

The most important mortality factors are host resistance in the first instars, competition for food in the leaves, bad synchronisation with leaf senescence in autumn and winter mortality (Girardoz et al. 2007a). Predation rates usually vary between 2 to 4% (Grabenweger et al. 2005b) although, in some cases, avian predation may reach 20% (Girardoz et al. 2007a). Mortality due to parasitism is limited (0 to 20%), probably because the parasitoids of *C. ohridella* are not well adapted to this new host (Girardoz et al. 2006; Grabenweger et al. 2005a).

The predator complex of *C. ohridella* includes mainly birds but also grasshoppers, ants and lacewings. Indeed, a predatory behaviour of workers of *Crematogaster scutellaris* (Olivier), acrobat ant, on larvae and pupae of horse-chestnut leaf miner was observed for the first time in Northern Italy (Radeghieri 2004). However, references on ants predating on leaf miners are scarce. Grabenweger et al. (2005b) studied the predator complex of *C. ohridella* in Europe. Of all potential predators observed, only blue tits, *Parus caeruleus* L., great tits, *Parus major* L., marsh tits, *Parus palustris* L. and the southern oak bushcricket *Meconema meridionale* (Costa) were found to prey on the pre-imaginal leaf miner stages. According to Grabenweger et al. (2005b) bushcrickets have never before been recorded preying on leaf miners; moreover *M. meridionale* showed a measurable negative impact on the *C. ohridella* population.

All parasitoids attacking *C. ohridella* are supposed to be polyphagous species (i.e. they parasitise all kinds of leaf miners) from Europe (Noyes 2002). They belong to two super-families of Hymenoptera: Ichneumonoidea and Chalcidoidea (Freise et al. 2002). Most are Chalcidoidea, mainly Eulophidae whereas Ichneumonoidea (Ichneumonidae and Braconidae) are scarce. The major species, in Switzerland and in other countries in Central Europe, are four Eulophidae (Girardoz et al. 2006): *Minotetrastichus frontalis* Nees, *Pnigalio agraules* Walker, *Chrysocharis nephereus* Walker and *Closterocerus trifasciatus* Westwood. These four species of parasitoids attack preferentially fourth instar larvae, spinning stages and pupae of *C. ohridella*. Most parasitoids of *C. ohridella* are optional hyperparasitoids, i.e. they also develop at the expense of primary parasitoids.

The main host-plant of *C. ohridella* is the white flowering horse-chestnut, *A. hippocastanum*, but experiments in field cages showed that the moth also develops very successfully on the Japanese species, *Aesculus turbinata*, and, to a lesser extend, on others *Aesculus* species such as *Aesculus* x *carnea*, *A. flava* and *A. glabra* (Freise et al. 2004; Dimić et al. 2005; Straw & Tilbury 2006). In addition, the horse-chestnut leaf miner is occasionally found attacking and developing on maple trees (*Acer pseudoplatanus* and *A. platanoides*), in the vicinity of heavily infested horse-chestnut trees (e.g., Pschorn-Walcher 1997).

Hypotheses and objective of this thesis

This thesis mainly aimed to investigate the present and potential impact of *C. ohridella* on the native fauna and flora. The two general hypotheses of the present thesis were:

Hypothesis 1 *– Indirect impact on native fauna: Cameraria ohridella has a negative impact on the population density of native leaf miners through the enhanced production and population build-up of a high quantity of polyphagous natural enemies.*

The first aim of this thesis was to assess the interactions of *C. ohridella* with native leaf miner species through their shared natural enemies, especially parasitoids. This indirect impact known as apparent competition is defined as a negative effect between species at the same trophic level that do, or do not share resources, mediated through the action of shared natural enemies (Morris et al. 2004; Van Nouhuys & Hanski 2000). Despite the low parasitism rates observed in *C. ohridella*, populations are so high that an unusual number of polyphagous parasitoids is produced, two to four times per year. These parasitoids then have the opportunity to attack other leaf miners and, hence, affect their density, especially in spring. Indeed, after overwintering, the parasitoids emerge from horse-chestnut dead leaves at the same time or earlier as their host, usually at least five weeks before suitable *C. ohridella* larvae or pupae are available (Girardoz et al. 2006; Grabenweger 2004). Similar observations were made by Péré (2005) during a preliminary study (Figs 6 & 7). According to Girardoz et al. (2006), when reared in captivity under natural conditions, the majority of the adult parasitoids matures in a few days and dies within five weeks after emergence, which suggests that they will attack early-occurring leaf miners. High populations of *C. ohridella* may also enhance populations of polyphagous predators which may as well affect the native fauna.

Chapter 2 provides a comparison of species richness and population densities of native leaf miners in the presence and absence of *C. ohridella*. Parallel studies were carried out in Bulgaria, where the host tree, *Aesculus hippocastanum*, is indigenous, and in Switzerland and France, where it has been introduced. Chapter 3 compares the parasitism and predation of the early-occurring native leaf miner species, *Orchestes fagi* L. (Coleoptera, Curculionidae) in the presence and absence of the invasive horse-chestnut leaf miner.

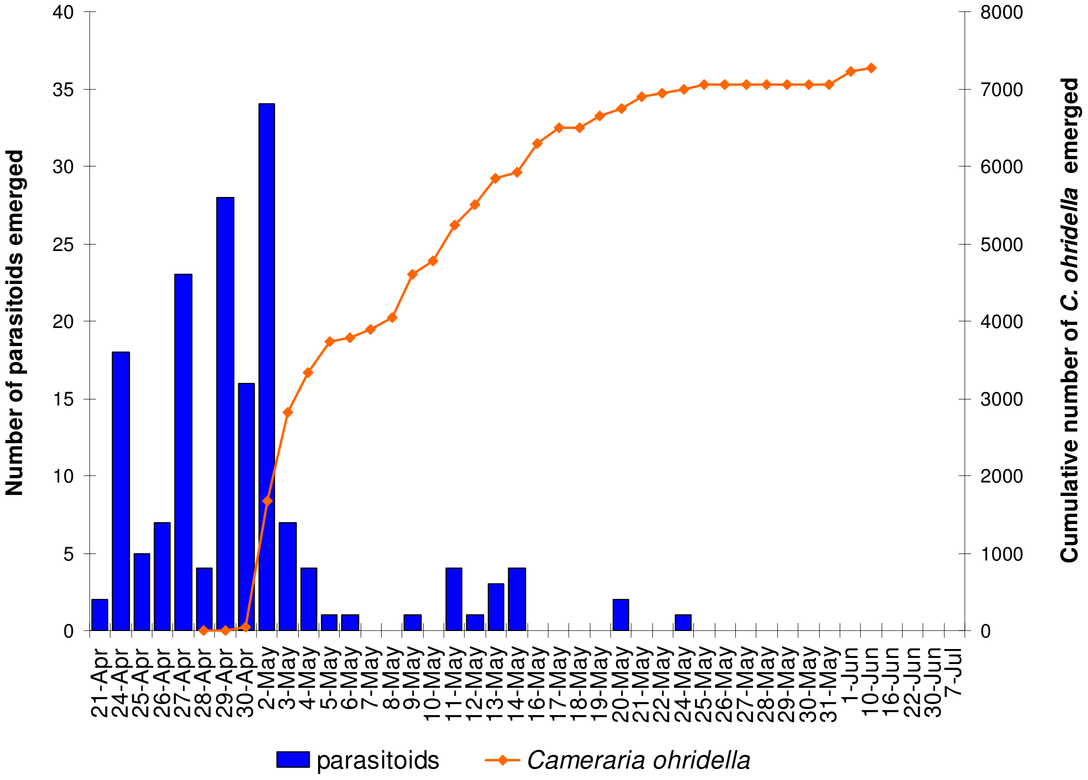

Fig. 6: Emergence of overwintering pupae of *Cameraria ohridella* and its parasitoid complex, Switzerland- spring 2005 (Péré 2005).

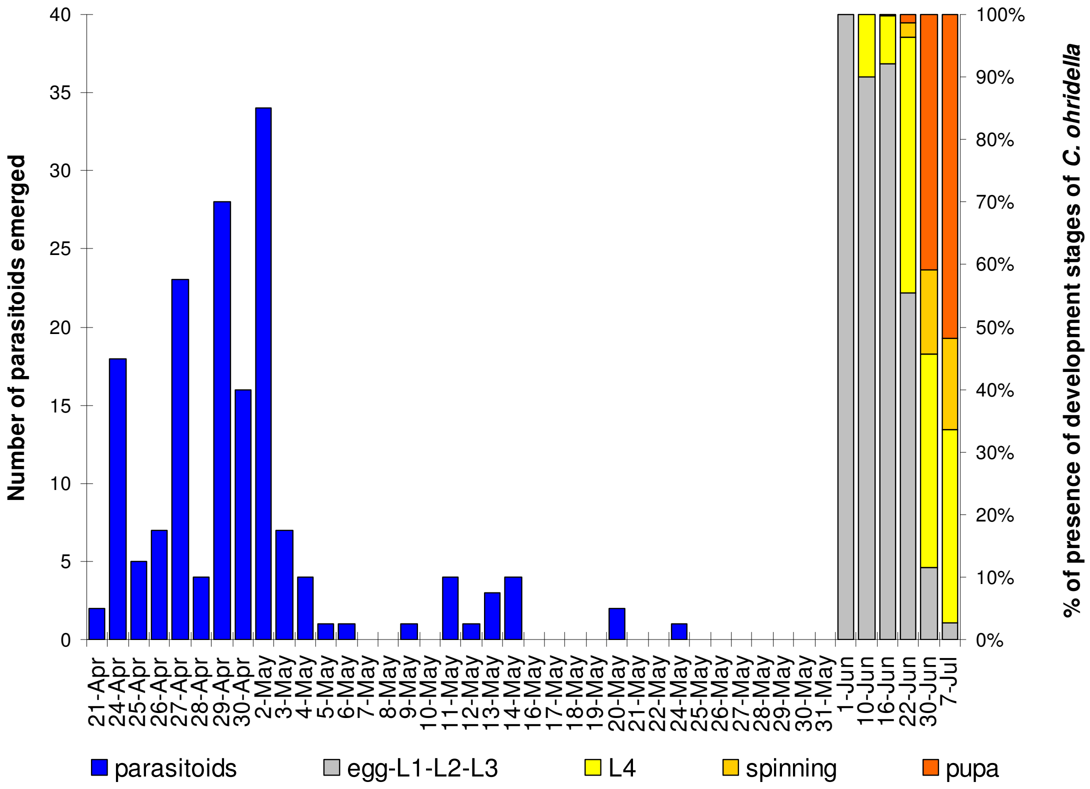

Fig. 7: Emergence of the parasitoid complex of *Cameraria ohridella* and percentage of presence of development stages of *C. ohridella*, Switzerland- spring 2005 (Péré 2005).

Hypothesis 2 *- Direct impact on native flora: Cameraria ohridella, primarily a pest of an ornamental, non-indigenous tree in Central Europe, is increasingly attacking a native tree, Acer pseudoplatanus and represents a potential risk for this and other Acer species.*

In Europe, mines of *C. ohridella* have also been recorded on two maple species, *A. pseudoplatanus* and *A. platanoides* (Tomiczek & Krehan 1998), but attacks are highly variable and seem to depend on the physiology of individual trees (Kenis et al. 2005). Preliminary observations carried out in 2005 showed that most maple trees are attacked in the vicinity of horse-chestnut, in contrast to other tree genera. However, only in some occasions *C. ohridella* larvae are able to develop whereas, in most cases, larvae die as early instars. In chapter 4, the aims of the study were to provide the first general overview of the relationship between *C. ohridella* and *A. pseudoplatanus* and, in particular, to understand whether variations in development success of *C. ohridella* are due to maple varieties that may show varying resistance levels, or to *C. ohridella* populations that have become adapted to maple. Furthermore, the objective was also to determine whether attacks on *A. pseudoplatanus* increase with time and whether there is a risk that *C. ohridella* will become a serious pest of this valuable European tree species.

References

Augustin S., Guichard S., Heitland W., Freise J., Svatoš A. & M. Gilbert (2009). Monitoring and dispersal of the invading Gracillariidae *Cameraria ohridella. Journal of Applied Entomology* In press.

Askew R.R. & Shaw M.R. (1974). An account of the Chalcidoidea (Hymenoptera) parasitising leaf-mining insects of deciduous trees in Britain. *Biological journal of the Linnean society* **6**(4): 289-335.

Deschka G. & Dimić N. (1986). *Cameraria ohridella* sp. n. (Lep., Lithocolletidae) aus Mazedonien, Jugoslawien. *Acta Entomologica Jugoslavica* **22**(1-2): 11-23.

Dimić N., Dautbasic M. & Peric P. (2005). Host plants of *Cameraria ohridella* Deschka & Dimić. *Entomofauna* **26**(10): 193-204.

Freise J.F., Heitland W. & Sturm A. (2004). Host plant range of the horse-chestnut leaf miner, *Cameraria ohridella* (Lepidoptera: Gracillariidae), a pest of the white flowering horse-chestnut, *Aesculus hippocastanum. Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie* **14**(1-6): 351-354.

Freise J.F., Heitland W. & Toshevski I. (2002). Parasitism of the horse-chestnut leaf miner, *Cameraria ohridella*, Deschka and Dimic (Lep., Gracillariidae), in Serbia and Macedonia. *Anzeiger für Schädlingskunde* **75**: 152-157.

Gilbert M., Guichard S., Freise J., Grégoire J.-C., Heitland W., Straw N., Tilbury C. & Augustin S. (2005). Forecasting *Cameraria ohridella* invasion dynamics in recently invaded countries: from validation to prediction. *Journal of Applied Ecology* **42**: 805-813.

Girardoz S., Kenis M. & Quicke D.L.J. (2006). Recruitment of native parasitoids by an exotic leaf miner, *Cameraria ohridella*: host-parasitoid synchronisation and influence of the environment. *Agricultural and Forest Entomology* **8**: 49-56.

Girardoz S., Quicke D.L.J. & Kenis M. (2007a). Factors favouring the development and maintenance of outbreaks in an invasive leaf miner *Cameraria ohridella* (Lepidoptera: Gracillariidae): a life table study. *Agricultural and Forest Entomology* **9**: 141-158.

Grabenweger G. (2004). Poor control of the horse chestnut leafminer, *Cameraria ohridella* (Lepidoptera: Gracillariidae), by native European parasitoids: a synchronisation problem. *European Journal of Entomology* **101**(1): 189-192.

Grabenweger G., Avtzis N., Girardoz S., Hrasovec B., Tomov R. & Kenis M. (2005a). Parasitism of *Cameraria ohridella* (Lepidoptera, Gracillariidae) in natural and artificial horse-chestnut stands in the Balkans. *Agricultural and Forest Entomology* **7**(4): 291-296.

Grabenweger G., Kehrli P., Schlick-Steiner B., Steiner F., Stolz M. & Bacher S. (2005b). Predator complex of the horse chestnut leafminer *Cameraria ohridella*: identification and impact assessment. *Journal of Applied Entomology* **129**(7): 353-362.

Hering E.M. (1951). Biology of the leaf miners. Netherlands, Junk, W.

Hespenheide H.A. (1991). Bionomics of leaf-mining insects. *Annual Review of Entomology* **36**: 535-560.

Kenis M. & Forster B. (1998). Die Rosskastanien-Miniermotte: neu in der Schweiz. *Der Gartenbau* **39**: 16-17.

Kenis M. & Péré C. (2007). Ecological impact of invasive insects in forest ecosystems. *In* Evans, H.F. and Oszako, T. (*eds.*). Alien Invasive Species and International Trade. IUFRO UNIT 7.03.12 Monograph. pp. 118-122.

Kenis M., Tomov R., Svatos A., Schlienslog P., Lopez Vaamonde C., Heitland W., Grabenweger G., Girardoz S., Freise J. & Avtzis N. (2005). The horse-chestnut leaf miner in Europe - Prospects and contraints for biological control. Pages 77-90 *In:* Proceedings of the Second International Symposium on Biological Control of Arthropods, 12-16 September 2005, Davos, Switzerland, Forest Health Technology Enterprise Team-Morgantown, WV.

Morris R.J., Lewis O.T. & Godfray H.C.J. (2004). Experimental evidence for apparent competition in a tropical forest food web. *Nature* **428**: 310-313.

Noyes J.S. (2002). Interactive catalogue of world Chalcidoidea 2001. Compact disk. Taxapad, Vancouver, Canada.

Péré C. (2005). Impact de la mineuse du marronnier d'Inde, *Cameraria ohridella*, sur les mineuses indigènes. MSc thesis, Tours, France, University François-Rabelais**:** 45 pages [in French].

Pimentel D. (2002). Biological invasions: economics and environmental costs of alien plant, animal, and microbe species. CRC Press, Boca Ratton, USA.

Pschorn-Walcher H. (1994). Freiland-Biologie des eingeschleppten Rosskastanien- Miniermotte *Cameraria ohridella* Deschka et Dimic (Lep., Gracillariidae) im Wienerwald. *Linzer biologische Beiträge* **26**(2): 633-642.

Pschorn-Walcher H. (1997). Biology and population dynamics of the horse-chestnut leaf miner, *Cameraria ohridella*. *Forstschutz Aktuell* **21**: 7-10.

Radeghieri P. (2004). *Cameraria ohridella* (Lepidoptera Gracillariidae) predation by *Crematogaster scutellaris* (Hymenoptera Formicidae) in Northern Italy (Preliminary note). *Bulletin of Insectology* **57**(1): 63-64.

Salleo S., Nardini A., Raimondo F., Lo Gullo M.A., Pace F. & Giacomich P. (2003). Effects of defoliation caused by the leaf miner *Cameraria ohridella* on wood production and efficiency in *Aesculus hippocastanum* growing in North-Eastern Italy. *Trees-Structure and Function* **17**: 367-375.

Straw N.A. & Tilbury C. (2006). Host plants of the horse-chestnut leaf-miner (*Cameraria ohridella*), and the rapid spread of the moth in the UK 2002-2005. *Arboricultural Journal* **29**: 83–99.

Tomiczek C. & Krehan H. (1998). The horse-chestnut leafmining moth (*Cameraria ohridella*): a new pest in Central Europe. *Journal of Arboriculture* **24**(3): 144-148.

Van Nouhuys S. & Hanski I. (2000). Apparent competition between parasitoids mediated by a shared hyperparasitoid. *Ecology Letters* **3**: 82-84.

Chapter 2

Species richness and abundance of native leaf miners are affected by the presence of the invasive horse-chestnut leaf miner

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Biological Invasions (in press)

Abstract

The effect of the alien horse-chestnut leaf miner, *Cameraria ohridella*, on native fauna was studied by comparing the species richness of native leaf miner communities and the abundance of selected native leaf miner species in the presence and absence of horse-chestnut trees infested by *C. ohridella*, in various environments in Europe. The species richness of native leaf miner communities in Switzerland was lower at sites where *C. ohridella* was present than at control sites. In Switzerland, France and Bulgaria, several native leaf miner species were significantly less abundant in the vicinity of infested horse-chestnuts. The native species most affected by the presence of the invasive alien species were those occurring early in the year and sharing their parasitoid complex with *C. ohridella.* These results suggest apparent competition mediated by shared natural enemies because these are the only link between *C. ohridella* and native leaf miners using other food resources.

