Published in Biodiversity and Conservation 20, issue 13, 3003-3016, 2011
which should be used for any reference to this work

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

Christelle Péré · Robert Bell · Ted C. J. Turlings · Marc Kenis

Abstract Apparent competition, through the action of shared natural enemies, is frequently suggested as a possible mechanism underlying the impact of invasive alien species on native species, but examples are rare, particularly in insects. A previous study showed that the beech leaf mining weevil, *Orchestes fagi*, was significantly less abundant close to horse-chestnut trees infested by the invasive horse-chestnut leaf mining moth, *Cameraria ohridella*, compared to control sites. Apparent competition through the sharing of natural enemies was proposed as a potential mechanism underlying this effect. To test the occurrence of apparent competition between the two leaf miner species, three observational studies and one experimental manipulation were carried out in Switzerland during 3 years. The total mortality, parasitism, predation and parasitoid diversity of larvae and pupae of *O. fagi* were compared between sites with and without horse-chestnut trees severely attacked by *C. ohridella*. Total mortality and predation rates of *O. fagi* were not significantly different between sites with and sites without *C. ohridella*. Despite a large overlap between the parasitoid complexes of the two leaf miners, parasitism of *O. fagi* was found to be positively influenced by the presence of horse-chestnuts infested by *C. ohridella* in only one of the four studies and only for 1 year. Similarly, parasitoid diversity was not higher near infested horse-chestnut trees compared to control sites. Thus, little evidence for apparent competition was found. Possible reasons, including possible insufficiencies in the experimental circumstances and design, are discussed.

Keywords Apparent competition · Indirect interactions · Invasive alien insect · Leaf miner · Mortality rate · Native insect species · Parasitism · Parasitoid complex · Predation

---

C. Péré · R. Bell · M. Kenis (✉)
CABI Europe-Switzerland, 1 Rue des grillons, 2800 Delémont, Switzerland
e-mail: m.kenis@cabi.org

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

R. Bell
School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK

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 1977; Morris et al. 2004). Apparent competition may be short-term, observed over a single generation or long-term, sustained over several generations (Holt and 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 and Godfray 1997; Morris et al. 2001, 2004), including through field population manipulations (Van Nouhuys and 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 *Erythroneura elegantula*, whose decline was associated with augmented levels of a shared egg parasitoid in the presence of *E. variabilis* (Settle and Wilson 1990). Recently, a seed feeder introduced in Australia as a weed biological control agent was found to affect native seed herbivores, most likely through apparent competition (Carvalheiro et al. 2008).

The invasion of the horse-chestnut leaf miner, *Cameraria ohridella* (Lepidoptera: Gracillariidae), in Europe, provides an excellent opportunity to investigate apparent competition between an invasive insect and native insects that do not share resources. *C. ohridella*, which probably originates from the Balkans (Valade et al. 2009), is the first leaf miner in Europe known to attack horse-chestnut, *Aesculus hippocastanum*, also of Balkan origin, which has been planted throughout the continent as an amenity tree. *C. ohridella* feeds nearly exclusively on horse-chestnut. It may also attack maple (*Acer* spp.) in the vicinity of horse-chestnut but on maples larvae usually die before pupation (Péré et al. 2010a). The moth 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, allow it to maintain outbreak densities after invasion (Girardoz et al. 2007a, b). In Europe, about 30 species of parasitoids have been recorded from *C. ohridella,* mostly Chalcidoidea with some Ichneumonoidea. All these parasitoids are known as generalists and, thus, are shared with many other leaf miners of various families. In Central Europe, the main parasitoid species are the eulophids *Minotetrastichus frontalis*, *Pnigalio agraules*, *Chrysocharis nephereus* and *Closterocerus trifasciatus* and the braconid *Colastes braconius* (Hellrigl 2001; Freise et al. 2002; Grabenweger et al. 2005; Girardoz et al. 2006). These are all very common European species that can be found on other leaf miners of different families (Askew and Shaw 1974; Noyes 2002; Yu et al. 2005). Despite low parasitism rates in Central Europe (Girardoz et al. 2007a, b), *C. ohridella* are so numerous compared to native leaf miner species that an unusually large number of polyphagous parasitoids is produced in the surroundings of infested horse-chestnut trees (Girardoz et al. 2007c; Grabenweger et al. 2010). The impact of these additional parasitoids could be particularly high on early-occurring leaf miner species because, as shown by Grabenweger (2004) and Girardoz et al. (2006), in spring (April–May), most parasitoids of *C. ohridella* emerge at least 5 weeks before the first suitable *C. ohridella* larvae or pupae are available. *C. ohridella* larvae and pupae are also attacked by generalist predators such as birds, bush-crickets, lacewings or ants, which could also be involved in apparent competition (Grabenweger et al. 2005; Girardoz et al.

