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ORIGINAL PAPER

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

Christelle Péré · Sylvie Augustin · Rumen Tomov · Long-hui Peng · Ted C. J. Turlings · Marc Kenis

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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 and 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,

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C. Péré (✉) · M. Kenis
CABI Europe-Switzerland, 1 Rue des Grillons,
2800 Delémont, Switzerland
e-mail: c.pere@cabi.org

S. Augustin · L. Peng
Unité de Zoologie Forestière, INRA, 45075 Orléans
Cedex 2, France

R. Tomov
University of Forestry, 1756 Sofia, Bulgaria

C. Péré · T. C. J. Turlings
Institute of Zoology, University of Neuchâtel,
2009 Neuchâtel, Switzerland

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 and Godfray 1997; Morris et al. 2001, 2004). Mechanisms have been demonstrated in laboratory studies (e.g., Bonsall and Hassell 1997, 1998) or through field population manipulations (Van Nouhuys and 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 and Crawley 2000; Kenis et al. 2009), but there are very few examples of invasive 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 and 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 and Dimić (Lepidoptera: 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 and 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 5 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 hypothesize 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 preda tion 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 50 km 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, 4–7 years in Switzerland and 2–4 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 2 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 19–22 sites in 2005 and 28–33 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

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[a] (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[a] (Hering)	*A. hen*	Dip: Agromyzidae		x	
Aulagromyza cornigera (Griffiths)	*A. cor*	Dip: Agromyzidae			x
Chromatomyia periclymeni[a] (Hendel)	*C. per*	Dip: Agromyzidae			x
Phyllonorycter emberizaepennella (Bouché)	*P. emb*	Lep: Gracillariidae		x	
Quercus robur L.					
Orchestes quercus[a] (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		

BG Bulgaria, *CH* Switzerland, *FR* France

[a] Leaf miner species whose mature larvae were found at least 2 weeks before those of *C. ohridella*

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.

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 it 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, 500 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 a 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 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).

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 3 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) (Fig. 3c, d). In Switzerland, no leaf miner species was found to be significantly more abundant in the presence of

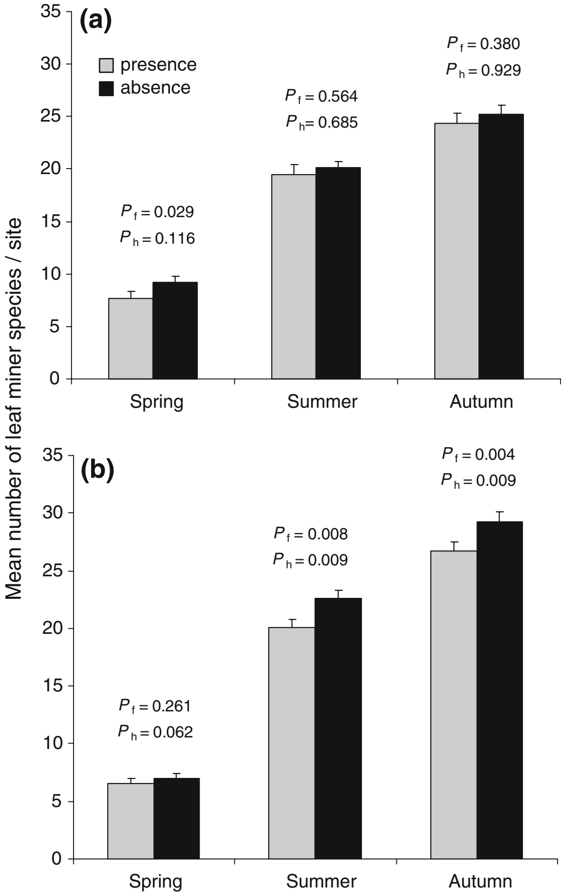

Fig. 1 Species richness of native leaf miners in the presence and absence of *Cameraria ohridella*, in Switzerland in **a** 2005 and **b** 2006 (Spring: collection in May; Summer: cumulative collections of May and July; Autumn: cumulative collections of May, July and September). P_f = confidence level 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

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.