Keywords: Invasive alien species; ecological impact; apparent competition; *Cameraria ohridella*; leaf miners; native communities

Introduction

Invasive alien species affect native biodiversity and ecosystems through various direct and indirect mechanisms, such as interspecific competition for resource or space, predation or habitat alteration (Parker et al. 1999; Levine et al. 2003). Insects are among the most numerous invasive species but their ecological impact remains largely unknown (Kenis et al. 2009). Among the least studied ecological effects of invasive species are those that occur indirectly, through a third species, e.g. when the invasive species is a vector of a disease or by apparent competition. Apparent competition refers to a negative effect between two or more species at the same trophic level that may or may not share resources, mediated through the action of shared natural enemies (Holt 1977; Van Nouhuys & Hanski 2000; Morris et al. 2004). It can occur in a variety of systems and may affect taxa at different trophic levels (Tompkins et al. 2000; Prenter et al. 2004). In insects, apparent competition has been often cited as a potential process structuring communities in which ordinary competition is not pervasive (Van Veen et al. 2006). It has been observed in various communities of native insect species (e.g. Müller & Godfray 1997; Morris et al. 2001, 2004). Mechanisms have been demonstrated in laboratory studies (e.g. Bonsall & Hassell 1997, 1998) or through field population manipulations (Van Nouhuys & Hanski 2000; Morris et al 2004). Apparent competition has also been cited as a mechanism by which invasive alien species affect populations of native species (Schönrogge & Crawley 2000; Kenis et al. 2009) but there are very few examples of alien insects causing long-term changes in natural insect communities due to shared natural enemies. Settle and Wilson (1990) showed that the invasion of the variegated leafhopper, *Erythroneura variabilis* in California led to the decline of its native congener *E. elegantula*, and that this decline was correlated with increased levels of a shared egg parasitoid. More recently, Carvalheiro et al. (2008) reported evidence that a seed feeder introduced as weed biological control agent may affect populations of several seed herbivores in Australia through apparent competition.

Leaf miners are among the insects most heavily attacked by parasitoids (Askew & Shaw 1979; Hawkins 1994). Most of these parasitoids are polyphagous, attacking leaf miners belonging to various genera, families and orders (Askew 1994). This high flexibility enables parasitoids to incorporate exotic leaf miners into their host spectrum and, in many cases, may provide substantial control (Godfray et al. 1995; Girardoz et al. 2007c).

The invasion of the horse-chestnut leaf miner, *Cameraria ohridella* Deschka & Dimić (Lep.: Gracillariidae) in Europe, presents an excellent opportunity to assess the occurrence of apparent competition between an alien leaf miner and native species using other food resources, mediated by polyphagous natural enemies. This moth, probably originating from the Balkan Mountains, is the first leaf miner known to attack the horse-chestnut, *Aesculus hippocastanum* L., in Europe. It has two to four generations per year, a rather high fecundity and low parasitism and predation rates, which allows it to build and maintain high outbreak densities throughout the continent (Girardoz et al. 2007a, b). Nevertheless, it shares over twenty species of parasitoids with native leaf miners (Grabenweger & Lethmayer 1999; Grabenweger 2003; Girardoz et al. 2006) and, despite low parasitism rates, populations of *C. ohridella* are so high that an unusually large number of polyphagous parasitoids are produced around infested trees, at each generation of the moth. *Cameraria ohridella* may also increase the local abundance of predators, such as birds, bush-crickets, ants and lacewings (Grabenweger et al. 2005b; Girardoz et al. 2007a, b). This enhanced production of parasitoids and, perhaps, predators, has the potential to significantly affect populations of native leaf miners living in the surroundings on other host plants. This effect may be particularly important in spring because the bulk of the parasitoids emerge from dead horse-chestnut leaves at least five weeks before suitable *C. ohridella* larvae or pupae are available (Grabenweger 2004; Girardoz et al. 2006) and thus are likely to attack the native leaf miners, which occur early in the season. The effect on leaf miner species occurring at the same time as *C. ohridella* is less predictable. If the native species are preferred compared to *C. ohridella*, parasitism may also increase in the presence of *C. ohridella*. On the other hand, it cannot be ruled out that locally, the high abundance of *C. ohridella* may divert parasitoids and predators from less abundant, co-occurring native leaf miners, relieving them from natural enemy pressures and increasing their population densities. This indirect but positive effect is sometimes referred to as apparent mutualism (Abrams et al. 1998).

In this study, we test the hypothesis that *C. ohridella* may locally affect populations and communities of native leaf miners through the sharing of natural enemies. We hypothesise that species occurring before *C. ohridella* in spring are negatively affected by the presence of the invasive species, whereas the effect on species occurring later during the season may be either negative or positive. The assessment of apparent competition will be done in two steps. In a first study, described herein, we will assess whether the presence of *C. ohridella* has an effect on native leaf miners, by comparing population levels of a large number of European leaf miner species in the presence and absence of *C. ohridella*, in Switzerland and France, where horse-chestnut is planted as ornamental tree, and in Bulgaria in a natural horse-chestnut forest. If these observational studies suggest the possibility of apparent competition, in a following step we will investigate the causal mechanisms by assessing parasitism and predation through field observations and experimental manipulations.

Materials and Methods

Field sites

The study was carried out in Switzerland, France and Bulgaria. In Switzerland, field experiments were conducted from May to September 2005 and 2006 and again in May 2008. Thirty-three pairs of sites were selected in the North-West of the country (Cantons Jura, Basel-Landschaft, Solothurn and Aargau), within a radius of 50km from the city of Delémont (47°22'N – 7°21'E), at altitudes between 300 and 700 m, in or at the edge of a broad-leaved forest. Thirty-three sites consisted of trees and shrubs less than 50 m from one or more horse-chestnut trees infested by *C. ohridella*. For each of these sites, a twin site with similar characteristics (altitude, soil type, vegetation cover, absence/presence of a stream, etc.) was selected at 2-5 km distance from the paired site and at a minimum of two km from any horse-chestnut trees. The experimental design in France was similar, with 30 pairs of sites in the Centre Region (Departments Loiret and Loir & Cher) within a radius of 50 km from the city of Orléans (47°54'N – 1°54'E), compared in May-June 2006, at altitudes between 90 and 141 m, in parks, in or at the edge of a broad-leaved forest. In contrast, in Bulgaria, we compared the situation in the only natural horse-chestnut stand, in the nature reserve of Dervisha (Veliki Preslav, Shumen District, alt.: 317-500 m area: 70.2 ha, coord: 41°56'N – 26°21'E) with ecologically similar forests without horse-chestnut 15-30 km from Dervisha. Two sets of ten sites were randomly selected in and outside the Dervisha forest. The ten sites outside the Dervisha forest were chosen to be as ecologically similar as possible to the Dervisha forest: similar altitude, similar vegetation cover (except for the absence of horse-chestnut) and similar relief and hygrometry (dark and humid valleys along a stream). Horse-chestnut does not grow naturally outside the Dervisha forest and all the ten sites outside the forest were at least at 10 km from the nearest planted horse-chestnut tree. Field surveys in Bulgaria were carried out in May-September 2005 and 2006. However, no leaf miner species was found in May and early June and, thus, samples were taken in July and September only.

All horse-chestnut trees occurring at the sites had been heavily and continuously infested by *C. ohridella* (i.e. a minimum of 20 mines per leaf) for at least 12 years in Bulgaria, 4 to 7 years in Switzerland and 2 to 4 years in France.

Species richness of native leaf miners in the presence and absence of *C. ohridella*

Species richness is defined here as the number of leaf miner species found in a given time at a particular site. Observations on species richness were carried out in Switzerland only. To compare species richness of native leaf miners at sites with and without horse-chestnut infested by *C. ohridella*, we measured leaf miners' richness at 22 pairs of sites in 2005 and 33 pairs of sites in 2006, three times per year: in spring (May-early June), summer (July) and autumn (September). We collected all leaf miner species found during a random search of precisely one man-hour on all trees and shrubs encountered in a 50 m radius from the central point of the site. The leaf miners were determined in the laboratory based on the identification of their host-plant, the characteristics of their mines, and the morphology of larvae or pupae, using Hering's (1957) and Ellis's (2008) keys. The number of different species found per site was counted. As leaf miner species vary in their life cycle, development time and number of generations and, because it is not always possible to determine the age of a mine after the larva has left it, the leaf miner species in summer and autumn collections were pooled with those of the previous collections at the same site.

Abundance of native leaf miners in the presence and absence of *C. ohridella*

The abundance of a leaf miner species is defined here as the number of leaves attacked by this leaf miner for a given number of leaves. The abundance of native leaf miners in the presence and absence of horse-chestnut infested by *C. ohridella* were compared in the three countries. In Switzerland, three times a year for two years (May, July and September 2005 and 2006), 11 leaf mining species feeding on *Lonicera xylosteum* L., *Corylus avellana* L. and *Fagus sylvatica* L. and known from previous surveys by the authors to be abundant in the region, were selected (Table 1). In addition, mines of the beech leaf mining weevil, *Orchestes fagi* L., were sampled on *F. sylvatica* in 2008 at the same sites as in 2006. At 19 to 22 sites in 2005 and 28 to 33 sites in 2006 and 2008, one thousand leaves per tree or shrub species were randomly selected within 50 m from the central point and preferably on at least five shrubs or major tree branches. All selected leaves were inspected for leaf-miners and those mined by the 11 pre-selected leaf miner species were counted and collected.

In France, a similar method was used. Five leaf miner species were collected on *Quercus robur* L. (30 pairs of sites) and *Lonicera periclymenum* L. (14 sites with horse-chestnut infested by *C. ohridella* and 25 unpaired sites without horse-chestnut) in May-June 2006 (Table 1). In Bulgaria, the abundance of 17 leaf miner species from five tree and shrub species (Table 1) was compared between the ten sites in the same natural horse-chestnut forest and the ten sites outside the forest. One thousand leaves per tree or shrub species were randomly selected and the number of leaves containing a given leaf miner species was counted.

Table 1: List of native leaf miners species whose abundance was studied in the three countries.

Host plants / Leaf miners	Abbrev.	Order: Family	BG	CH	FR
Carpinus betulus L.					
Parornix carpinella (Frey)	*P. car*	Lep: Gracillariidae	x		
Phyllonorycter esperella (Goeze)	*P. esp*	Lep: Gracillariidae	x		
Phyllonorycter tenerella (de Joannis)	*P. ten*	Lep: Gracillariidae	x		
Stigmella carpinella (von Heinemann)	*S. car*	Lep: Nepticulidae	x		
Stigmella microtheriella (Stainton)	*S. mic*	Lep: Nepticulidae	x		
Cornus mas L.					
Antispila treitschkiella (Fischer von Röslerstamm)	*A. tre*	Lep: Heliozelidae	x		
Phytomyza agromyzina Meigen	*P. agr*	Dip: Agromyzidae	x		
Corylus avellana L.					
Parornix devoniella (Stainton)	*P. dev*	Lep: Gracillariidae	x		
Phyllonorycter coryli (Nicelli)	*P. cor*	Lep: Gracillariidae	x	x	
Phyllonorycter nicellii (Stainton)	*P. nic*	Lep: Gracillariidae	x	x	
Stigmella microtheriella (Stainton)	*S. mic*	Lep: Nepticulidae	x	x	
Fagus sylvatica L.					
*Orchestes fagi** (L.)	*O. fag*	Col: Curculionidae		x	
Phyllonorycter maestingella (Müller)	*P. mae*	Lep: Gracillariidae	x	x	
Stigmella hemargyrella (Kollar)	*S. hem*	Lep: Nepticulidae	x	x	
Stigmella tityrella (Stainton)	*S. tit*	Lep: Nepticulidae	x	x	
Lonicera spp.					
*Aulagromyza hendeliana** (Hering)	*A. hen*	Dip: Agromyzidae		x	
Aulagromyza cornigera (Griffiths)	*A. cor*	Dip: Agromyzidae			x
*Chromatomyia periclymeni** (Hendel)	*C. per*	Dip: Agromyzidae			x
Phyllonorycter emberizaepennella (Bouché)	*P. emb*	Lep: Gracillariidae		x	
Quercus robur L.					
*Orchestes quercus** (L.)	*O. que*	Col: Curculionidae			x
Phyllonorycter quercifoliella (Zeller)	*P. que*	Lep: Gracillariidae			x
Phyllonorycter roboris (Zeller)	*P. rob*	Lep: Gracillariidae			x
Ulmus glabra Hudson					
Phyllonorycter schreberella (Fabricius)	*P. sch*	Lep: Gracillariidae	x		
Stigmella lemniscella (Zeller)	*S. lem*	Lep: Nepticulidae	x		
Stigmella ulmivora (Fologne)	*S. ulm*	Lep: Nepticulidae	x		

Notes: BG = Bulgaria, CH = Switzerland, FR = France
*Leaf miner species whose mature larvae were found at least two weeks before those of *C. ohridella*.

Confounding environmental variables

Various environmental variables may influence species richness and abundance of insects (Ricketts et al. 2001). Confounding factors were minimized in the Swiss and French samples by selecting paired sites with similar characteristics. In addition, at the Swiss sites, the influence of two potential confounding factors was assessed: woody plant richness and soil cover. Woody plant richness directly influences leaf miners' richness and may also affect species abundance, e.g. through natural enemies. Woody plant richness was measured as the number of tree and shrub species found per site in a radius of 50 m from the central point of the site. Soil cover may also influence species richness and abundance, as if can be expected that leaf miners and their natural enemies may react either positively or negatively to the level of urbanization or the amount of forested areas in the surroundings. On the other hand, it is not clear at which distance soil cover may affect species richness and abundance. Thus, it was assessed by measuring, on a 1:25,000 map, the percentage of soil (± 5%) covered by forests and constructed habitats (buildings, roads, parking places, etc.), in circles of 250 m, 500 m and 1000 m radii around each site (i.e. six measures of soil cover per site).

Statistical analysis

For the Swiss data, generalized linear models (GzLM) were used to test the effect of presence/absence of *C. ohridella*, woody plant richness and soil cover on the abundance and species richness of native leaf miners. Counts of abundance were analyzed using a negative binomial distribution with log link function, and counts of species richness were analyzed using a Poisson distribution with a log link function, and Pearson chi-square as the method for estimating the scale parameter. Presence/absence of *C. ohridella* was entered as fixed factor and woody plant richness and soil cover as covariates. The six measures of soil cover were entered separately in different analyses because they are highly correlated. For the French and Bulgarian data, comparisons of abundance of native leaf miners were performed using the Mann-Whitney test for unpaired sites or the Wilcoxon paired samples test. All statistical analyses were performed using SPSS software (SPSS Inc., version 16.0, Chicago, USA).

Results

Species richness of native leaf miners in the presence and absence of *C. ohridella*

In Switzerland, species richness of native leaf miners (Annex 1) was higher at sites where *C. ohridella* was absent than at those where it was present, in both 2005 and 2006 (Fig. 1). In 2005, the difference was significant in the spring collection when the woody plant richness and the percentage of forest cover (all distances) were included as covariates, but not when we replaced the percentage of forest cover by the percentage of constructed habitat (all distances) as a measure of land use (Fig. 1). In 2006, differences were significant after the summer and autumn collections, for all combinations of environmental variables included as covariates in the GzLM (Fig. 1).

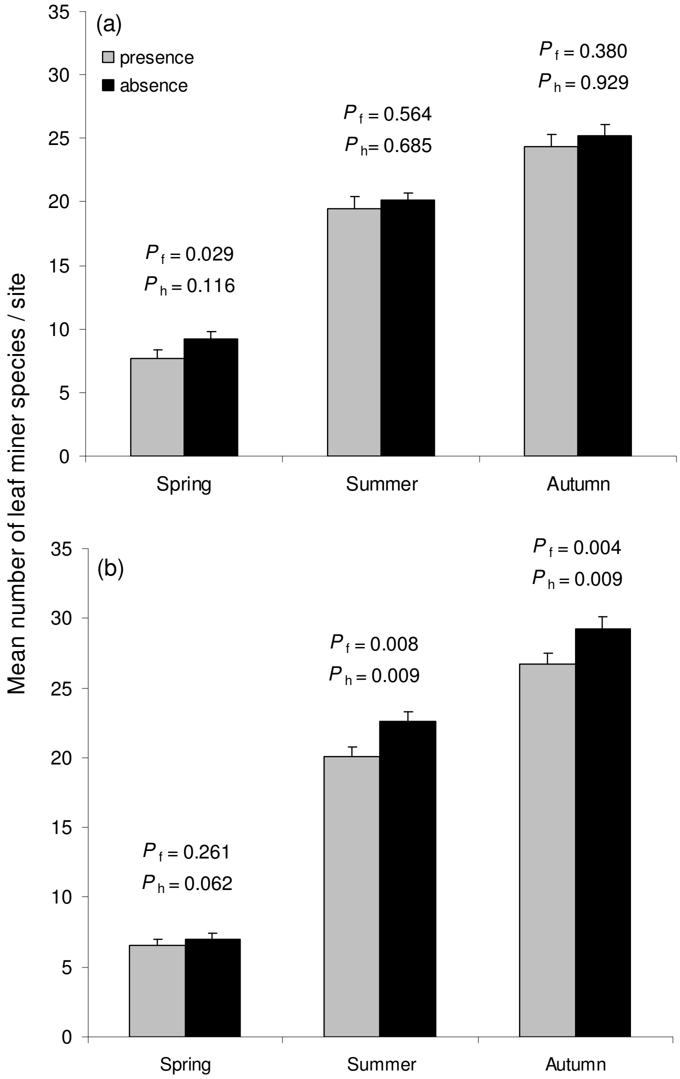

Fig. 1: Species richness of native leaf miners in the presence and absence of *Cameraria ohridella*, in Switzerland in (a) 2005 and (b) 2006 (Spring: collection in May; Summer: cumulative collections of May and July; Autumn: cumulative collections of May, July and September). P_f = confidence level in the GzLM when the % of forest cover in a radius of 250 m was included as covariate. P_h = confidence level in the GzLM when the % of constructed habitat in a radius of 250 m was included as covariate. Error bars represent standard errors.

Abundance of native leaf miners in the presence and absence of *C. ohridella*

In Switzerland, several leaf miner species were found to be statistically less abundant in the presence of *C. ohridella* than in its absence (Figs. 2-3). In particular, the beech leaf mining weevil, *O. fagi*, was strongly affected by the presence of *C. ohridella*, in the three years of sampling, despite high yearly variations in weevil density (Fig. 2a). The agromyzid fly, *Aulagromyza hendeliana,* was not significantly more abundant in the presence of *C. ohridella* (Fig. 2b). In 2006, population levels were significantly lower in the presence of *C. ohridella* than in its absence for *Stigmella tityrella* (Stainton) (1st and 2nd generations) and *S. microtheriella* (Stainton) and *S. hemargyrella* (Kollar) (2nd generation) (Figs. 3c and 3d). In Switzerland, no leaf miner species was found to be significantly more abundant in the presence of *C. ohridella*, except for the 2nd generation of *Phyllonorycter coryli*, in 2005 (Fig. 3b).

A comparable situation was found in France, with the two spring species, the oak leaf mining weevil, *Orchestes quercus* L., and the fly *Chromatomyia periclymeni* (Hendel) being significantly lower in abundance in the vicinity of horse-chestnut infested by *C. ohridella* compared to the control sites (Fig. 4). However, another oak leaf miner, *Phyllonorycter roboris* (Zeller), occurring slightly later in the season than the two other species (Ellis 2008), was significantly more abundant in the presence of *C. ohridella* than in its absence.

In Bulgaria, in the July samples, four (2005) and six (2006) leaf miner species were found to be significantly less abundant in the horse-chestnut forest, respectively (Table 2). No species was significantly more abundant in the horse-chestnut forest in 2005, and only one in 2006 (Table 2). In contrast, in September 2005 three species were significantly more abundant in the horse-chestnut forest and, in September 2006, four species were found significantly more abundant and three less abundant (Table 2). On average, leaf miner species were significantly less abundant in the horse-chestnut forest in July 2005 (Wilcoxon rank test, $P = 0.010$) but not at the other sampling dates.