2007b). 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.

In Switzerland, France and Bulgaria the species richness and the abundance of native leaf miners feeding on various deciduous tree and shrub species were lower in the vicinity of horse-chestnut trees infested by *C. ohridella* compared to control sites (Péré et al. 2010b). In particular, a notable variation in abundance was observed in the native beech leaf mining weevil, *Orchestes fagi* (syn. *Rhynchaenus fagi*) (Coleoptera: Curculionidae), in Switzerland. In a comparative observation involving 19–30 pairs of sites with beech trees, the weevil was four to seven times less abundant in presence than in absence of horse-chestnut trees attacked by *C. ohridella*, both at low (2005) and high (2006 and 2008) weevil density (Péré et al. 2010b). Natural enemies are possibly one of the causes of changes in the population level of *O. fagi* because they are the most obvious link between *C. ohridella* and *O. fagi*, which exclusively attacks the European beech, *Fagus sylvatica* (Bale 1984). *O. fagi* is a univoltine species. Adults overwinter in the litter and females oviposit in young beech leaves very soon after bud burst in mid-late April, with the new generation adults emerging 30–43 days later in early-mid June (Bale 1981, 1984; Bale and Luff 1978; Pullin 1985). The larval and pupal stages are thus perfectly synchronized with the flight period of the parasitoids of *C. ohridella*. *O. fagi* has a large parasitoid complex in Europe (Askew and Shaw 1974; Tsankov and Stalev 1992; Noyes 2002; Tóth and Lukáš 2005; Yu et al. 2005). In particular, it is known to be attacked by all the main parasitoids of *C. ohridella* in Switzerland (Girardoz et al. 2006, 2007a, b) (Table 1). 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.

The study described in Péré et al. (2010b) on population changes in *O. fagi* and other native leaf miners in the presence and absence of *C. ohridella* did not focus on natural enemies. Thus, the role of natural enemies in the low abundance of native leaf miners in the vicinity of infested horse-chestnut trees needs confirmation. In the current paper, we report on the comparison of overall mortality, parasitism and predation of larvae and pupae of *O. fagi*, in the presence and absence of *C. ohridella*. We tested the hypothesis that mortality, parasitism and predation rates in *O. fagi* are higher in the vicinity of horse-chestnut attacked by *C. ohridella* than at sites away from infested horse-chestnut trees. Various observational studies and experimental manipulations were used, some of which were made in parallel and at the same sites as those used in Switzerland by Péré et al. (2010b).