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 (miner-induced) plant volatiles, or the large quantities of pheromone released by *C. ohridella*

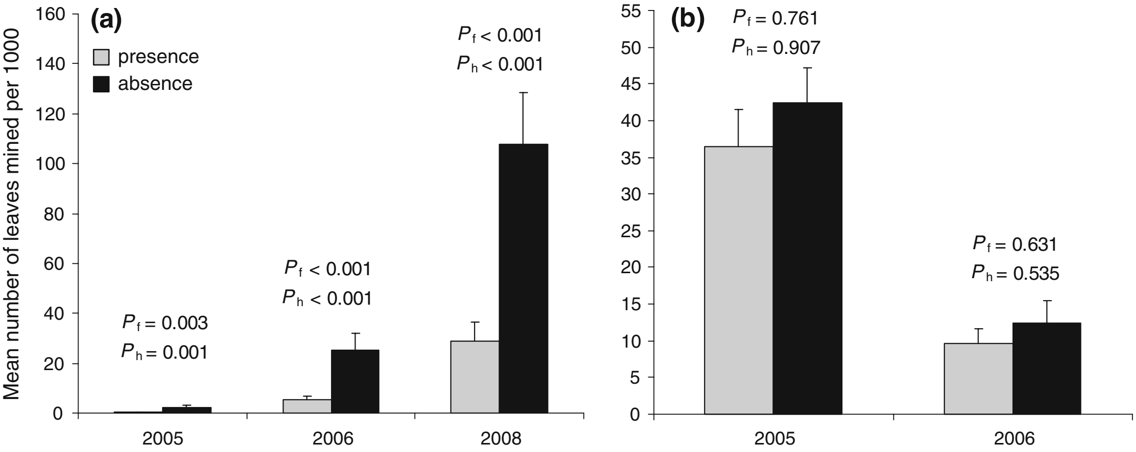

Fig. 2 Abundance of **a** *Orchestes fagi* (2005, 2006 and 2008) and **b** *Aulagromyza hendeliana*, (2005 and 2006) in presence and absence of *Cameraria ohridella*, in Switzerland. *Error bars* represent standard errors. For the significance of P_{f} and P_{h}, see Fig. 1