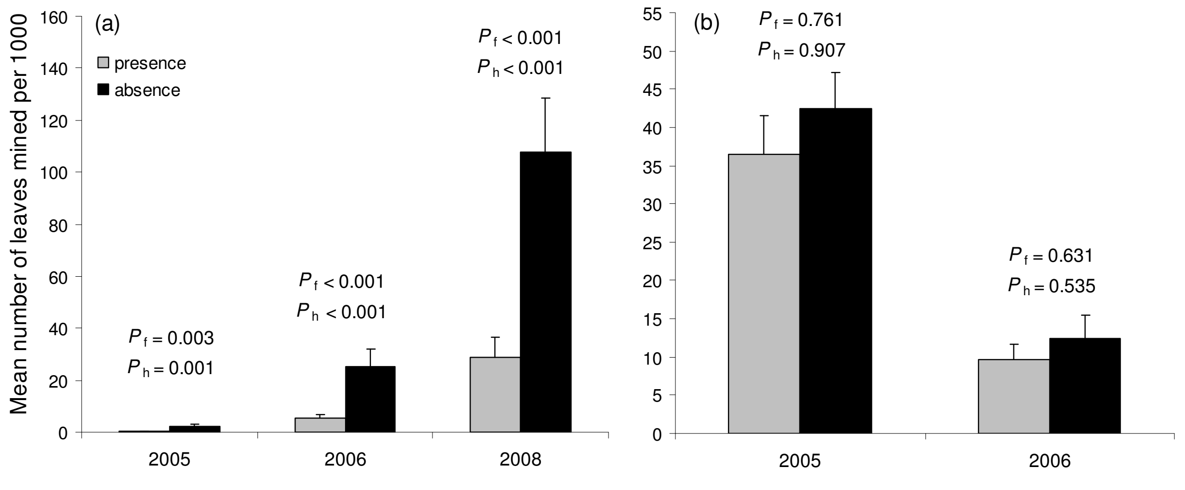

Fig. 2: Abundance of (a) *Orchestes fagi* (2005, 2006 and 2008) and (b) *Aulagromyza hendeliana*, (2005 and 2006) in presence and absence of *Cameraria ohridella*, in Switzerland. Error bars represent standard errors. For the significance of P_f and P_h, see Figure 1.

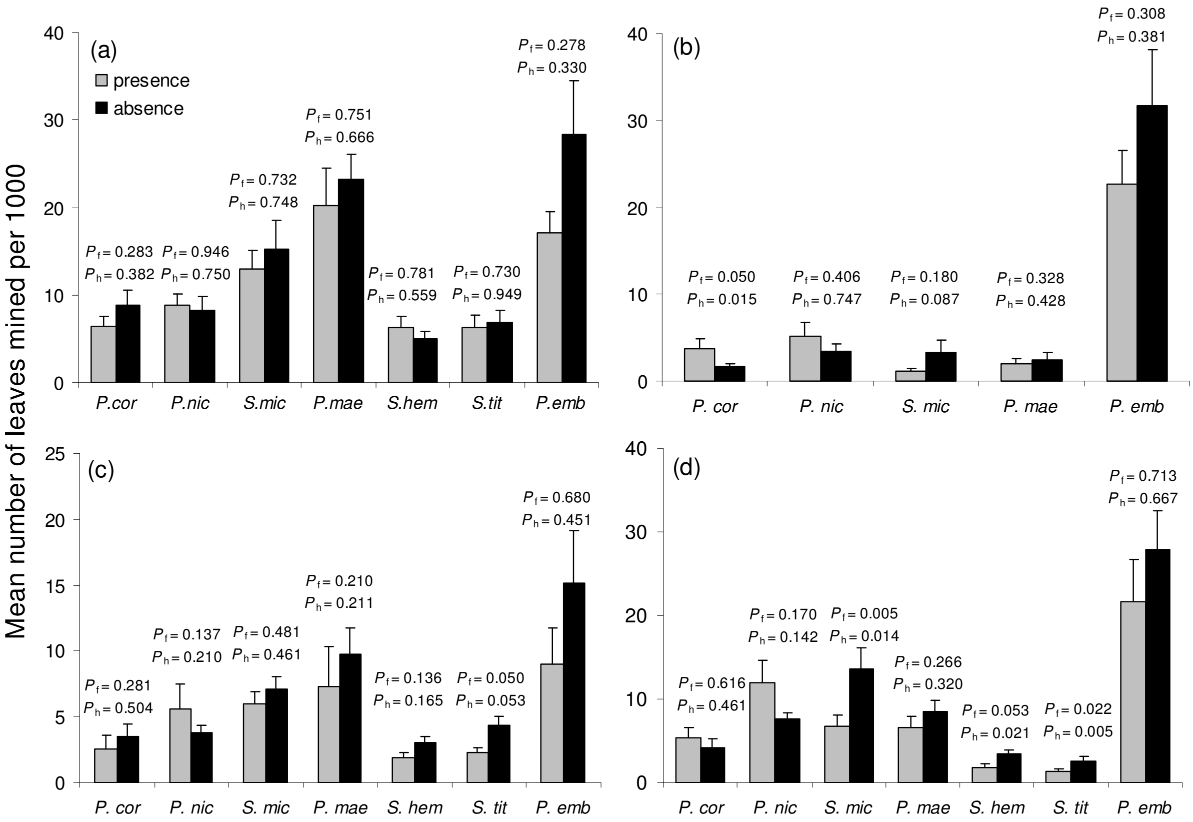

Fig. 3: Abundance of native leaf miners in Switzerland, in (a) July 2005, (b) September 2005, (c) July 2006 and (d) September 2006. Error bars represent standard errors. For the significance of P_f and P_h, see Figure 1. Abbreviations of leaf miner species are given in Table 1.

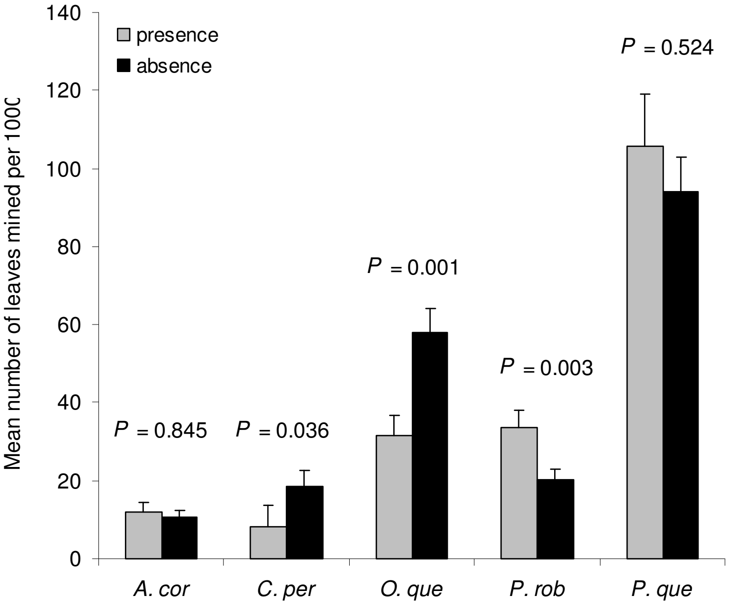

Fig. 4: Abundance of native leaf miners in May-June 2006 (France). Error bars represent standard errors. Abbreviations of leaf miner species are given in Table 1.

Table 2: Median abundance of the number of native leaf miner species found in Bulgaria, in presence (Pres.) and absence (Abs.) of *Cameraria ohridella* and *P*-value of the Mann-Whitney test. Significant *P*-values ($P \leq 0.05$) are in bold-face.

	2005						2006					
Host plants	July			September			July			September		
Leaf miner species	Pres.	Abs.	*P*-value	Pres.	Abs.	*P*-value	Pres.	Abs.	*P*-value	Pres.	Abs.	*P*-value
Carpinus betulus												
Parornix carpinella	0.50	1.70	0.284	2.60	3.00	0.789	5.30	6.60	0.381	24.60	12.20	**0.041**
Phyllonorycter esperella	0.20	1.60	0.108	1.50	2.00	0.319	3.40	1.60	**0.041**	3.20	15.40	**0.027**
Phyllonorycter tenerella	1.80	2.20	0.295	13.20	11.80	0.909	20.20	25.60	0.596	29.80	24.80	0.426
Stigmella carpinella	-	-	-	2.10	1.70	0.350	7.80	4.50	0.149	8.80	6.80	0.378
Stigmella microtheriella	2.20	2.00	0.416	2.90	2.80	0.489	3.40	4.10	0.674	11.20	29.00	**0.010**
Cornus mas												
Antispila treitschkiella	0.80	1.40	0.574	13.20	10.30	0.137	28.00	29.00	0.677	66.00	46.00	0.112
Phytomyza agromyzina	3.60	2.70	0.906	3.50	2.70	0.727	21.10	15.40	0.288	18.80	0.70	**<0.001**
Corylus avellana												
Parornix devoniella	0.60	8.50	**0.015**	15.90	7.70	**0.028**	31.10	22.80	0.173	9.60	6.10	**0.023**
Phyllonorycter coryli	0.10	1.60	**0.045**	1.80	8.10	0.061	4.30	18.80	**0.028**	11.70	7.70	0.761
Phyllonorycter nicellii	4.50	5.90	0.819	1.50	3.70	0.627	0.30	2.70	**0.011**	2.60	2.80	0.701
Stigmella microtheriella	2.60	3.20	0.786	6.80	12.50	0.253	14.00	43.00	**<0.001**	14.00	27.50	0.185
Fagus sylvatica												
Phyllonorycter maestingella	4.20	15.70	**0.001**	26.90	21.50	1.000	27.30	88.30	**0.001**	20.00	82.50	**0.001**
Stigmella hemargyrella	4.80	9.60	**0.049**	10.90	4.80	**0.013**	19.70	15.60	0.271	10.20	9.90	0.762
Stigmella tityrella	-	-	-	-	-	-	1.70	2.30	0.847	1.00	3.20	0.129
Ulmus glabra												
Phyllonorycter schreberella	1.20	1.60	0.807	12.80	6.40	0.095	5.20	3.30	0.126	7.70	2.90	**0.048**
Stigmella lemniscella	1.90	1.80	0.703	4.10	0.50	**0.001**	4.90	14.80	**0.008**	1.40	0.80	0.803
Stigmella ulmivora	0.30	1.40	0.084	-	-	-	0.90	7.20	**0.003**	-	-	-

Discussion

In all three countries, our results show a general tendency towards a lower abundance and species richness of native leaf miner communities in the vicinity of horse-chestnut trees attacked by *C. ohridella*. This suggests apparent competition mediated by natural enemies because parasitoids or predators are the only link between *C. ohridella* and native leaf miners. None of the native leaf miners feed on horse-chestnut and, therefore, an effect of direct competition for space or food can be excluded. Furthermore, the observation that early-occurring leaf miner species, in particular the two *Orchestes* spp., are more strongly affected by the presence of *C. ohridella* strengthens the notion that apparent competition via natural enemies occurs. Indeed, these early-occurring species are particularly exposed to parasitoids of *C. ohridella* that emerge too early in spring to attack the alien species (Grabenweger 2004). Although apparent competition is a likely causal mechanism driving the observed differences in native leaf miner communities that has not been proven, it cannot be ruled out that differences in some unmeasured environmental or biotic variables between sites with horse-chestnut and without horse-chestnut could also have had an effect on the observed pattern, especially in France and Bulgaria. Furthermore, other mechanisms of indirect impact should be considered. For example, horse-chestnut could have an impact on the oviposition behavior of native leaf miner species, e.g. through plant volatiles, or the large quantities of pheromone released by *C. ohridella* may also confuse other leaf miners. Thus, apparent competition between *C. ohridella* and native leaf miners needs to be confirmed by specific observations on parasitism and predation rates or, better, through manipulative field experiments in which the role of natural enemies could be ascertained.

The Swiss results are most robust because of larger sample sizes over several years, the use of paired sites and the potential environmental confounding factors that were taken into account in the analyses. Of particular interest is the case of the beech leaf mining weevil, *O. fagi*, which was considerably less abundant in the presence of *C. ohridella* in all three years investigated. Populations of the weevil increased more than 20 fold between 2005 and 2008, but differences remained. This univoltine species is one of the few European leaf miner species whose larvae occur very early in the year and are well synchronised with the presence of adult parasitoids emerging from overwintering mines of *C. ohridella* (Bale 1981; Girardoz et al. 2006). In addition, *O. fagi* is known to be attacked by the main parasitoids of *C. ohridella*, i.e. *Minotetrastichus frontalis* Nees, *Chrysocharis nephereus* Walker, *Closterocerus trifasciatus* Westwood, *Pnigalio agraules* Walker and *Colastes braconius* Haliday (Noyes 2002; Yu et al. 2005; Girardoz et al. 2006). Thus, *O. fagi* would be the best target for studies into the mechanisms of apparent competition mediated by *C. ohridella*. Another candidate may be *O. quercus*, whose populations were found to be negatively affected by the presence of *C. ohridella* in France. *Orchestes quercus* has similar phenological characteristics and a similar natural enemy complex to *O. fagi* (Hering 1957; Noyes 2002; Yu et al. 2005). However, observations in France were done for a single generation of the weevil and more data are needed to confirm that the presence of *C. ohridella* is associated with lower population densities of *O. quercus*.

Other early-occurring species in Europe include several agromyzid flies, but, in contrast to leaf mining weevils, their parasitoid complex shows less overlap with leaf mining Lepidoptera (Noyes 2002; Yu et al. 2005). Nevertheless, the two early-season agromyzid flies investigated during this study, *A. hendeliana* and *C. periclymeni*, showed tendencies of reduced abundance in the presence of *C. ohridella*, the difference being significant for *C. periclymeni*.

Nearly all European leaf mining Lepidoptera are attacked by at least one of the major parasitoids of *C. ohridella* (Askew & Shaw 1979; Grabenweger et al. 2005a), but their phenology based on Hering's key (1957) largely overlaps with *C. ohridella*. Thus, parasitoids or predators of *C. ohridella* may be less inclined to attack native leaf miners when high numbers of *C. ohridella* larvae or pupae are also present. On the other hand, we did not find clear evidence that populations of native leaf miners occurring at the same time as *C. ohridella* increased in the presence of the invader and, thus, it is unlikely that high densities of *C. ohridella* divert parasitoids and predators from native leaf miners.

In western and central Europe, the impact of *C. ohridella* will remain local because horse-chestnut is an ornamental tree planted mainly in urban areas (Gilbert et al. 2005). However, its impact may dramatically increase should the moth become adapted to native trees such as the sycamore maple, *Acer pseudoplatanus*, which is already commonly attacked in the vicinity of infested horse-chestnuts (Freise et al. 2004). In principle, the impact of *C. ohridella* on local leaf miner communities should be higher in natural horse-chestnut stands in the Balkans. Our observations in the horse-chestnut forest in Bulgaria suggest some effects on native leaf miners. However, our sampling method in Bulgaria, consisting of comparing ten samples in a single forest with various sites in surrounding forests implies pseudoreplications and does not rule out confounding effects of environmental variables related to this single forest. The fact that naturally growing horse-chestnut is very rare in the Balkans precludes a more suitable sampling method. The forest investigated in this study is the only site in Bulgaria where horse-chestnut grows naturally and the few other remaining natural horse-chestnut stands in Macedonia, Albania and Greece are very difficult to access and investigate. Perhaps the most important observation in Bulgaria is that we were unable to find any species of leaf miner occurring in spring before *C. ohridella*. No early-season species were found at the surrounding sites either, but this may be due to the effect of this large horse-chestnut forest being observed over greater distances. Parasitoids are known to spread quickly and far if necessary (Quednau 1990), and the billions of parasitoids (or predators) emerging in spring from dead horse-chestnut leaves may fly long distances to find suitable hosts. Further studies on *C. ohridella* and native leaf miners should include an assessment of the spatial scale of the effects of apparent competition on natural leaf miner communities (Morris et al. 2005).

Should the role of natural enemies be confirmed in further studies, this case would provide the first evidence for indirect ecological effects between an invasive leaf miner and native leaf miners. More generally, it would be one of the first observed cases of an invasive herbivorous insect indirectly affecting native herbivores that do not share the same resource. To our knowledge, the only other convincing case is that of Carvalheiro et al. (2008), who observed that an introduced seed feeder affects native communities of seed herbivores in Australia. Leaf miners are ideal models to study apparent competition between alien and native species because, firstly, leaf miners are among the most successful invaders and become particularly abundant compared to native species (Godfray et al. 1995; Girardoz et al. 2007c); secondly, because leaf miners share many polyphagous natural enemies, particularly parasitoids (Askew 1994; Memmott et al. 1994; Morris et al. 2004); and, thirdly, because leaf miners are easy to sample and parasitism can be easily assessed using standard methods (Girardoz et al. 2007b). The approach used in this study to investigate the effect of *C. ohridella* on native leaf miners through apparent competition could be applied to other invasive leaf miners, in particular those attacking host plants that are widespread in natural ecosystems. In Europe, three invasive gracillariid leaf miners may be particularly suitable for such studies, the Asian *Phyllonorycter issikii*, which mines leaves of lime, *Tilia* spp. (Šefrova 2002), and the North American *Phyllonorycter robiniella* and *Parectopa robiniella*, two pests of black locust, *Robinia pseudoacacia*, an exotic but widespread tree in central Europe (Whitebread 1989).

More generally, we hope that this study will encourage further research on the ecological impact of invasive alien insects. In a recent review, Kenis et al. (2009) identified only 72 alien insects worldwide for which an ecological impact had been investigated, and evidence for impact was found for 54 of them. It is likely that among the tens of thousands of alien insects occurring in all continents, many more affect native biodiversity and ecosystem processes in ways that remain to be discovered.

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References

Abrams P.A., Holt R.D. & Roth J.D. (1998). Apparent competition or apparent mutualism? Shared predation when populations cycle. *Ecology* **79**: 201-212.

Askew R.R. (1994). Parasitoids of leaf-mining Lepidoptera: what determines their host ranges? Pages 177-204 *In*: B.A. Hawkins and W. Sheehan (*eds*). *Parasitoid community ecology*. Oxford University Press, New York, USA.

Askew R.R. & Shaw M.R. (1979). Mortality factors affecting the leaf-mining stages of *Phyllonorycter* (Lepidoptera: Gracillariidae) on oak and birch. 1. Analysis of the mortality factors. *Zoological Journal of the Linnean Society* **67**:31-49.

Bale J.S. (1981). Seasonal distribution and migratory behaviour of beech leaf mining weevil, *Rhynchaenus fagi* L. *Ecological Entomology* **6**:109-118.

Bonsall M.B. & Hassell M.P. (1997). Apparent competition structures ecological assemblages. *Nature* **388**:371-373.

Bonsall M.B. & Hassell M.P. (1998). Population dynamics of apparent competition in a host-parasitoid assemblage. *Journal of Animal Ecology* **67**:918-929.

Carvalheiro L.G., Buckley Y.M., Ventim R., Fowler S.V. & Memmott J. (2008). Apparent competition can compromise the safety of highly specific biocontrol agents. *Ecology Letters* **11:**690-700.

Ellis W.N. (2008). Nederlandse bladmineerders/Dutch leafminers. URL http://www.bladmineerders.nl

Freise J.F., Heitland W. & Sturm A. (2004). Host-plant range of the horse-chestnut leaf miner, *Cameraria ohridella* Deschka & Dimic (Lepidoptera, Gracillariidae), a pest of the white flowering horse-chestnut, *Aesculus hippocastanum.* In German. *Mitteilung der Deutschen Gesellschaft Angewandter Entomologie* **14**:351-354.