Materials and methods

Field sites

The experiment was conducted and observations were made in 2006–2008 in north-west Switzerland in Cantons Jura, Basel-Landschaft, Solothurn and Aargau. Sites were within 50 km of Delémont (47°22′N–7°21′E), between 300 and 700 m elevation, in or at the edge of a broad-leaved forest. In this region, horse-chestnut is an exotic tree found mainly in urban areas, but single trees, or small groups of trees, are occasionally planted in forested areas. In such areas, all trees are permanently infested by *C. ohridella*. Since sustainable populations of *C. ohridella* occur only on horse-chestnut (Péré et al. 2010a), the presence or absence of *C. ohridella* in an area can thus be determined by the presence or absence of horse-chestnut. The horse-chestnut sites (i.e. in presence of *C. ohridella*) were characterized by the presence of beech trees located less than 50 m from one or more horse-chestnut

Table 1 List of parasitoid species shared by *C. ohridella* and *O. fagi* based on a literature review and their basic biological characteristics

Superfamily Family: subfamily	Parasitoid species	Mode of development	Mode of parasitism	Ecto/endo parasitism	Life stage attacked	Relative proportion of parasitism on *C. ohridella*
Chalcidoidea						
Eulophidae: Entedoninae	***Chrysocharis nephereus***	Solitary	I*	Endo	L, P	10%
	Chrysocharis nitetis	Solitary	I*	Endo	L, P	
	Closterocerus trifasciatus	Solitary	I*	Endo	L, P	1%
	Pediobius saulius	Solitary	K*	Endo	L, P	
Eulophidae: Eulophinae	***Pnigalio agraules***	Solitary	I	Ecto	L, P	14%
	Pnigalio longulus	Solitary	I	Ecto	L, P	
	Pnigalio pectinicornis	Solitary	I	Ecto	L	
	Pnigalio soemius	Solitary	I	Ecto	L	
	Cirrospilus diallus	Solitary	I	Ecto	L, P	
	Cirrospilus vittatus	Solitary	I*	Ecto	L	
Eulophidae: Tetrastichinae	***Minotetrastichus frontalis***	Gregarious	I*	Ecto	L, P	40%
Ichneumonoidea						
Braconidae: Exothecinae	***Colastes braconius***	Solitary	I	Ecto	L, P	35%
Ichneumonidae: Pimplinae	*Itoplectis alternans*	Solitary	I*	Endo	P	

Table combined using the following references: Askew and Shaw (1974), Girardoz et al. (2006), Grabenweger et al. (2003), Noyes (2002), Tóth and Lukáš (2005), Tsankov and Stalev (1992), and Yu et al. (2005). In bold are the five parasitoid species commonly found on *C. ohridella* in Switzerland. The last column indicates the relative proportion of the parasitoids of *C. ohridella* reared from single overwintering mines collected at four of the investigated sites in winter 2007–2008 (C. Péré, unpublished data, based on 265 parasitism events, broods of the gregarious *M. frontalis* counting as a single parasitism event)

* Facultatively hyperparasitic, *K* koinobiont, *I* idiobiont, *L* larva, *P* pupa

trees that had been heavily and continuously infested by *C. ohridella* (i.e. a minimum of 20 mines per leaf) for at least 4–7 years. For each of these sites, a control site, without any horse-chestnut (i.e. in absence of *C. ohridella*) within a radius of 2 km, but with otherwise similar characteristics (relative abundance of beech tree, altitude, soil type, percentage of forest cover, absence/presence of a stream) was selected at 2–5 km from the paired site.

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

Four different methods (one experiment and three observational studies) were used to measure and compare the total mortality, parasitism and predation rates and, parasitoid diversity of *O. fagi* between sites with and without horse-chestnut infested by *C. ohridella*. Only larval and pupal mortality was recorded. Egg mortality was not considered since *C. ohridella* is subject to little egg predation (Girardoz et al. 2007a) and has no recorded egg parasitoids (Grabenweger et al. 2007). Similarly, none of the two leaf miners is known to be attacked by parasitoids at the adult stage. Adult predation has never been studied but, since both species have a totally different adult life history, their predator complexes at the adult stage may be expected to be very different.