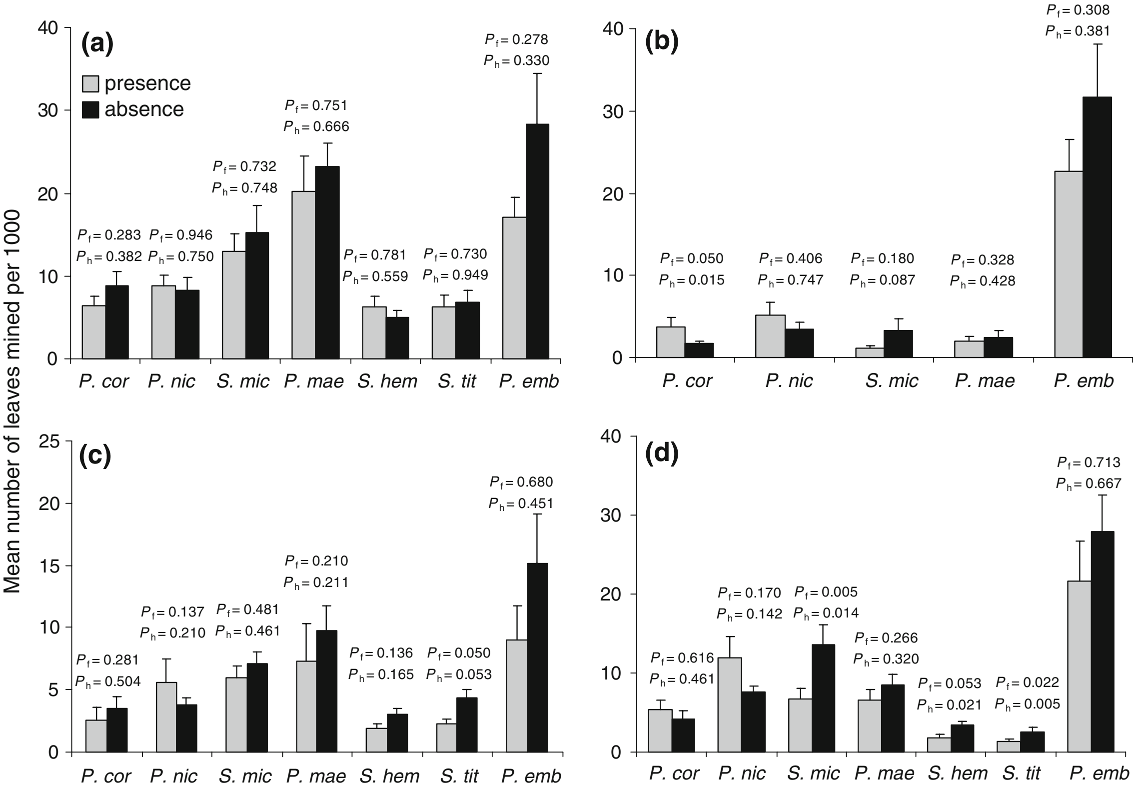

Fig. 3 Abundance of native leaf miners in Switzerland, in **a** July 2005, **b** September 2005, **c** July 2006 and **d** September 2006. *Error bars* represent standard errors. For the significance of P_{f} and P_{h}, see Fig. 1. Abbreviations of leaf miner species are given in Table 1

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.

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

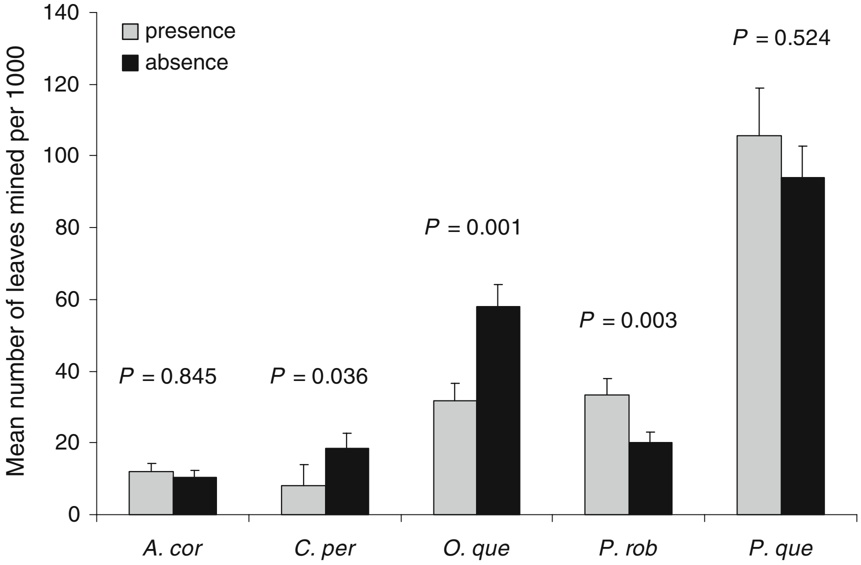

Table 2 Median abundance of the number of native leaf miner species found in Bulgaria, in presence (Pres.) and absence (Abs.) of *Cameraria ohridella* and *P* value of the Mann–Whitney test

Host plants/leaf miner species	2005						2006					
	July			September			July			September		
	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**	–	–	–

Significant *P* values ($P \leq 0.05$) are in bold-face

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 3 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 synchronized 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 and 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 *Phyllonotycter 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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