Gilbert M., Guichard S., Freise J., Grégoire J.C., Heitland W., Straw N., Tilbury C. & Augustin S. (2005). Forecasting *Cameraria ohridella* invasion dynamics in recently invaded countries: from validation to prediction. *Journal of Applied Entomology* **42**:805-813.

Girardoz S., Kenis M. & Quicke D.L.J. (2006). Recruitment of native parasitoids by an exotic leaf miner, *Cameraria ohridella*: host-parasitoid synchronisation and influence of the environment. *Agricultural and Forest Entomology* **8:**49-56.

Girardoz S., Quicke D.L.J. & Kenis M. (2007a). Factors favouring the development and maintenance of outbreaks in an invasive leaf miner *Cameraria ohridella* (Lepidoptera: Gracillariidae): a life table study. *Agricultural and Forest Entomology* **9:**141-158.

Girardoz S., Tomov R., Eschen R., Quicke D.L.J. & Kenis M. (2007b). Two methods assessing the mortality factors affecting the larvae and pupae of *Cameraria ohridella* in the leaves of *Aesculus hippocastanum* in Switzerland and Bulgaria. *Bulletin of Entomological Research* **97:**445-453.

Girardoz S., Volter L., Tomov R., Quicke D.L.J. & Kenis M. (2007c). Variations in parasitism in sympatric populations of three invasive leaf miners. *Journal of Applied Entomology* **131:**603-612.

Godfray H.C.J., Agassiz D.L.J., Nash D.R. & Lawton J.H. (1995). The recruitment of parasitoid species to two invading herbivores. *Journal of Animal Ecology* **64:**393-402.

Grabenweger G. (2003). Parasitism of different larval stages of *Cameraria ohridella. Biocontrol* **48:**671-684.

Grabenweger G. (2004). Poor control of the horse chestnut leafminer, *Cameraria ohridella* (Lepidoptera: Gracillariidae), by native European parasitoids: a synchronisation problem. *European Journal of Entomology* **101:**189-192.

Grabenweger G., Avtzis N., Girardoz S., Hrasovec B., Tomov R. & Kenis M (2005a). Parasitism of *Cameraria ohridella* (Lepidoptera, Gracillariidae) in natural and artificial horse-chestnut stands in the Balkans. *Agricultural and Forest Entomology* **7:**291-296.

Grabenweger G., Kehrli P., Schlick-Steiner B., Steiner F., Stolz M. & Bacher S. (2005b). Predator complex of the horse chestnut leafminer *Cameraria ohridella*: identification and impact assessment. *Journal of Applied Entomology* **129:**353-362.

Grabenweger G. & Lethmayer C. (1999). Occurrence and phenology of parasitic Chalcidoidea on the horse chestnut leafminer, *Cameraria ohridella* Deschka & Dimic (Lep., Gracillariidae). *Journal of Applied Entomology* **123:**257-260.

Hawkins B.A. (1994). Host mortality and parasitoid impact. Pages 111-131 *In*: Pattern & Process in host-parasitoid interactions. Cambridge University Press.

Hering E.M. (1957). Bestimmungstabellen der blattminen von Europa. Junk, W, Netherlands

Holt R.D. (1977). Predation, apparent competition, and the structure of prey communities. *Theoretical Population Biology* **12:**197-229.

Kenis M., Auger-Rozenberg M.A., Roques A., Timms L., Péré C., Cock M.J.W., Settele J., Augustin S. & Lopez-Vaamonde C. (2009). Ecological effects of invasive alien insects. *Biological Invasions* **11:**21-45.

Levine J.M., Vilà M., D'Antonio C.M., Dukes J.S., Grigulis K. & Lavorel S. (2003). Mechanisms underlying the impacts of exotic plant invasions. *Proceeding of the Royal Society London B: Biological Sciences* **270:**775-781.

Memmott J., Godfray H.C.J. & Gauld I.D. (1994). The structure of a tropical host-parasitoid community. *Journal of Animal Ecology* **63:**521-540.

Morris R.J., Lewis O.T. & Godfray H.C.J. (2004). Experimental evidence for apparent competition in a tropical forest food web. *Nature* **428:**310-313.

Morris R.J., Lewis O.T. & Godfray H.C.J. (2005). Apparent competition and insect community structure: a spatial perspective. *Annales Zoologici Fennici* **42**: 449-462.

Morris R.J., Müller C.B. & Godfray H.C.J. (2001). Field experiments testing for apparent competition between primary parasitoids mediated by secondary parasitoids. *Journal of Animal Ecology* **70:**301-309.

Müller C.B. & Godfray H.C.J. (1997). Apparent competition between two aphid species. *Journal of Animal Ecology* **66:**57-64.

Noyes J.S. (2002). Interactive catalogue of world Chalcidoidea 2001. Compact disk. Taxapad, Vancouver, Canada.

Parker I.M., Simberloff D., Lonsdale W.M., Goodell K., Wonham M., Kareiva P.M., Williamson M.H., Von Holle B., Moyle P.B., Byers J.E. & Goldwasser L. (1999). Impact: toward a framework for understanding the ecological effects of invaders. *Biological Invasions* **1:**3-19.

Prenter J., MacNeil C., Dick J.T.A. & Dunn A.M. (2004). Roles of parasites in animal invasions. *Trends in Ecology and Evolution* **19:**385-390.

Quednau F.W. (1990). Introduction, permanent establishment, and dispersal in Eastern Canada of *Olesicampe geniculatae* Quednau and Lim (Hymenoptera: Ichneumonidae), an important biological control agent of the mountain ash sawfly, *Pristiphora geniculata* (Hartig) (Hymenopttera: Tenthredinidae). *Canadian Entomologist* **122:**921-934.

Ricketts T.H., Daily G.C., Ehrlich P.R. & Fay J.P. (2001). Countryside Biogeography of Moths in a Fragmented Landscape: Biodiversity in Native and Agricultural Habitats. *Conservation Biology* **15:**378-388.

Schönrogge K. & Crawley M.J. (2000). Quantitative webs as a means of assessing the impact of alien insects. *Journal of Animal Ecology* **69:**841-868.

Settle W.H. & Wilson T.L. (1990). Invasion by the variegated leafhopper and biotic interactions: parasitism, competition, and apparent competition. *Ecology* **71:**1461-1470.

Šefrova H. (2002). *Phyllonorycter issikii* (Kumata, 1963) - bionomics, ecological impact and spread in Europe (Lepidoptera, Gracillariidae). *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* **3:**99-104.

Tompkins D.M., Draycott R.A.H. & Hudson P.J. (2000). Field evidence for apparent competition mediated via the shared parasites of two gamebird species. *Ecology Letters* **3:**10-14.

Van Nouhuys S. & Hanski I. (2000). Apparent competition between parasitoids mediated by a shared hyperparasitoid. *Ecology Letters* **3:**82-84.

Van Veen F.J.F., Morris R.J. & Godfray H.C.J. (2006). Apparent competition, quantitative food webs, and the structure of phytophagous insect communities. *Annual Review of Entomology* **51:**187-208.

Whitebread S.E. (1989). *Phyllonorycter robiniella* (Clemens, 1859) in Europe (Lepidoptera, Gracillariidae). *Nota Lepidopterologica* **12:**344-353.

Yu D., Van Achterberg C. & Horstmann K. (2005). World Ichneumonoidea 2004. Taxonomy, Biology, Morphology and Distribution. CD/DVD, Taxapad, Vancouver, Canada.

Annex 1: List of the native leaf miner species found in north-west Switzerland on various shrub and tree species between 2005 and 2006.

Host-plants leaf miner species	Order	Family
***Acer* spp.**		
Caloptilia rufipennella (Hübner)	Lepidoptera	Gracillariidae
Heterarthrus aceris (Kaltenbach)	Hymenoptera	Tenthredinidae
Heterarthrus wuestneii (Konow) (syn. *H. healyi*)	Hymenoptera	Tenthredinidae
Hinatara recta (CG Thomson)	Hymenoptera	Tenthredinidae
Phyllonorycter acerifoliella (Zeller)	Lepidoptera	Gracillariidae
Phyllonorycter geniculellan (Ragonot)	Lepidoptera	Gracillariidae
Phyllonorycter platanoidella (De Joannis)	Lepidoptera	Gracillariidae
Stigmella aceris (Frey)	Lepidoptera	Nepticulidae
Stigmella speciosa (Frey)	Lepidoptera	Nepticulidae
***Alnus* spp.**		
Agromyza alnivora Spencer	Diptera	Agromyzidae
Coleophora sp.	Lepidoptera	Coleophoridae
Fenusa dohrnii (Tischbein)	Hymenoptera	Tenthredinidae
Heterarthrus vagans (Fallén)	Hymenoptera	Tenthredinidae
Orchestes testaceus (Müller)	Coleoptera	Curculionidae
Phyllonorycter sp.	Lepidoptera	Gracillariidae
Stigmella alnetella (Stainton)	Lepidoptera	Nepticulidae
***Carpinus betulus* L.**		
Coleophora sp.	Lepidoptera	Coleophoridae
Eriocrania sp.	Lepidoptera	Eriocraniidae
Incurvaria sp.	Lepidoptera	Incurvariidae
Parornix carpinella (Frey)	Lepidoptera	Gracillariidae
Phyllonorycter esperella (Goeze)	Lepidoptera	Gracillariidae
Phyllonorycter sp.	Lepidoptera	Gracillariidae
Phyllonorycter tenerella (de Joannis)	Lepidoptera	Gracillariidae
Stigmella microtheriella (Stainton)	Lepidoptera	Nepticulidae
Stigmella sp.	Lepidoptera	Nepticulidae
***Coronilla* spp.**		
Liriomyza congesta (Becker)	Diptera	Agromyzidae
***Cornus* spp.**		
Antispila metallella (Denis & Schiffermüller)	Lepidoptera	Heliozelidae
Antispila treitschkiella (Fischer von Rösslerstamm)	Lepidoptera	Heliozelidae
Coleophora sp.	Lepidoptera	Coleophoridae
Phytomyza agromyzina Meigen	Diptera	Agromyzidae
***Corylus avellana* L.**		
Coleophora sp.	Lepidoptera	Coleophoridae
Incurvaria sp.	Lepidoptera	Incurvariidae
Paracrania chrysolepidella (Zeller)	Lepidoptera	Eriocraniidae
Parornix devoniella (Stainton)	Lepidoptera	Gracillariidae
Phyllonorycter coryli (Nicelli)	Lepidoptera	Gracillariidae
Phyllonorycter nicellii (Stainton)	Lepidoptera	Gracillariidae
Stigmella floslactella (Haworth)	Lepidoptera	Nepticulidae
Stigmella microtheriella (Stainton)	Lepidoptera	Nepticulidae
Trachys minutus (Linnaeus)	Coleoptera	Buprestidae

***Crataegus* spp.**		
Coleophora sp.	Lepidoptera	Coleophoridae
Incurvaria masculella (Denis & Schiffermüller)	Lepidoptera	Incurvariidae
Lyonetia clerkella (Linnaeus)	Lepidoptera	Lyonetiidae
Parornix anglicella (Stainton)	Lepidoptera	Gracillariidae
Phyllonorycter corylifoliella (Hübner)	Lepidoptera	Gracillariidae
Phyllonorycter oxyacanthae (Frey)	Lepidoptera	Gracillariidae
Stigmella sp.	Lepidoptera	Nepticulidae
***Fagus sylvatica* L.**		
Incurvaria sp.	Lepidoptera	Incurvariidae
Orchestes fagi (Linnaeus)	Coleoptera	Curculionidae
Parornix fagivora (Frey)	Lepidoptera	Gracillariidae
Phyllonorycter maestingella (Müller)	Lepidoptera	Gracillariidae
Stigmella hemargyrella (Kollar)	Lepidoptera	Nepticulidae
Stigmella tityrella (Stainton)	Lepidoptera	Nepticulidae
***Fraxinus excelsior* L.**		
Gracillaria syringella (Fabricius)	Lepidoptera	Gracillariidae
***Ilex aquifolium* L.**		
Phytomyza ilicis Curtis	Diptera	Agromyzidae
***Laburnum* sp.**		
Leucoptera laburnella (Stainton)	Lepidoptera	Lyonetiidae
***Ligustrum vulgare* L.**		
Gracillaria syringella (Fabricius)	Lepidoptera	Gracillariidae
***Lonicera xylosteum* L.**		
Aulagromyza hendeliana (Hering)	Diptera	Agromyzidae
Chromatomyia periclymeni (Hendel)	Diptera	Agromyzidae
Phyllonorycter emberizaepennella (Bouché)	Lepidoptera	Gracillariidae
Stigmella lonicerarum (Frey)	Lepidoptera	Nepticulidae
***Lonicera periclymenum* L.**		
Chromatomyia aprilina (Goureau)	Diptera	Agromyzidae
***Malus* spp.**		
Coleophora sp.	Lepidoptera	Coleophoridae
Stigmella sp.	Lepidoptera	Nepticulidae
***Prunus avium* L.**		
Coleophora sp.	Lepidoptera	Coleophoridae
Lyonetia clerkella (Linnaeus)	Lepidoptera	Lyonetiidae
Parornix sp.	Lepidoptera	Gracillariidae
Phyllonorycter messaniella (Zeller)	Lepidoptera	Gracillariidae
***Prunus spinosa* L.**		
Phyllonorycter spinicolella (Zeller)	Lepidoptera	Gracillariidae
Stigmella plagicolella (Stainton)	Lepidoptera	Nepticulidae
***Pyrus communis* L.**		
Stigmella pyri (Glitz)	Lepidoptera	Nepticulidae
***Quercus robur* L.**		
Coleophora sp.	Lepidoptera	Coleophoridae
Dyseriocrania subpurpurella (Haworth)	Lepidoptera	Eriocraniidae
Orchestes pilosus (Fabricius)	Coleoptera	Curculionidae
Orchestes quercus (Linnaeus)	Coleoptera	Curculionidae
Phyllonorycter kuhlweiniella (Zeller)	Lepidoptera	Gracillariidae
Phyllonorycter sp. 1	Lepidoptera	Gracillariidae
Phyllonorycter sp. 2	Lepidoptera	Gracillariidae
Profenusa pygmaea (Klug)	Hymenoptera	Tenthredinidae
Stigmella ruficapitella (Haworth)	Lepidoptera	Nepticulidae
Stigmella samiatella (Zeller)	Lepidoptera	Nepticulidae
Stigmella sp.	Lepidoptera	Nepticulidae
Tischeria decidua (Wocke)	Lepidoptera	Tischeriidae
Tischeria ekebladella (Bjerkander)	Lepidoptera	Tischeriidae

***Robinia pseudoacacia* L.**		
Phyllonorycter robiniella (Clemens)	Lepidoptera	Gracillariidae
***Rosa* spp.**		
Agromyza idaeiana (Hardy)	Diptera	Agromyzidae
Coptotriche angusticollella (Duponchel)	Lepidoptera	Tischeriidae
Stigmella anomalella (Goeze)	Lepidoptera	Nepticulidae
Stigmella sp.	Lepidoptera	Nepticulidae
***Rubus* spp.**		
Agromyza sp.	Diptera	Agromyzidae
Coptotriche marginea (Haworth)	Lepidoptera	Tischeriidae
Metallus pumilus (Klug)	Hymenoptera	Tenthredinidae
Metallus sp.	Hymenoptera	Tenthredinidae
Stigmella aurella (Fabricius)	Lepidoptera	Nepticulidae
Stigmella sp.	Lepidoptera	Nepticulidae
Stigmella spendidissimella (Herrich-Schaeffer)	Lepidoptera	Nepticulidae
***Salix* spp.**		
Coleophora sp.	Lepidoptera	Coleophoridae
Isochnus sp.	Coleoptera	Curculionidae
Phyllonorycter sp.	Lepidoptera	Gracillariidae
Stigmella salicis (Stainton)	Lepidoptera	Nepticulidae
Trachys minutus (Linnaeus)	Coleoptera	Buprestidae
***Sambucus* spp.**		
Liriomyza amoena (Meigen)	Diptera	Agromyzidae
***Sorbus aria* (L.) Crantz.**		
Coleophora sp.	Lepidoptera	Coleophoridae
Incurvaria sp.	Lepidoptera	Incurvariidae
Phyllonorycter corylifoliella (Hübner)	Lepidoptera	Gracillariidae
Phyllonorycter sorbi (Frey)	Lepidoptera	Gracillariidae
Stigmella sp.	Lepidoptera	Nepticulidae
***Syringa vulgaris* L.**		
Gracillaria syringella (Fabricius)	Lepidoptera	Gracillariidae
***Tilia platyphyllos* Scop.**		
Bucculatrix thoracella (Thunberg)	Lepidoptera	Bucculatricidae
Parna sp.	Hymenoptera	Tenthredinidae
Stigmella tiliae (Frey)	Lepidoptera	Nepticulidae
***Ulmus campestris* auct. non L.**		
Coleophora limosipennella (Duponchel)	Lepidoptera	Coleophoridae
***Ulmus glabra* Hudson**		
Coleophora sp.	Lepidoptera	Coleophoridae
Fenusa ulmi Sundevall	Hymenoptera	Tenthredinidae
Orchestes sp.	Coleoptera	Curculionidae
Phyllonorycer sp.	Lepidoptera	Gracillariidae
Phyllonorycter schreberella (Fabricius)	Lepidoptera	Gracillariidae
Phyllonorycter tristrigella (Haworth)	Lepidoptera	Gracillariidae
Stigmella lemniscella (Zeller)	Lepidoptera	Nepticulidae
Stigmella sp.	Lepidoptera	Nepticulidae
Stigmella ulmivora (Fologne)	Lepidoptera	Nepticulidae
***Viburnum lantana* L.**		
Phyllonorycter lantanella (Schrank)	Lepidoptera	Gracillariidae

Chapter 3

Does the presence of the invasive horse-chestnut leaf miner, *Cameraria ohridella*, affect the native beech leaf mining weevil, *Orchestes fagi*, through apparent competition?

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In preparation

Abstract

A previous study had shown that the beech leaf mining weevil, *Orchestes fagi*, was significantly less abundant in the surroundings of horse-chestnut trees infested by the invasive horse-chestnut leaf miner, *C. ohridella*, compared to control sites. Apparent competition through the sharing of natural enemies was proposed as a potential mechanism underlying this effect. Several observational studies and experimental manipulations in Switzerland were used to test the occurrence of apparent competition between the two leaf miners. Total mortality, parasitism, predation and parasitoid species richness were compared between sites with and without horse-chestnut trees attacked by *C. ohridella*. No evidence for apparent competition was found between *C. ohridella* and the native beech leaf mining weevil, *Orchestes fagi*, as total mortality, parasitism and predation were not significantly different. Several parasitoid species commonly attacking *C. ohridella* were reared from *O. fagi* but, in general, their density was not higher in the vicinity of infested horse-chestnut trees. Possible explanations for these unexpected results are provided.

Keywords: invasive alien insect, leaf miner, *Cameraria ohridella*, *Orchestes fagi*, parasitoid complex, parasitism, apparent competition

Introduction

Apparent competition is a mechanism through which a species negatively affects another species, which may or may not share resources, at the same trophic level, mediated through the action of shared natural enemies (Holt 1987; Morris et al. 2004). Apparent competition may be short-term, observed over a single generation or long-term, sustained over several generations (Holt & Kotler 1987). It may occur in different taxonomic groups and at different trophic levels (Tompkins et al. 2000; Prenter et al. 2004). In particular, it has been observed in various insect communities (e.g. Müller & Godfray 1997; Morris et al. 2001, 2004), including through field population manipulations (Van Nouhuys & Hanski 2000; Morris et al. 2004). Apparent competition is also a mechanism by which an alien insect may affect populations and communities of native insects (Kenis et al. 2009). However examples of invasive insects affecting native species via the sharing of natural enemies are rare. The first reported case was that of the variegated leafhopper, *Erythroneura variabilis*, an invasive species in California, and its native congener *E. elegantula*, whose decline was correlated with increased levels of a shared egg parasitoid in the presence of *A. variabilis* (Settle & Wilson 1990). Recently, a seed feeder introduced in Australia as a weed biological control agent was found affecting populations of native seed herbivores, probably through apparent competition (Carvalheiro et al. 2008).

The invasion of the horse-chestnut leaf miner, *Cameraria ohridella* Deschka & Dimić (Lepidoptera: Gracillariidae), in Europe provides an excellent opportunity to investigate apparent competition between an invasive insect and native insects that do not share resources. *Cameraria ohridella*, which probably originates from the Balkans, is the first leaf miner in Europe known to attack horse-chestnut, *Aesculus hippocastanum* L., another Balkan species planted throughout the continent as an amenity tree. *Cameraria ohridella* has two to four generations a year and overwinters as a pupa in dead horse-chestnut leaves. Its high fecundity, multivoltinism and low parasitism rates compared to other leaf mining species allows it to develop permanent outbreaks (Girardoz et al. 2006; Girardoz et al. 2007a; Kenis et al. 2005; Tilbury & Evan 2003). In Europe, about 30 species of parasitoids have been recorded from *C. ohridella*, mostly Chalcidoidea with some Ichneumonoidea. All parasitoids are idiobiont and generalist and, thus, are shared with many other leaf miners of various families. In Central Europe, the main parasitoid species are the eulophids *Minotetrastichus frontalis* (Nees), *Pnigalio agraules* (Walker), *Chrysocharis nephereus* (Walker) and *Closterocerus trifasciatus* (Westwood) and the braconid *Colastes braconius* (Haliday) (Hellrigl 2001; Freise et al 2002; Grabenweger 2003; Girardoz et al. 2006). These are all very common European species that can be found on other leaf miners of different families (Askew & Shaw 1979; Noyes 2002; Yu et al. 2005). Despite low parasitism rates in Central Europe (Girardoz et al. 2007a, b), populations of *C. ohridella* are so high compared to native leaf miner species that an unusual number of polyphagous parasitoids is produced in the surroundings of infested horse-chestnut trees. The impact may be particularly high on early-occurring species because, in spring, most parasitoids of *C. ohridella* emerge at least five weeks before the first suitable *C. ohridella* larvae or pupae are available (Grabenweger 2004; Girardoz 2006). *Cameraria ohridella* is also attacked by generalist predators such as birds, bush-crickets, lacewings or ants (Grabenweger et al. 2005; Girardoz et al. 2007b). These could also be involved in apparent competition although, in leaf miners, predation has been much less investigated than parasitism. Mortality due to pathogens is rare in leaf miners

(Hespenheide 1991) and, thus, pathogens are unlikely to be the cause of apparent competition in this system.

A previous study (Chapter 2) had shown that species richness and abundance of native leaf miners were lower in the vicinity of horse-chestnut trees infested by *C. ohridella* compared to control sites. In particular, a dramatic variation in abundance was observed in the beech leaf mining weevil, *Orchestes fagi* L. (syn. *Rhynchaenus fagi* (L.)) (Coleoptera: Curculionidae), in Switzerland. Natural enemies are likely to be the cause of changes in the population level of *O. fagi* because they are the only link between *C. ohridella* and the native leaf miner, which attacks exclusively the European beech, *Fagus sylvatica* L. (Bale 1984). *Orchestes fagi* is a univoltine species. Females oviposit in young beech leaves very soon after bud burst in late April, with the new generation adults emerging 30 to 43 days later in mid June (Bale 1981; Bale 1984; Bale & Luff 1978; Pullin 1985). The larval and pupal stages are thus perfectly synchronized with the flight period of the parasitoids of *C. ohridella*. *Orchestes fagi* is known to be attacked by the main parasitoids of *C. ohridella* (Table 1). In particular, *C. braconius* and *C. nephereus* are two important parasitoids of *O. fagi* in Great Britain (Woodcock and Vanbergen 2008). Predation has been less studied (Nielsen 1968; Pullin 1985) but it is likely that both leaf miners share similar polyphagous predators when occurring at the same site.

Table 1: Lists of parasitoid species shared by *C. ohridella* and *O. fagi* based on a literature review. In bold are the five parasitoid species commonly found on *C. ohridella* in Switzerland.

SUPERFAMILY Family: Subfamily	Parasitoids species
CHALCIDOIDEA	
Eulophidae: Entedoninae	***Chrysocharis nephereus* (Walker)**
	Chrysocharis nitetis (Wlk.)
	***Closterocerus trifasciatus* (Westwood)**
	Pediobius saulius (Wlk.)
Eulophidae: Eulophinae	***Pnigalio agraules* (Wlk.)**
	Pnigalio longulus (Zetterstedt)
	Pnigalio pectinicornis (L.)
	Pnigalio soemius (Wlk.)
	Cirrospilus diallus (Wlk.)
	Cirrospilus vittatus (Wlk.)
Eulophidae: Tetrastichinae	***Minotetrastichus frontalis* (Nees)**
ICHNEUMONOIDEA	
Braconidae: Exothecinae	***Colastes braconius* (Haliday)**
Ichneumonidae: Pimplinae	*Itoplectis alternans* (Gravenhorst)

Table combined using the following references: Askew & Shaw 1974; Girardoz et al. 2006; Grabenweger et al. 2003; Noyes 2002; Schindler 1966; Tóth & Lukáš 2005; Tsankov & Stalev 1992; Yu et al. 2005

The preliminary study on population changes in *O. fagi* in the presence and absence of *C. ohridella* was purely observational and did not particularly focus on natural enemies (Chapter 2). Thus, the role of natural enemies in the decrease of *O. fagi* abundance in the vicinity of horse-chestnut trees needs confirmation. In this paper, we report on the comparison of total mortality, parasitism and predation in *O. fagi*, in the presence and absence of *C. ohridella*. We tested the hypothesis that total mortality, parasitism and predation in *O. fagi* are higher in the vicinity of horse-chestnut trees infested by *C. ohridella* than at sites away from horse-chestnut trees. Both observational studies and experimental manipulations were used.

Material and methods

Field sites

All experiments were carried out in Switzerland, at sites previously described in Chapter 2. The sites were selected in the north-west of the country (Cantons Jura, Basel-Landschaft, Solothurn and Aargau), within a radius of 50 km of the city of Delémont (47°22'N – 7°21'E), at altitudes between 300 and 700 m, in or at the edge of a broad-leaved forest. The sites consisted of beech trees less than 50 m from one or more horse-chestnut trees infested by *C. ohridella*. For each of these sites, a twin site with similar characteristics (beech trees, altitude, soil type, vegetation cover, absence/presence of a stream, etc.) was selected at 2-5 km distance from the paired site and at a minimum of 2 km from any horse-chestnut trees. All horse-chestnut trees occurring at the sites had been heavily and continuously infested by *C. ohridella* (i.e. a minimum of 20 mines per leaf) for at least 4 to 7 years.

Comparison of mortality factors in the presence and absence of *C. ohridella*

Four different experiments were carried out to measure and compare total mortality, parasitism, predation and parasitoid species richness between sites with and without *C. ohridella*.

Experiment 1: Exposure of <u>Fagus sylvatica</u> saplings

In mid-late April 2006 and 2008, as soon as beech bud burst began, about one hundred potted *F. sylvatica* saplings (ca. 70 ± 10cm high) were placed in gauze cages (100 cm x 50 cm x 50 cm) and kept outdoors under natural conditions but protected from direct rain and sun. Adults of *O. fagi* were collected in the forest by beating beech branches and then put in the cages in groups of 15-20 to oviposit on the saplings, without being sexed. In late-April, soon after egg laying, the *F. sylvatica* saplings infested by *O. fagi* were transferred to five (2006) and six (2008) pairs of sites. At sites with *C. ohridella*, the saplings were placed within 3 meters of an infested horse-chestnut. The saplings were regularly checked for weevil development and returned to the laboratory when the majority of the weevils were in the pupal stage, i.e. after about 20 days in 2006 and 15 days in 2008. Mines were dissected in the laboratory, the development stage was recorded and mortality attributed to one of these three categories: parasitism, predation or unknown cause. While parasitism was easily noticed, predation was more difficult to estimate. We used the criteria of Pullin (1985) but may have overestimated predation because all mines opened before maturity, with or without larval remains inside, were considered as preyed. In addition, suspected cases of host feeding by parasitoids were also classified as predation. Predation was assessed in 2008 only.

In 2006, the ten sites were chosen at random among our 33 pairs of sites (Chapter 2). All sites had beech in the vicinity. In contrast, in 2008, we selected 12 sites with no beech within a radius of 300 m, to assess parasitism and predation on locally low populations of *O. fagi*.

Experiment 2: Tagged cohort samples

In 2007, parasitism, predation and total mortality of natural populations of *O. fagi* were assessed in the presence and absence of *C. ohridella* at a selected number of sites. A previous study (Chapter 2) had shown all of these selected sites contained abundant populations of *O. fagi*. Five *O. fagi* populations existing sympatrically with *C. ohridella* populations (separation of less than 50 metres from an infested horse-chestnut) were compared with five *O. fagi* populations inhabiting areas where *C. ohridella* was not present (separation greater than two kilometres). At each site, one hundred *O. fagi* leaf mines were randomly selected. *Orchestes fagi* began oviposition in late-April and leaf tagging commenced soon after. The mines of *O. fagi* were tagged and numbered using red tape attached approximately 10 centimetres away from the mine. Where possible a maximum of 30 mines per tree was used in order to reduce the importance of individual trees within a site. Once per week, leaf mine size and condition was recorded. Mines were revisited from the time at which they were located up until the *O. fagi* beetle emerged from the mine. If larval development stopped or the mine was successfully attacked, the leaf was removed and returned to the laboratory, where it was dissected under a stereomicroscope. Egg mortality was not recorded by this study, since *C. ohridella* is subject to little egg predation (Girardoz et al. 2007a) and has no recorded egg parasitoids (Grabenweger et al. 2007).

The larval development stage was recorded and mortality attributed to one of three categories: parasitism, predation or an unknown cause.

Experiment 3: Grab samples

In 2007, the grab sample approach consisted of the random collection of an extra 100 leaf mines from all sites of experiment 2, at the end of the development period. Samples were also taken from an additional two pairs of sites. Unless stated otherwise, the same protocol developed for experiment 2 was followed.

Experiment 4: Mass sampling

In late May 2006 and 2008, at 30 pairs of sites, one thousand leaves of *F. sylvatica* per site were randomly selected within 50 m from the central point of the site, preferably on at least five shrubs or major tree branches (see experiment "Abundance" in Chapter 2). All leaves mined by *O. fagi* were collected and inspected in the laboratory. Parasitism and total mortality of *O. fagi* were measured. This sampling method surely underestimated parasitism because, in contrast to the other three experiments, collections were not necessarily made at the end of the development of *O. fagi*, i.e. when parasitism is likely to be the highest. Instead, in these mines, all developmental stages were found, from young instars (i.e. before being parasitized) to emergence holes of hosts and parasitoids. Nevertheless, the parasitism rates and total mortality rates collected in this experiment were suitable for comparisons between sites with and without horse-chestnut trees.

Parasitoid identification

For each experiment, all parasitoid larvae, pupae and cocoons found in the mines of *O. fagi* were placed singly in a Petri dish stored in a humid chamber at room temperature. Parasitoids were reared through to the adult stage for their identification. Parasitoids were preliminarily classified into morphospecies, compared to parasitoids of *C. ohridella* maintained in the collection of the senior author and, when possible, identified using various identification keys. Specimens are presently being examined by taxonomists for confirmation.

Statistical analysis

To compare the total mortality, parasitism and predation of *O. fagi* in the presence and absence of *C. ohridella*, a Mann-Whitney U-test (two-tailed) was used for experiments 1, 2 and 3 and, a Wilcoxon test for paired samples was used for experiment 4. The species richness of the parasitoid community of *O. fagi* was compared between sites with and without *C. ohridella*, using Mann-Whitney U-test on Shannon-Wiener diversity indices (Mouillot & Lepretre 1999) calculated for the parasitoid communities at each site. Within sites, each parasitoid species was represented as a proportion of the total number of parasitoids emerged at that site. Broods of gregarious parasitoids such as *M. frontalis* were counted as one parasitoid. The calculation of the Shannon-Wiener diversity index was made using data from experiments 2, 3 and 4 (data from 2008 only), which had provided the highest number of parasitoid species. Data from experiments 2 and 3, collected at the same sites in 2007, were combined. For the calculation of the index on data of experiment 4, only sites providing parasitoids were included in the analysis i.e. 15 sites with *C. ohridella* and 22 sites without *C. ohridella*.

Detailed testing of hypotheses was carried out using binary logistic regression with the data of experiment 4 performed in 2008, which included the highest number of sites and individuals of *O. fagi*. The dependant variable examined was parasitism and the predictors were the presence/absence of *C. ohridella* and the host density (number of mines per 1000 leaves) of *O. fagi* at each site.

Results

Total mortality, parasitism and predation

Total mortality, parasitism and predation of *O. fagi* are presented for all four experiments in Table 2. Parasitism was above 30% for experiments 2 and 3, but lower than 20% in the two others. Predation varied between 14 and 33% and total mortality between 46 and 91%. The highest total mortality was observed in experiment 2. Mortality, parasitism and predation were not significantly different in the presence and in the absence of *C. ohridella*, for any of the experiments (Table 2). Table 3 shows the parameter estimates for the logistic regression model predicting the parasitism rates of *O. fagi* from the two factors investigated, using data from the mass sampling in 2008 (experiment 4). A significant positive association between parasitism rate and host density was found, but not between parasitism and the presence of *C. ohridella*.

Table 2: Comparison of the proportion of total mortality, parasitism and predation of *Orchestes fagi*, in presence (Pres.) and absence (Abs.) of *Cameraria ohridella*. Mann-Whitney test for experiments 1, 2 and 3 and Wilcoxon test for paired samples for experiment 4. No significant difference was observed at P <0.05 for any of the comparison.

Experiment	Year	Number of sites		Mortality (%)		Parasitism (%)		Predation (%)	
		Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.
1	2006	5	5	58.49	51.23	11.79	9.02	-	-
1	2008	6	6	61.44	58.61	14.09	17.22	16.22	14.17
2	2007	5	5	85.53	91.42	30.78	33.40	21.13	25.19
3	2007	7	7	73.01	81.64	32.53	30.56	26.84	32.99
4	2006	18	26	52.47	45.99	8.02	6.17	-	-
4	2008	25	28	83.78	84.64	13.44	6.92	-	-

Table 3: Parameter estimates for the logistic regression model predicting parasitism of *Orchestes fagi*, in leaf mines sampled from the factors presence of *Cameraria ohridella* and host density of *O. fagi*, using data from experiment 4 in 2008.

Parameter	B	S.E.	Wald	*P*
Presence-Absence of *C. ohridella*	-0.085	1.036	0.007	0.935
Host density of *O. fagi*	0.071	0.028	6.420	0.011
Presence-Absence of *C. ohridella* x Host density of *O. fagi*	0.009	0.036	0.058	0.810
Constant	-0.650	0.843	0.595	0.441

Table 4: Percentage contribution of each parasitoid species of *Orchestes fagi* towards the parasitoid community recorded in the four experiments, in presence (Pres.) and absence (Abs.) of *Cameraria ohridella*. In bold are the five main parasitoids of *C. ohridella* in Switzerland. Significant differences in the proportion in presence and absence of *C. ohridella* are indicated by an asterisk (Chi-square test, $P < 0.05$).

		Exp. 1				Exp. 2		Exp. 3		Exp. 4				Total	
SUPERFAMILY		2006		2008		2007		2007		2006		2008			
Family: Subfamily	Parasitoids species	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.
CHALCIDOIDEA															
Eulophidae: Entedoninae	***Chrysocharis nephereus* (Walker)**			2.25		15.69	18.97	24.26	28.50		3.23	21.60	23.90	15.80	17.99
	***Closterocerus trifasciatus* (Westwood)**						1.72							0.00	0.39
	Pediobius saulius (Wlk.)						0.86						0.53	0.00	0.39
Eulophidae: Eulophinae	***Pnigalio agraules* (Wlk.)**			7.87		0.98	1.72	1.48	1.14			6.82	0.53*	3.61	0.77
	Pnigalio soemius (Wlk.)	85.71	44.44*	55.56	22.67*	1.97	15.52*	2.94	3.50	28.57	38.80	4.55	6.35	17.83	13.54
	Pnigalio pectinicornis L.							0.74						0.23	0.00
	Cirrospilus diallus (Nees)											2.27	2.65	0.45	0.97
	Cirrospilus pictus (Nees)												0.53	0.00	0.19
Eulophidae: Tetrastichinae	***Minotetrastichus frontalis* (Nees)**					14.76	13.79	2.26	23.86*			2.27	11.64*	4.51	11.41
	Baryscapus sp.	4.76				11.76		8.88	1.14*				1.59	5.42	0.77
Pteromalidae: Pteromalinae	*Trichomalus inscitus* (Wlk.)					0.98		0.74				16.00	14.81	3.61	5.42
ICHNEUMONOIDAE															
Braconidae: Brachistinae	*Eubazus minutus* L.					5.88	2.59	3.68	3.50	14.29	6.45*	14.77	9.52	5.64	5.03
	Triaspis sp.						0.86	2.26	6.82			3.50	14.29*	1.35	6.58
Braconidae: Exothecinae	***Colastes braconius* (Haliday)**	9.52	55.56*	34.83	77.33*	48.39	43.97	53.68	31.82*	57.14	51.61	28.50	13.76*	41.53	36.56
	TOTAL parasitoid species	3	2	4	2	8	9	10	8	3	4	9	12	11	13

Parasitoid richness

Overall, 13 parasitoid species belonging to three taxonomic families were reared from *O. fagi* (Table 4). More parasitoids were obtained from samples collected from natural populations (experiments 2, 3 and 4 providing 11, 10 and 12 parasitoid species, respectively) than from mines exposed on saplings in experiment 1 (five species). The five parasitoid species most commonly collected from *C. ohridella* in Switzerland, *C. nephereus*, *C. trifasciatus*, *P. agraules*, *M. frontalis* and *C. braconius* were all reared from *O. fagi*, the latter being the most abundant parasitoid of *O. fagi* in this study (Table 4).

The Shannon-Wiener diversity index of parasitoid communities at sites where *C. ohridella* was present did not differ from sites where *C. ohridella* was absent ($U_{7,7} = 20$, $P = 0.565$ and $U_{15,22}=148.50$, $P = 0.606$ in 2007 and 2008 respectively).

Discussion

Parasitoid complex and mortality of *Orchestes fagi*

A total of 13 parasitoid species was collected from *O. fagi* in the four experiments. More parasitoid species were collected from natural populations of *O. fagi* than when mines were exposed on *F. sylvativa* saplings in the field. The absence of *Eubazus minutus* and *Triaspis* sp. in the mines on saplings can be explained by the fact that these two braconids are egg-larval parasitoids of beetles (Shaw & Huddleston 1991) and, although eggs were still present on saplings the day of exposure, they may have been too old to attract parasitoid females. The total absence of *M. frontalis* in mines on saplings is less understandable, particularly since it was frequently found in the three other experiments and it is the most abundant parasitoid of *C. ohridella* in Switzerland. Saplings were small and it is possible that *M. frontralis*, being a parasitoid of arboreal leaf miners (Askew & Shaw 1974) does not forage for mines situated close to the ground.

For practical reasons, the four experiments could not be carried out in the same year, which prevents a thorough comparison of the efficiency of the methods in assessing mortality factors. However, the tagged cohort samples and the grab samples (experiments 2 and 3) provided the highest rates of parasitism, predation and mortality and are probably the most reliable methods to assess mortality factors in natural populations. The same methods have recently been used to assess mortality factors of *C. ohridella*, and their respective advantages and disadvantages have been discussed in Girardoz et al. (2007b). The mass sampling at many sites at once (experiment 4) did not allow the collection of all mines precisely at the end of their development, which led to an underestimation of mortality rates, in particular parasitism. The exposure of saplings (experiment 1) also provided lower mortality rates, most probably because parasitoids and, perhaps predators, of arboreal leaf miners are less used to foraging in the herbaceous layer.

Apparent competition between *Cameraria ohridella* and *Orchestes fagi*

In a previous study, Péré et al. (Chapter 2) had observed that *O. fagi* was far less abundant in the surroundings of horse-chestnut infested by *C. ohridella* than at a minimum of 2 km from a horse-chestnut. They attributed that to apparent competition through shared natural enemies, most likely parasitoids but perhaps also predators, whose populations can be enhanced by the presence of *C. ohridella*. In this study, however, we did not find any evidence for apparent competition in any of our four observational and experimental approaches used to measure variations in parasitism and predation. Total mortality, parasitism and predation were not significantly different between sites with *C. ohridella* and sites without *C. ohridella*, despite the fact that the same sites were used as in the previously cited study. Many parasitoid species of *C. ohridella* were reared from *O. fagi* but, in general, their density was not higher in the vicinity of infested horse-chestnut trees. Three explanations for these unexpected results may be envisaged: (1) natural enemies of *C. ohridella* play a role in the observed difference in population densities of *O. fagi*, but we were not able to measure them with our experimental designs; (2) horse-chestnut trees and *C. ohridella* affect populations of *O. fagi* through another mechanism; (3) variations are due to other confounding factors that were not considered in Péré et al. (Chapter 2).

It is possible that our observations and experiments failed to detect a true effect of natural enemies. Woodcock & Vanbergen (2008) showed that some parasitoids of *O. fagi*, in particular the most abundant parasitoid in Switzerland, *C. braconius*, are density-dependent. The logistic regression analysis on the data collected during mass sampling in 2008 also showed a positive relationship between total parasitism rates and host populations. Since populations of *O. fagi* were lower at sites with *C. ohridella* compared to sites without *C. ohridella*, a difference in parasitism may have been masked by the density-dependent relationship. However, had this been the case, it would have appeared in the logistic regression, and, possibly, in experiment 1 (2008), in which all saplings were exposed at a 300m distance from the first beech tree. A more likely explanation is the unfortunate timing of the experiments. These were all made between 2006 and 2008, a period during which populations of *O. fagi* were very high throughout the investigated region. Populations of *O. fagi* are known to fluctuate dramatically from very low densities to outbreak situations and the population dynamics of the pest are regulated by its synchronisation with host plant phenology (Bale 1984; Day & Watt 1989). It cannot be ruled out that apparent competition with *C. ohridella* occurs only at very low *O. fagi* densities. The presence of parasitoids of the invasive moth may locally prevent the increase of *O. fagi* populations when these are very low, and local variations in leaf miner densities may remain for some years. During outbreak years, since beech is far more abundant than horse-chestnut at a regional level, a local production of high numbers of *C. ohridella* parasitoids probably has very little influence on the large number of *O. fagi* in the region. To verify this hypothesis, saplings artificially infested with *O. fagi* should be exposed when natural populations are in the latency phase.

Parasitoids of *C. ohridella* may not have any influence on neighbouring *O. fagi*, either because their local dispersal behaviour has been underestimated, a common feature in spatial parasitoid ecology (Antolin & Strong 1987), or because of the occurrence of sibling species in the supposedly shared parasitoids. It has long been thought that most parasitoids of leaf miners are polyphagous species attacking a wide taxonomic and ecological range of species, but recent molecular studies suggest that sibling species with restricted host ranges do occur in leaf miner parasitoids (Bernardo et al. 2008). Nevertheless, the presence of *C. ohridella* and horse-chestnut may influence *O. fagi* through other mechanisms. For example, horse-chestnut is attacked by an unusually low number of herbivores and screening tests to assess the food range of *O. fagi* adults showed that horse-chestnut was one of the only two host trees rejected among the 20 offered (Bale & Luff 1978). Thus, it is possible that horse-chestnut also acts as a repellent against *O. fagi* adults. Dead leaves of horse-chestnut may also repel *O. fagi* adults that overwinter in the litter. The pheromone released by the high number of *C. ohridella* adults flying around horse-chestnut trees in spring may confuse other leaf miners, particularly gracillariids, but it should not affect *O. fagi*, which mates and oviposits at least two or three weeks earlier than *C. ohridella.* The impact of volatiles and pheromones from horse-chestnut and *C. ohridella* on populations of *O. fagi* is not easy to test because, in natural or semi-natural environments, i.e. where both leaf miners may co-occur, all horse-chestnut trees are permanently infested by the invasive moth.

In their comparison of population densities of *O. fagi* at sites with and without *C. ohridella*, Péré et al. (Chapter 2) considered several confounding factors, such as plant diversity, percentage of forest and constructed habitat at various distances. Also, the use of paired sites in the analysis was meant to reduce the influence of abiotic factors such as climate, soil, exposition, etc. However, it cannot be ruled out that other confounding factors may have caused these variations. One potential confounding factor that remains to be verified is the abundance of beech trees that was variable between sites and could have possibly been different between sites with and without *C. ohridella*.

Despite these negative results, we believe that *C. ohridella* and invasive leaf miners in general provide good models for testing apparent competition between invasive and indigenous species. Perhaps an invasive leaf miner feeding on a more widely distributed host, such as the black locust leaf miners *Phyllonorycter robiniella* and *Parectopa robiniella* (Whitebread 1989) or the lime leaf miner *Phyllonorycter issikii* (Šefrova 2002) would represent more suitable models in Europe.

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References

Antolin M.F. & Strong D.R. (1987). Long distance dispersal by a parasitoid (*Anagrus delicatus*, Mymaridae) and its host. *Oecologia* **73:** 288-292.

Askew R.R. & Shaw M.R. (1974). "An account of the Chalcidoidea (Hymenoptera) parasitising leaf-mining insects of deciduous trees in Britain." *Biological journal of the Linnean society* **6**(4): 289-335.

Askew R.R. & Shaw M.R. (1979). Mortality factors affecting the leaf-mining stages of *Phyllonorycter* (Lepidoptera: Gracillariidae) on oak and birch. 1. Analysis of the mortality factors. *Zoological Journal of the Linnean Society* **67**:31-49.

Bale J.S. (1981). Seasonal distribution and migratory behaviour of beech leaf mining weevil, *Rhynchaenus fagi* L. *Ecological Entomology* **6**:109-118.

Bale J.S. (1984). Bud burst and success of the beech weevil, *Rhynchaenus fagi*: feeding and oviposition. *Ecological Entomology* **9**:139-148.

Bale J.S. & Luff M.L. (1978). The food plants and feeding preferences of the beech leaf mining weevil, *Rhynchaenus fagi* L. *Ecological Entomology* **3**:245-249.

Bernardo U., Monti M.M., Nappo A.G., Gebiola M., Russo A., Pedata P.A. & Viggiani, G. (2008). Species status of two populations of *Pnigalio soemius* (Hymenoptera: Eulophidae) reared from two different hosts: an integrative approach. *Biological Control* **46:**293-303.

Boucek Z. & Askew R.R. (1968). Paleoarctic Eulophidae (Excl. Tetrastichinae). (Hym. Chalcidoidea). Index of entomophagous insects. La Francois, Paris.

Carvalheiro L.G., Buckley Y.M., Ventim R., Fowler S.V. & Memmott J. (2008). Apparent competition can compromise the safety of highly specific biocontrol agents. *Ecology Letters* **11:**690-700.

Day K.R. & Watt A.D. (1989). Population studies of the beech leaf mining weevil (*Rhynchaenus fagi*) in Ireland and Scotland. *Ecological Entomology* **14**:23-30.

Freise J., Heitland W. & Tosevski I. (2002). Parasitism of the horse chestnut leaf miner, *Cameraria ohridella* Deschka and Dimic (Lep., Gracillariidae), in Serbia and Macedonia. *Journal of Pest Science* **75**:152-157.

Girardoz S., Kenis M. & Quicke D.L.J. (2006). Recruitment of native parasitoids by an exotic leaf miner, *Cameraria ohridella*: host-parasitoid synchronisation and influence of the environment. *Agricultural and Forest Entomology* **8:**49-56.

Girardoz S., Quicke D.L.J. & Kenis M. (2007a). Factors favouring the development and maintenance of outbreaks in an invasive leaf miner *Cameraria ohridella* (Lepidoptera: Gracillariidae): a life table study. *Agricultural and Forest Entomology* **9:**141-158.

Girardoz S., Tomov R., Eschen R., Quicke D.L.J. & Kenis M. (2007b). Two methods assessing the mortality factors affecting the larvae and pupae of *Cameraria ohridella* in the leaves of *Aesculus hippocastanum* in Switzerland and Bulgaria. *Bulletin of Entomological Research* **97:**445-453.

Girardoz S., Volter L., Tomov R., Quicke D.L.J. & Kenis M. (2007c). Variations in parasitism in sympatric populations of three invasive leaf miners. *Journal of Applied Entomology* **131:**603-612.

Grabenweger G., Stolz M. & Jeziorny K. (2003). A key to the parasitoids of *Cameraria ohridella* (Leipdoptera, Gracillariidae). CD-Rom version 1.0. University of Natural Resources and Applied Life Science, Vienna.

Grabenweger G. (2004). Poor control of the horse chestnut leafminer, *Cameraria ohridella* (Lepidoptera: Gracillariidae), by native European parasitoids: a synchronisation problem. *European Journal of Entomology* **101**(1): 189-192.

Grabenweger G., Avtzis N., Girardoz S., Hrasovec B., Tomov R. & Kenis M (2005a). Parasitism of *Cameraria ohridella* (Lepidoptera, Gracillariidae) in natural and artificial horse-chestnut stands in the Balkans. *Agricultural and Forest Entomology* **7:**291-296.

Grabenweger G., Hopp H., Jackel B., Balder H., Koch T. & Schmolling S. (2007). Impact of poor host-parasitoid synchronisation on the parasitism of *Cameraria ohridella* (Lepidoptera: Gracillariidae). *European Journal of Entomology* **104**:153-158.

Hellrigl K. (2001). Neue Erkenntnisse und Untersuchungen über die Roßkastanien-Miniermotte. *Gredleriana* **1**: 9-81.

Hespenheide H.A. (1991). Bionomics of leaf mining insects. *Annual Review of Entomology* **36**: 535-560.

Holt R.D. (1977). Predation, apparent competition, and the structure of prey communities. *Theoretical Population Biology* **12**(2): 197-229.

Holt R.D. & Kotler B.P. (1987). Short-term apparent competition. *The American Naturalist* **130**(3):412-430.

Kenis M., Auger-Rozenberg M.A., Roques A., Timms L., Péré C., Cock M.J.W., Settele J., Augustin S. & Lopez-Vaamonde C. (2009). Ecological effects of invasive alien insects. *Biological Invasions* **11:**21-45.

Kenis M., Tomov R., Svatos A., Schlienslog P., Lopez Vaamonde C., Heitland W., Grabenweger G., Girardoz S., Freise J. & Avtzis N. (2005). The horse-chestnut leaf miner in Europe - Prospects and contraints for biological control. Pages 77-90 *In:* Proceedings of the Second International Symposium on Biological Control of Arthropods, 12-16 September 2005, Davos, Switzerland, Forest Health Technology Enterprise Team-Morgantown, WV.

Morris R.J., Lewis O.T. & Godfray H.C.J. (2004). Experimental evidence for apparent competition in a tropical forest food web. *Nature* **428:**310-313.

Morris R.J., Müller C.B. & Godfray H.C.J. (2001). Field experiments testing for apparent competition between primary parasitoids mediated by secondary parasitoids. *Journal of Animal Ecology* **70:**301-309.

Mouillot D. & Lepretre A. (1999). A comparison of species diversity estimators. *Researches on Population Ecology* **41**:203-215.

Müller C.B. & Godfray H.C.J. (1997). Apparent competition between two aphid species. *Journal of Animal Ecology* **66:**57-64.

Nielsen B.O. (1968). Studies on the fauna of beech foliage 2. Observations on the mortality and mortality factors of the beech weevil [*Rhynchaenus* (Orchestes) *fagi* L.] (*Coleoptera, Curculionidae*). *Natura Jutlandica* **14**: 99-125.

Noyes J.S. (2002). Interactive catalogue of world Chalcidoidea 2001. *In:* Compact disk, Taxapad, Vancouver, Canada.

Prenter J., MacNeil C., Dick J.T.A. & Dunn A.M. (2004). Roles of parasites in animal invasions. *Trends in Ecology and Evolution* **19:**385-390.

Pullin A.S. (1985). A simple life table study based on development and mortality in the beech weevil, *Rynchaenus fagi* L. *Journal of Biological Education* **19**:152-156.

Settle W.H. & Wilson T.L. (1990). Invasion by the variegated leafhopper and biotic interactions: parasitism, competition, and apparent competition. *Ecology* **71:**1461-1470.

Šefrova H. (2002). *Phyllonorycter issikii* (Kumata, 1963) - bionomics, ecological impact and spread in Europe (Lepidoptera, Gracillariidae). *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* **3:**99-104.

Shaw M. R. & Huddleston T. (1991). Classification and biology of braconid wasps. Royal Entomological Society of London, London.

Schindler U. (1966). Zum massenwechsel des Buchenspringrusslers 1963 and 1964. *Forst- und Holzwirt* **20**:1-5.

Tilbury C. & Evan H. (2003). Exotic pest alert: horse chestnut leaf miner, *Cameraria ohridella* Desch & Dem. (Lepidoptera: Gracillariidae). Forestry Commission.

Tompkins D.M., Draycott R.A.H. & Hudson P.J. (2000). Field evidence for apparent competition mediated via the shared parasites of two gamebird species. *Ecology Letters* **3:**10-14.

Tóth, P. & Lukáš J. (2005). Parasitic Ichneumonoidea on the horse chestnut leaf miner, *Cameraria ohridella* (Lepidoptera: Gracillariidae) in Slovakia. Journal of Pest Science **78**:151-154.

Tsankov, G., and Z. Stalev. 1992. Studies of parasitoids of the larvae and pupae of the of *Rhynchaenus fagi* L. (Curculionidae, Coleoptera) in the region of Strandja mountain. *Nauka za Gorata* **29**:45-49.

Van Nouhuys S. & Hanski I. (2000). Apparent competition between parasitoids mediated by a shared hyperparasitoid. *Ecology Letters* **3:**82-84.

Whitebread S.E. (1989). *Phyllonorycter robiniella* (Clemens, 1859) in Europe (Lepidoptera, Gracillariidae). *Nota Lepidopterologica* **12:**344-353.

Woodcock B.A. & Vanbergen A.J. (2008). Parasitism of the beech leaf-miner weevil in a woodland: patch size, edge effects and parasitoid species indentity. *Insect Conservation and Diversity* **1**: 180-188.

Yu D., Van Achterberg C. & Horstmann K. (2005). World Ichneumonoidea 2004. Taxonomy, Biology, Morphology and Distribution. CD/DVD, Taxapad, Vancouver, Canada.

Chapter 4

The invasive alien leaf miner, *Cameraria ohridella* and the native maple, *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 this study was to provide an overview of the relationship between *C. ohridella* and *A. pseudoplatanus* and determine whether *C. ohridella* may 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 showed 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. As yet, there is little evidence that *C. ohridella* represents a major risk for *A. pseudoplatanus*.

Keywords: Invasive alien insect, leaf miner, *Cameraria ohridella*, *Acer pseudoplatanus*, host shift, ecological impact

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 in New Zealand (Wise 1953).

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 (R. Valade unpublished data), 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*, but not all. For example, the leaf miner is able to develop on *A. turbinata*, *A. flava* and *A. glabra*, but rarely or never on *A.* x *carnea* (hybrid *A. hippocastanum* x *A. pavia*), *A. indica*, *A. chinensis*, *A. californica*, and *A. 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, *A. 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 though 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 (e.g. 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 favorable on the American maple, *A. 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, one could expect that the total defoliation of horse-chestnut would produce selective pressure for the utilisation 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 since no sign of decline or dieback on mature trees has been observed (Salleo et al. 2003). 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 utilised 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 (Chapter 2).

The objective of this 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 following questions: (1) How common 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? (2) Does *C. ohridella* oviposit "at random" on *A. pseudoplatanus* or is it specifically attracted by this species compared to other non-*Aesculus* hosts? (3) Do *C. ohridella* populations vary in their ability to attack and develop on *A. pseudoplatanus*? (4) Is there variation among individual *A. pseudoplatanus* in their susceptibility to *C. ohridella*? (5) Can host plant chemistry, in particular concentration of phenolic compounds, explain variation in tree resistance? (6) 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

Oviposition preference and 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 thirteen indigenous tree and shrub species that are commonly found with horse-chestnut in Central Europe (listed in 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 meters from the trunk of heavily infested horse-chestnut trees 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. Since 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 10 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 dm² of leaf area. These leaf areas were measured using the Scion Image program (Scion Image, Alpha 4.0.3.2., Scion Corporation, Frederick, Maryland, USA). The number of eggs and larvae found per leaf was compared among host plant species. Since 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 *Cameraria ohridella* on *Acer pseudoplatanus*

Twenty random leaf samples were collected from the lower half of the canopy of *A. pseudoplatanus*. The leaves were sampled at distances of 0-3m, 10-20m and 50-100m 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 history of invasion by *C. ohridella*, which appeared in Upper Austria in 1989, in N-W Switzerland in 1998, and in France from 2000 to 2003.

About 40 samples 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 the both years. Leaves were returned to the laboratory in a cool box to avoid development during transport and there they were inspected under a stereomicroscope. The following information was collected for each sample: (1) number of eggs and mines of *C. ohridella* per leaf and per site; (2) proportion of respective developmental stages; (3) mortality rate; (4) 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. The correlation between the level of attack on *A. pseudoplatanus* and the nearest infested horse-chestnut tree was calculated.

Intraspecific variation in *Cameraria ohridella* and *Acer pseudoplatanus*

In 2007 and 2008, ten *A. pseudoplatanus* saplings (ca. 120 ± 10cm high) originating from 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 and 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 and Berlincourt 47°19'37''N, 7°13'23"E) where no development beyond the second instar had been observed on maple (i.e., in total 50 *Acer* 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*. Since 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.

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 (ca. 120cm ± 10cm high) 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. Then, pupae were collected from the dead leaves, sexed using the method of Freise & Heitland (1999) and left at room temperature in plastic containers (20 cl). At emergence (i.e. in mid May) ten adults (five males and five females) were put in gauze cages (200 cm x 60 cm x 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.

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

Results

Oviposition preference and development success on various host-plants

Eggs or larvae were found on all the fourteen tree and shrub species investigated, but their numbers varied greatly among species (Fig. 1). *Aesculus hippocastanum* was more heavily

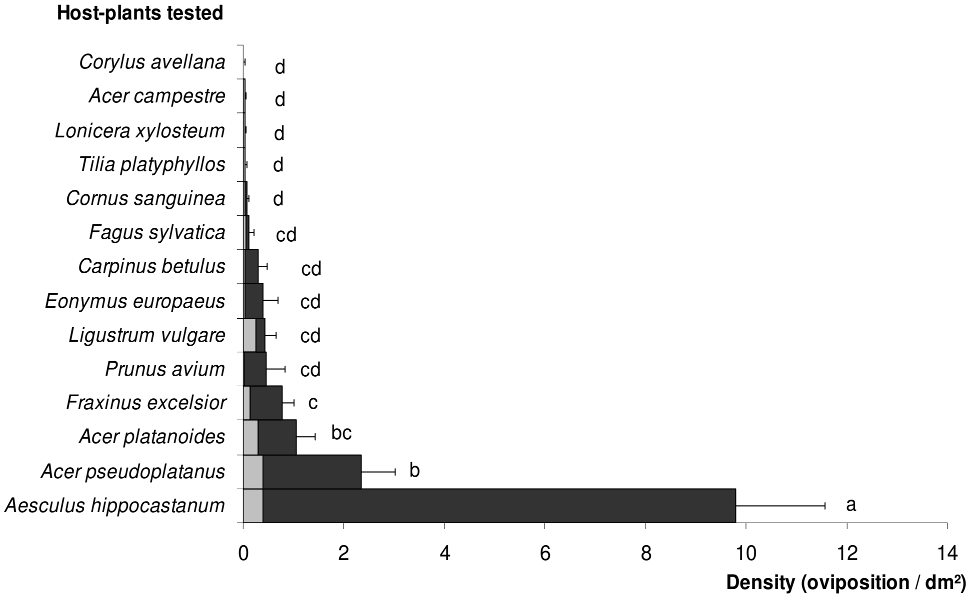

Fig. 1: Mean number of eggs (light grey) and larvae (dark grey) of *Cameraria ohridella* per dm^2 of leaf on various potential host plants found within 0-3 meters of an infested *Aesculus hippocastanum*. Error bars represent standard errors of the mean number of oviposition (eggs + larvae). Different letters indicate significant difference ($P < 0.05$).

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 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 *Cameraria ohridella* on *Acer pseudoplatanus*

The proportion of sites in Austria, Switzerland and France where mines of *C. ohridella* were found on *A. pseudoplatanus* varied from 53% to 87% in the two years, most of the mines being found within 0-3m of *A. hippocastanum* trees (Fig. 2). Few mines were found at 10-20m and almost none at 50-100m. The mean number of mines per *A. pseudoplatanus* leaf varied between 2 and 14 across the two years and the three countries over 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, but 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. 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 ($P = 0.204$ and $P = 0.765$ 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 to 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).

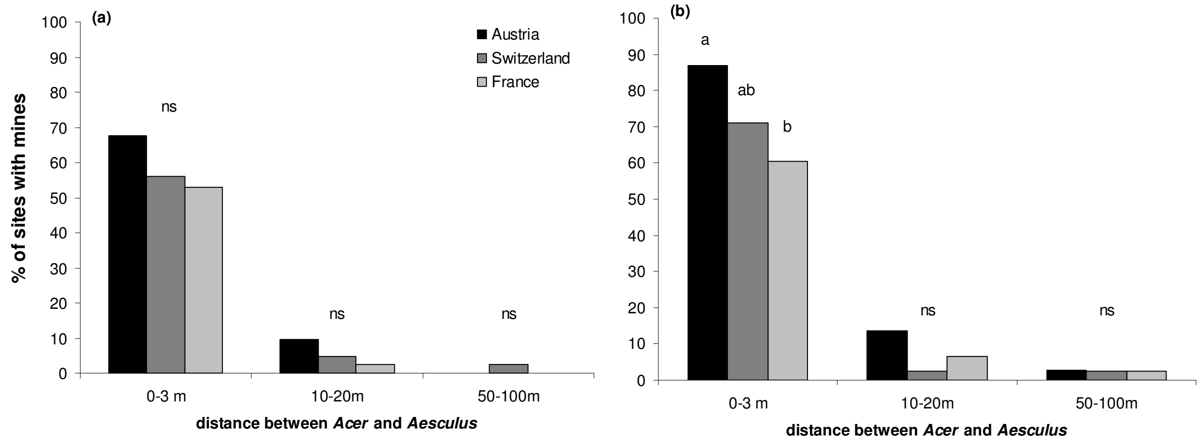

Fig. 2: Percentage of sites with mines on *Acer pseudoplatanus* in the three countries at three distances from an infested *Aesculus hippocastanum* in 2005 (a) and 2006 (b). Different letters above bars indicate significant differences between countries ($P < 0.05$).

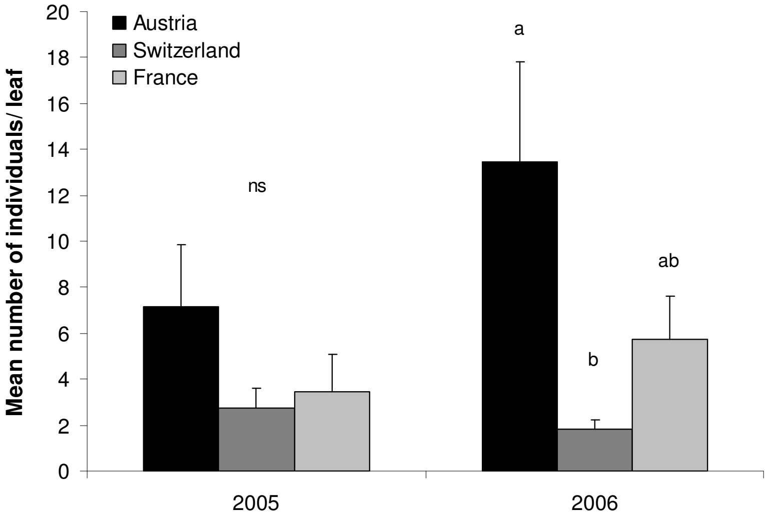

Fig. 3: Mean number of individuals (eggs + larvae) of *Cameraria ohridella* on *Acer pseudoplatanus* situated within 0-3 meters of an infested *Aesculus hippocastanum*, in 2005 and 2006, in the three countries. Error bars represent standard errors. Different letters above bars indicate significant differences among countries ($P < 0.05$).

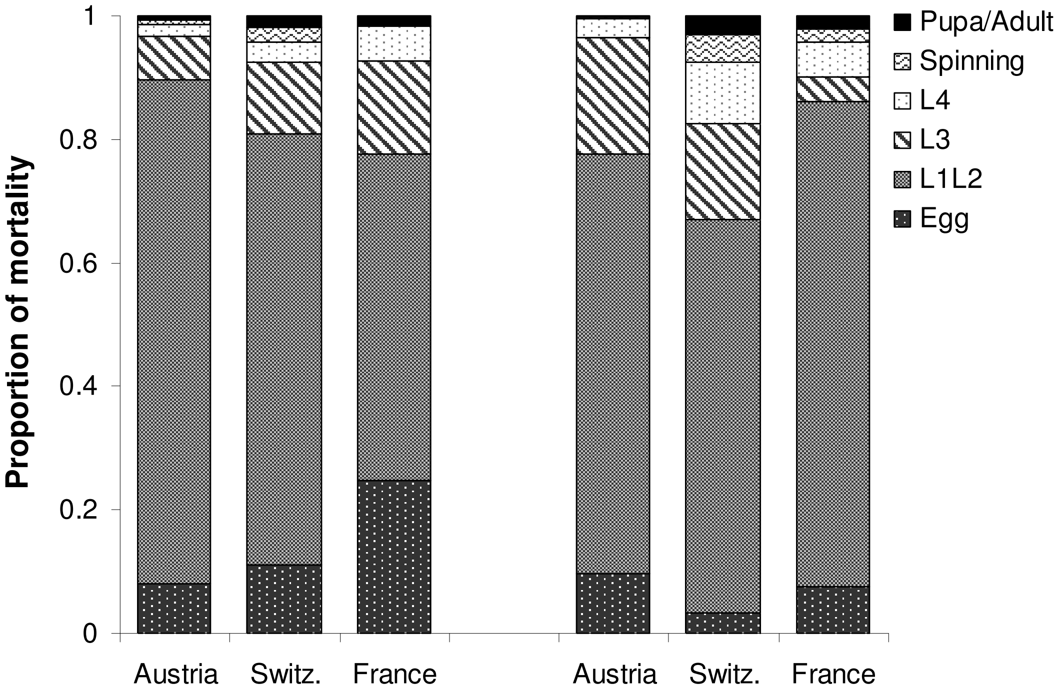

Fig. 4: Proportional mortality of *Cameraria ohridella* at different development stages on *Acer pseudoplatanus* in Austria, Switzerland and France (0-3m from an infested horse-chestnut), in 2005 (left) and 2006 (right).

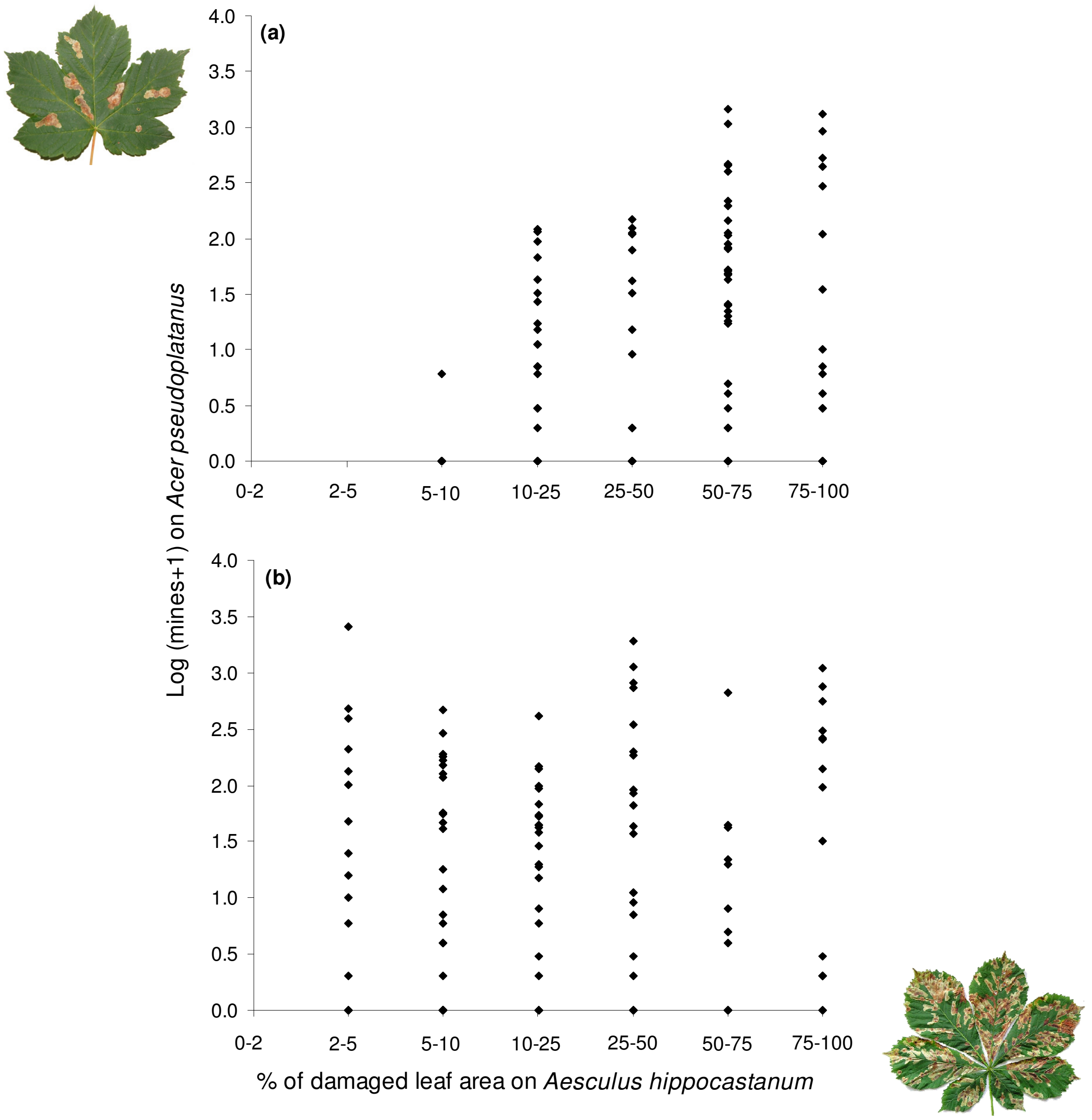

Fig. 5: Relationship between log-transformed mine numbers [$\log_{10}$(number of mines + 1)] on *Acer pseudoplatanus* and the percentage of damaged leaf area on *Aesculus hippocastanum*, at 0-3m in Austria, Switzerland and France in 2005 (a) and 2006 (b). Data on mine counts were log-transformed in order to eliminate heteroscedasticity observed at sites.

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 (r = 0.607, n = 33, $P < 0.001$; r = 0.736, n = 38, $P < 0.001$; r = 0.720, n = 31, $P < 0.001$ in Austria, Switzerland and France respectively). 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 *Cameraria ohridella* and *Acer 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 ($P \leq 0.001$ in 2007 and 2008) and among the five sites in 2007 ($P = 0.002$) but not among four sites in 2008 ($P = 0.190$, 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 ($P = 0.224$ and $P = 0.260$ 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. In contrast, the development success significantly differed among saplings ($P = 0.003$ and $P = 0.001$ 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* ($P = 0.883$) but it was significantly different among the different saplings ($P = 0.028$).

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	Sept.	June	Sept.
Berlincourt	482	10371	198	0*
Birse	149	2981	266	1882
Cabi	17	715	56	1899
Munchenstein	122	2642	77	2027
Lignières	17	4131	0	264

*At the site Berlincourt, the saplings were accidentally destroyed during the summer 2008

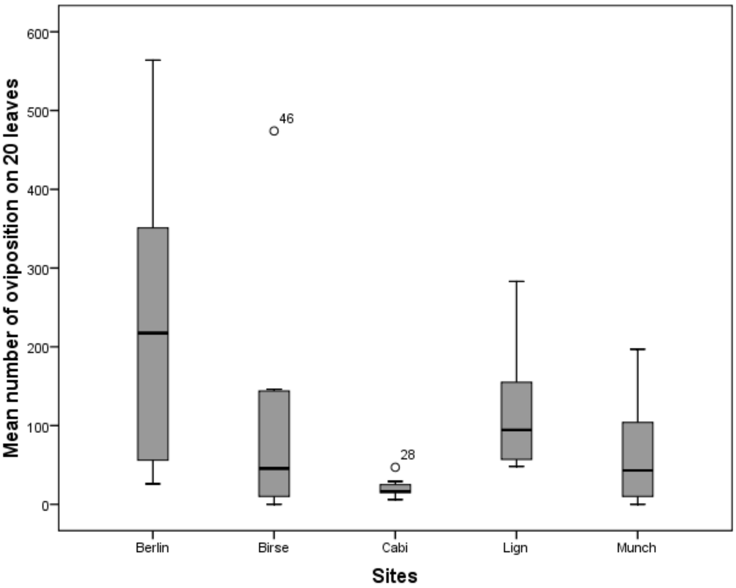

Fig. 6: Box plots of the mean number of ovipositions of *C. ohridella* on 20 leaves of *Acer pseudoplatanus* saplings planted in the field at five sites in Switzerland (September, 2007). Boxes depict 25th, 50th (median) and 75th percentiles, whiskers depict 10th and 90th percentiles, and points depict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

Discussion

This study provides the first comprehensive information on *A. pseudoplatanus* as host of *C. ohridella* and allows us to answer some questions and provide preliminary responses to others.

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

To our surprise, 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 maple die in the first two instars, leaving only very small mines that are not easily visible to the naked eyes. 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, but some mines were found at 50-100 m. Thus, in contrast to previous conclusions (e.g. Pschorn-Walcher 1997; Avtzis & Avtzis 2003), the utilisation of *A. pseudoplatanus* by *C. ohridella* is not limited to situations when horse-chestnut foliage becomes unavailable due to defoliation. Attacks on maple were commonly observed in the first generation when the level of attack on horse-chestnut was still 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 to 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 fourteen deciduous tree and shrub species investigated. Although horse-chestnut was the preferred host plant with the highest number of mines per dm^2, 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*. Furthermore, successful development 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 and S. Augustin unpublished data). *Acer* and *Aesculus* are in the same family, Sapindacea, and thus it is not surprising that these two genera may share common pest herbivores (Stevens 2001 onwards). Lopez-Vaamonde et al. (2003) found in gracillarid leaf miners of the genus *Phyllonorycter* that host shifts 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).

Ours results also suggest that females of *C. ohridella* preferentially select the host plant where offspring survival is higher, 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* in its origin area, although Avtzis & Avtzis (2003) found *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 (this study and, C. Péré and M. Kenis unpublished data). This suggests either that *C. ohridella* populations vary in their ability to attack and develop on maple, or that individual maple trees vary in their attractiveness and susceptibility to the moth. Comparisons in a common garden among *C. ohridella* populations collected at sites with different success of attack on maple failed to show any significant differences in their performance. However, more tests should be made, for example 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*?

Our results 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, but there is little doubt that the moth develops more successfully on some trees than on others, as observed in our experiments. We observed a positive year to year correlation in the number of large mines at our sample sites in the three countries. There are also empirical observations that the same maple 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). Host resistance also occurs on *A. hippocastanum*. Girardoz et al. (2007b) observed that about 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).

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?

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 to the two other countries. Since *C. ohridella* arrived in Austria about 10 to 12 years earlier, it may suggest that damage on *A. pseudoplatanus* is increasing. However, since individual *A. pseudoplatanus* trees vary in their attack rates, it may also be that 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 pers. comm.). Our results showing that high variation in level of attack arises from variability among trees rather than among moth populations is encouraging. 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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References

Augustin S., Guichard S., Heitland W., Freise J., Svatoš A. & Gilbert M. (2009). Monitoring and dispersal of the invading Gracillariidae *Cameraria ohridella. Journal of Applied Entomology* In press.

Avtzis N. & Avtzis D. (2003). *Cameraria ohridella* Deschka & Dimic (Lep.: Gracillariidae): A new pest on *Aesculus hippocastanum* in Greece. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft* **394**: 199-202.

Avtzis N. & Avtzis D. (2006). Zusammenfassende Betrachtung über die Verbreitung und die Biologie von *Cameraria ohridella* Deschka & Dimic (Lep., Gracillariidae) in Griechenland**.** *Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie* **15**: 177-182.

Craig T.P., Itami J., Shantz C., Abrahamson W.G., Horner J. & Craig J.V. (2000). The influence of host plant variation and intraspecific competition on oviposition preference and offspring performance in the host races of *Eurosta solidaginis*. *Ecological entomology* **25**: 7-18.

Dimić N., Dautbašić M. & Perić P. (2005). Host plants of *Cameraria ohridella* Deschka & Dimić, 1986 (Lepidoptera, Gracillariidae). *Entomofauna* **26**: 193-204.

Faeth S.H. (1990). Aggregation of a leafminer, *Cameraria* sp. Nov. (Davis): consequences and causes. *Journal of Animal Ecology* **59**:569-586.

Faeth S.H., Mopper S. & Simberloff D. (1981). Abundances and diversity of leaf-mining insects on three oak host species: effects of host-plant phenology and nitrogen content of leaves. *Oikos* **37**: 238-251.

Freise J.F. & Heitland W. (1999). A brief note on sexual differences in pupae of the horse-chestnut leaf miner, *Cameraria ohridella* Deschka & Dimic (1986) (Lep., Gracillariidae), a new pest in Central Europe on *Aesculus hippocastanum. Journal of Applied Entomology* **123**: 191-192.

Freise J.F., Heitland W. & Sturm A. (2003). Das physiologische Wirtspflanzenspektrum der Rosskastanien-Miniermotte, *Cameraria ohridella* Deschka & Dimic (Lepidoptera: Gracillariidae). *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* **55**: 209-211.

Freise J.F., Heitland W. & Sturm A. (2004). Das Wirtspflanzenspektrum der Roßkastanien-Miniermotte, *Cameraria ohridella* Deschka & Dimic (Lepidoptera: Gracillariidae), einem Schädling der Roßkastanie, *Aesculus hippocastanum*. *Mitteilungen der Deutschen Gesellschaft fur Allgemeine und Angewandte-Entomologie* **14**: 351-354.

Gilbert M. & Grégoire J.C. (2003). Visual, semi-quantitative assessments allow accurate estimates of leafminer population densities: An example comparing image processing and visual evaluation of damage by the horse-chestnut leafminer *Cameraria ohridella*. *Journal of Applied Entomology* **127**: 354-359.

Girardoz S., Quicke D.L.J. & Kenis M. (2007a). Factors favouring the development and maintenance of outbreaks in an invasive leaf miner *Cameraria ohridella* (Lepidoptera: Gracillariidae): a life table study. *Agricultural and Forest Entomology* **9**: 141-158.

Girardoz S., Tomov R., Eschen R., Quicke D.L.J. & Kenis M. (2007b). Two methods to assess the mortality factors affecting larvae and pupae of *Cameraria ohridella* in leaves of *Aesculus hippocastanum* in Switzerland and Bulgaria. *Bulletin of Entomological Research* **97**: 445-453.

Guillén M., Davis D.R. & Heraty J.M. (2001). Systematics and biology of a new, polyphagous species of *Marmara* (Lepidoptera: Gracillariidae) infesting grapefruit in the Southwestern United States. *Proceedings of the Entomological Society of Washington*, **103**: 636-654.

Hellrigl K. (1998). Zum Auftreten der Robinien-Miniermotte, *Phyllonorycter robiniella* (Clemens) und der Roßkastanien-Miniermotte, *Cameraria ohridella* Desch. & Dimic (Lep., Gracillariidae) in Südtirol. — *Anz.Schädlingsk.*, *Pflanzenschutz*, *Umweltschutz* **71**: 65-68.

Hellrigl K. (2001). Neue Erkenntnisse und Untersuchungen über die Rosskastanien-Miniermotte *Cameraria ohridella* Deschka & Dimic, 1986 (Lepidoptera, Gracillariidae). *Gredleriana* **1**: 9-81.

Kenis M., Tomov R., Svatos A., Schlienslog P., Lopez Vaamonde C., Heitland W., Grabenweger G., Girardoz S., Freise J. & Avtzis N. (2005). The horse-chestnut leaf miner in Europe - Prospects and contraints for biological control. Pages 77-90 *In:* Proceedings of the Second International Symposium on Biological Control of Arthropods, 12-16 September 2005, Davos, Switzerland, Forest Health Technology Enterprise Team-Morgantown, WV.

Lopez-Vaamonde C., Godfray H.C. & Cook J.M. (2003). Evolutionary dynamics of host-plant use in a genus of leaf-mining moths. *Evolution* **57**: 1804-1821.

Pschorn-Walcher H. (1997). Biology and population dynamics of the horse-chestnut leaf miner, *Cameraria ohridella*. *Forstschutz Aktuell* **21**: 7-10.

Salleo S., Nardini A., Raimondo F., Lo Gullo A.M., Pace F. & Giacomich P. (2003). Effects of defoliation caused by the leaf miner *Cameraria ohridella* on wood production and efficiency in *Aesculus hippocastanum* growing in north-eastern Italy. *Trees* **17**: 367-375.

Singer M.C. & Thomas C.D. (1988). Heritability of oviposition preference and its relationship to offspring performance within a single insect population. *Evolution* **42**: 977-985.

Slinkard K. & Singleton L. (1977). Total phenol analysis: automation and comparison with manual methos. *American Journal of Enology and Viticulture* **28**: 49-55.

Stevens P.F. (2001 onwards). Angiosperm Phylogeny Website. Version 9, June 2008 [and more or less continuously updated since].http://www.mobot.org/MOBOT/research/APweb/

Straw N.A. & Tilbury C. (2006). Host plants of the horse-chestnut leaf-miner (*Cameraria ohridella*), and the rapid spread of the moth in the UK 2002-2005. *Arboricultural Journal* **29**: 83–99.

Tomiczek C. & Krehan H. (1998). The horsechestnut leafmining moth (*Cameraria ohridella*): a new pest in Central Europe. *Journal of Arboriculture* **24**: 144-148.

Wise K.A.J. (1953). Host plants of *Lithocolletis messaniella*. *New Zealand Journal of Science and Technology (A)* **35**: 172-174.

Chapter 5

Summary & Conclusions

Summary

The present thesis describes investigations on the current and potential ecological impact of the horse-chestnut leaf miner, *Cameraria ohridella*, an invasive alien species in Europe, on the native fauna and flora.

Chapter 1 is a short introduction presenting the issue of invasive species, the biology and ecology of leaf miners in general and the invasion of *C. ohridella* in Europe. It also presents and develops the two major hypotheses of the thesis, which are: (1) *C. ohridella* has a negative impact on the population density of native leaf miners through the enhanced production and population build-up of a high quantity of polyphagous natural enemies; (2) *C. ohridella*, which is primarily a pest of an ornamental, non-indigenous tree in Central Europe, is increasingly attacking a native tree, *Acer pseudoplatanus* and represents a potential risk for this and other *Acer* species.

Chapter 2 describes studies on the effect of the alien horse-chestnut leaf miner, *C. ohridella*, on native fauna. We compared the species richness of native leaf miner communities and the abundance of selected native leaf miner species in the presence and absence of horse-chestnut trees infested by *C. ohridella*, in various environments in Europe. The species richness of native leaf miner communities in Switzerland was lower at sites where *C. ohridella* was present than at sites where it was absent. In Switzerland, France and Bulgaria, several native leaf miners feeding on various woody species were significantly less abundant in the vicinity of infested horse-chestnuts. The native species most affected by the presence of the alien species were those occurring early in the year and sharing their parasitoid complex with *C. ohridella.*

These results described in Chapter 2 suggested apparent competition mediated by shared natural enemies because these are the only link between *C. ohridella* and native leaf miners feeding on other host-plants. However, since these were purely observational studies of leaf miner populations, apparent competition needed to be ascertained by measuring mortality factors, in particular parasitism. Therefore, in **Chapter 3,** we present several observational and experimental approaches to test the occurrence of apparent competition between *C. ohridella* and the native beech leaf mining weevil, *Orchestes fagi.* In the previous study in Switzerland, described in Chapter 2, *O. fagi* was shown to be significantly less abundant in the surroundings of horse-chestnut trees infested by the invasive horse-chestnut leaf miner, *C. ohridella*, compared to control sites. Total mortality, parasitism, predation and parasitoid species richness were compared between sites with and without horse-chestnut trees attacked by *C. ohridella*. No evidence for apparent competition was found. Total mortality, parasitism and predation were not significantly different between sites with *C. ohridella* and sites without *C. ohridella*. Several parasitoid species commonly attacking *C. ohridella* were reared from *O. fagi* but, in general, their density was not higher in the vicinity of infested horse-chestnut trees. Possible explanations for these unexpected results are provided in the discussion section of the chapter.

In **Chapter 4**, we present a study, of which the objective was to provide an overview of the relationship between *C. ohridella* and *A. pseudoplatanus* and determine whether *C. ohridella* may shift to this native tree. In the field, females oviposited on different deciduous tree species. Although less frequently attacked than *Aesculus hippocastanum*, *A. pseudoplatanus* was clearly preferred for oviposition over 12 other woody species investigated. Surveys in Europe showed 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.* 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*. As yet, there is little evidence that *C. ohridella* represents a major risk for *A. pseudoplatanus*.

Finally, **Chapter 5** is a general conclusion of the thesis, including recommendations for further work.

Conclusions

The impact of invasive species on native species, communities, and ecosystems is widely recognized (Williamson 1996; Wittenberg & Cock 2001; Pimentel 2002), and invasive species are now considered as one of the most important threat to natural ecosystems and biodiversity (Sakai et al. 2001). The important economic impact of these species is evident in agriculture, horticulture, aquaculture, stored products, and forestry (Pimentel 2002). In addition to economic impacts, invasive species have, in a multitude of ways, severe negative consequences for biodiversity (Sakai et al. 2001). Ecological interactions between native and invasive species may be direct (e.g., predation, herbivory, parasitism, competition, mutualism) or indirect (e.g., habitat alteration, apparent competition, cascading trophic interactions) and result in changes in the population biology and ecology of the native species. Vertebrates, plants, arthropods and pathogens represent the most important groups of invaders.

Although there is a worldwide concern about the ecological impact of invasive insects on native species, communities and ecosystems, the available information is still rather limited. In a recent review (Kenis et al. 2009), we found over 400 primary research publications describing studies on the ecological impact of invasive alien insect. However, these studies concerned only 72 alien species, and most publications described ecological impact by ants, bees, external-feeding herbivores or biological control agents. The present thesis is the first attempt to investigate the ecological impact by an invasive alien leaf miner on the native fauna and flora.

Our study provides one of the first examples suggesting that invasive insects may indirectly affect native species through apparent competition (Chapters 2 & 3). Very few studies have investigated such interactions in invertebrates, and even fewer in the context of invasive insects. Settle & Wilson (1990) showed that the variegated leafhopper, *Erythroneura variabilis* Beamer, an invasive species in Californian vineyards, contributes to an increasing proportion of the overall parasitoid population, which results in higher parasitism rates for the native grape leafhopper, *E. elegantula* Osborn (Homoptera: Cicadellidae). More recently, Carvalheiro et al. (2008) reported evidence that a seed feeder introduced as weed biological control agent may affect populations of several seed herbivores in Australia through apparent competition. Other studies failed to observe apparent competition between invasive and native insects. For example, Schönrogge & Crawley (2000) used quantitative linkage webs to investigate the effect of alien cynipid gall wasp on native gall wasps in the UK through shared native parasitoids and inquilines. They concluded that the recruitment of parasitoids and inquilines by the invading species was unlikely to have strong effect on the native species because the native parasitoids did not exhibit strong responses to the invasive wasps.

Although we failed to find evidence for apparent competition through shared natural enemies between *Cameraria ohridella* and *Orchestes fagi*, we still believe that the striking variations in weevil densities at sites with and without horse-chestnut infested by *C. ohridella* would merit further investigations. Firstly, since impact on the native species through apparent competition is more likely to occur when the native species is rarer than the invasive species, the manipulative experiments described in Chapter 3 should be repeated when, or where, populations of *O. fagi* will be in the latency phase. Among the different techniques used to measure mortality factors, the tagged cohort samples and the grab samples provided the highest rates of parasitism, predation and mortality and are probably the most reliable methods to assess mortality factors in natural populations. The same methods have recently been used to assess mortality factors of *C. ohridella*, and their respective advantages and disadvantages have been discussed in Girardoz et al. (2007). The mass sampling at many sites at once did not allow the collection of all mines precisely at the end of their development, which led to an underestimation of mortality rates, in particular parasitism. The exposure of saplings also provided lower mortality rates, most probably because parasitoids and, perhaps predators, of arboreal leaf miners are less used to forage in the herbaceous layer. Secondly, horse-chestnut is one of the few tree species rejected by adults of *O. fagi* for maturation feeding (Bale & Luff 1978). Thus, the effect of the presence of horse-chestnut, e.g. through plant volatiles, on the oviposition behaviour of the weevil, or the impact of dead leaves on overwintering adults, should be tested.

More generally, apparent competition between an invasive and a native leaf miner could be tested using the methods developed in this thesis, but rather on invasive leaf miners feeding on a more widely distributed host, such as the black locust leaf miners *Phyllonorycter robiniella* and *Parectopa robiniella* (Whitebread 1989) or the lime leaf miner *Phyllonorycter issikii* (Šefrova 2002).

Alien invertebrate herbivores can be particularly harmful to native plant populations, sometimes driving them to local extinction (Kenis et al. 2009). The best examples of direct effect are those caused by forest insects. For example, the balsam woolly adelgid, *Adelges piceae* (Ratzeburg) (Hemiptera: Adelpigae), and the hemlock woolly adelgid, *A. tsugae* (Annand) are threatening unique forest ecosystems in eastern North America by attacking Fraser fir (*Abies fraseri* (Pursh) Poir), Eastern and Carolina hemlock (*Tsuga canadensis* (L.) Carr. and *T. caroliniana* Engelm.) on a large scale (e.g. Smith & Nicholas 2000; Jenkins 2003; Small et al. 2005; Weckel et al. 2006).

Cameraria ohridella does not represent a direct ecological threat for its main host, the horse-chestnut, in Central Europe because it is an exotic ornamental species and it does not kill mature trees (Salleo et al. 2003). Its impact on the few remaining horse-chestnut stands in the Balkans, particularly through a potential impact on tree regeneration (Thalmann 2003) would merit further investigations, although recent molecular studies showed that the moth probably originates from at least some of these sites (R. Valade et al. personal communication).

However, *C. ohridella* may represent a risk to the native flora in Europe if the damage occasionally observed on maple, particularly *Acer pseudoplatanus*, was increasing. In this thesis we provide the first comprehensive information on *A. pseudoplatanus* as host of *C.*

ohridella and on the potential threat posed by the moth on this important native tree (Chapter 4). 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 in New Zealand (Wise, 1953).

Our results show little evidence that *C. ohridella* represents a major risk for *A. pseudoplatanus*. The outcomes of our study suggest that the high variation observed in the attack levels and the development success of *C. ohridella* on *A. pseudoplatanus* arises from variability among individual trees rather than among moth populations. Nevertheless, since outbreaks of *C. ohridella* on *A. hippocastanum* continue unabated and total defoliation occurs every year, it is possible that, under such a strong selection pressure, *C. ohridella* population may develop their ability to reproduce on *A. pseudoplatanus.* More tests should be made, for example comparing *C. ohridella* populations emerging from *A. hippocastanum* and populations emerging from *A. pseudoplatanus*, before excluding genetically-based variations among *C. ohridella* populations or individuals in their ability to overcome tree resistance. Mechanisms behind host tree variations in their susceptibility to *C. ohridella* should also be further investigated, in particular by comparing the chemical composition of susceptible and resistant varieties and species. Furthermore, it has sometimes been suggested that the low parasitism of *C. ohridella* may be due to the lack of attractiveness of the parasitoids to *A. hippocastanum*, a tree that does not host any native leaf miner. If possible, when large populations of *C. ohridella* are found on *A. pseudoplatanus*, the opportunity should be taken to rear parasitoids and compare the parasitoid complex of the moth on the two host trees. Although preliminary studies have shown that parasitism of *C. ohridella* is similar on the two tree species, it would be of utmost interest to carry out an olfactometry study on the tritrophic interactions between the parasitoids, *C. ohridella*, and the two host trees.

References

Bale J.S. & Luff M.L. (1978). The food plants and feeding preferences of the beech leaf mining weevil, *Rhynchaenus fagi* L. *Ecological Entomology* **3**:245-249.

Carvalheiro L.G., Buckley Y.M., Ventim R., Fowler S.V. & Memmott J. (2008). Apparent competition can compromise the safety of highly specific biocontrol agents. *Ecology Letters* **11:**690-700.

Girardoz S., Tomov R., Eschen R., Quicke D.L.J. & Kenis M. (2007). Two methods assessing the mortality factors affecting the larvae and pupae of *Cameraria ohridella* in the leaves of *Aesculus hippocastanum* in Switzerland and Bulgaria. *Bulletin of Entomological Research* **97:**445-453.

Guillén M., Davis D.R. & Heraty J.M. (2001). Systematics and biology of a new, polyphagous species of *Marmara* (Lepidoptera: Gracillariidae) infesting grapefruit in the Southwestern United States. *Proceedings of the Entomological Society of Washington*, **103**: 636-654.

Jenkins MA (2003) Impact of the balsam woolly adelgid (*Adelges piceae* Ratz.) on an *Abies fraseri* (Pursh) Poir. dominated stand near the summit of Mount LeConte, Tennessee. *Castanea* **68:**109–118.

Kenis M., Auger-Rozenberg M.A., Roques A., Timms L., Péré C., Cock M.J.W., Settele J., Augustin S. & Lopez-Vaamonde C. (2009). Ecological effects of invasive alien insects. *Biological Invasions* **11:**21-45.

Pimentel D. (ed) (2002). Biological invasions. Economic and environmental costs of alien plants, animal and microbe species. CRC Press, Boca Raton.

Sakai A.K., Allendorf F.W., Holt J.S., Lodge D.M., Molofsky J., With K.A., Baughman S., Cabin R.J., Cohen J.E., Ellstrand N.C., McCauley D.E., O'Neil P.O., Parker I.M., Thompson J.N. & Weller S.G. (2001). The population biology of invasive species. *Annual Review of Ecology and Systematics* **32:**305-332.

Salleo S., Nardini A., Raimondo F., Assunta Lo Gullo M., Pace F. & Giacomich, P. (2003) Effects of defoliation caused by the leaf miner *Cameraria ohridella* on wood production and efficiency in *Aesculus hippocastanum* growing in north-eastern Italy. *Trees* **17**: 367-375.

Šefrova H. (2002). *Phyllonorycter issikii* (Kumata, 1963) - bionomics, ecological impact and spread in Europe (Lepidoptera, Gracillariidae). *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* **3:**99-104.

Settle W.H. & Wilson T.L. (1990). Invasion by the variegated leafhopper and biotic interactions: parasitism, competition, and apparent competition. *Ecology* **71:**1461-1470.

Schönrogge K. & Crawley M.J. (2000). Quantitative webs as a means of assessing the impact of alien insects. *Journal of Animal Ecology* **69:**841-868.

Small MJ, Small CJ, Dreyer GD (2005) Changes in a hemlock-dominated forest following woolly adelgid infestation in southern New England. *Journal of the Torrey Botanical Society* **132:**458–470.

Smith G.F., Nicholas N.S. (2000). Size- and age-class distributions of Fraser fir following balsam woolly adelgid infestation. *Canadian Journal of Forest Research* **30:**948–957.

Thalmann, C. (2003) Effects of defoliation by horse chestnut leafminer (*Cameraria ohridella*) on its host *Aesculus hippocastanum*. PhD thesis, Univerity of Bern, Switzerland.

Weckel M, Tirpak JM, Nagy C, Christie R (2006) Structural and compositional change in an old-growth eastern hemlock Tsuga canadensis forest, 1965–2004. *Forest Ecology and Management* **231:**114–118.

Whitebread S.E. (1989). *Phyllonorycter robiniella* (Clemens, 1859) in Europe (Lepidoptera, Gracillariidae). *Nota Lepidopterologica* **12:**344-353.

Williamson M. (1996). Biological invasions. Chapman and Hall, London.

Wise K.A.J. (1953). Host plants of *Lithocolletis messaniella*. *New Zealand Journal of Science and Technology (A)* **35:** 172-174.

Wittenberg R. & Cock M.J.W. (2001). Invasive alien species: a toolkit of best prevention and management practices. CAB International, Wallingford.

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