Method 1: Experimental exposure of F. sylvatica saplings

In mid-late April 2006 and 2008, as soon as beech bud burst began, about one hundred potted *F. sylvatica* saplings (ca. 70 ± 10 cm high) were placed in gauze cages (100 cm × 50 cm × 50 cm) and kept outdoors under natural conditions but protected from direct rain and sun. Each cage contained from five to six saplings. Adults of *O. fagi* were collected in a forest by beating beech branches and then placed in each cage in groups of 10–15 individuals 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 m of an infested horse-chestnut. In 2006, an average of 46 mines (32–59) on three to four saplings was exposed at each site. In 2008, on approximately eight saplings, an average of 180 mines (146–232) was exposed at each site. The saplings were checked every 4–6 days 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 either parasitism, predation or unknown cause. While parasitism was easily noticed, predation was more difficult to estimate. We considered that a leaf miner had been preyed upon when, either the mine was ripped, usually by birds, and the content had disappeared, or the larva was sucked through the leaf surface by an invertebrate predator or a host-feeding parasitoid, leaving a characteristic flat larval skin (Pullin 1985). This may have overestimated predation because all mines opened before maturity, with or without larval remains inside, were considered as preyed upon. Predation was assessed in 2008 only.

In 2006, the ten sites were chosen at random among our 30 pairs of beech tree sites (Péré et al. 2010b). All sites had beech within 50 m from the central point of the site. In contrast, in 2008, we selected 12 sites with no beech within a radius of 300 m from the central point of the site, because natural populations of *O. fagi* were very high that year in the investigated region and we suspected that the influence of neighbouring populations of *C. ohridella* on parasitism and predation of *O. fagi* would be more easily detectable on locally low weevil populations.

Method 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 five pairs of sites. These selected sites had contained abundant populations of *O. fagi* in the two previous years (Péré et al. 2010b). Five *O. fagi* populations existing sympatrically with *C. ohridella* populations (separation of less than 50 m from an infested horse-chestnut) were compared with five *O. fagi* populations inhabiting areas where *C. ohridella* was not present (separation greater than 2 km). At each site, 100 *O. fagi* leaf mines were randomly selected, soon after oviposition had started, i.e. in late-April. The mines of *O. fagi* were tagged and numbered using red tape attached approximately 10 cm away from the mine. A maximum of 30 mines per tree were selected in order to reduce the importance of individual trees within a site. Mines were revisited every 4–6 days, 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.

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

Method 3: Grab samples

In 2007, the grab sampling involved randomly collecting an extra 100 mined leaves from the five pairs of sites of method 2, at the end of the development period. Samples were also taken from an additional two pairs of sites. Mines were examined in the laboratory as for methods 1 and 2.

Method 4: Mass sampling

In late May 2006 and 2008, at the 30 pairs of beech tree sites used by Péré et al. (2010b), one thousand beech leaves per site were inspected for the presence of *O. fagi* mines. The surveyed leaves were haphazardly selected from beech branches at a maximum of 2 m from the ground within 50 m from the central point of the site. The leaves were surveyed from at least three different beech trees. The population density of *O. fagi* was very variable between sites, the number of leaves mined per 1,000 leaves counted varying from 0 to 154 in 2006 and 0 to 330 in 2008. On all leaves surveyed, only those mined by *O. fagi* were collected and inspected in the laboratory. Parasitism and total mortality rates of *O. fagi* were measured as in the other methods. This sampling method surely underestimated parasitism because, in contrast to the other three methods, 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 and total mortality rates measured in this sampling method were suitable for comparisons between sites with and without horse-chestnut trees.

Parasitoid identification

For each method, all parasitoid larvae, pupae and cocoons found in the mines of *O. fagi* were placed singly in a Petri dish stored in a humid container 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 (Boucek and Askew 1968; Grabenweger et al. 2003; Shaw and Huddleston 1991). The identity of the Chalcidoidea was confirmed by Hannes Bauer, Natural History Museum of Bern, Switzerland.

Analyses

To compare the total mortality, parasitism and predation rates of *O. fagi* in the presence and absence of *C. ohridella*, a Mann–Whitney *U*-test (two-tailed) was used since the "paired" sites were not sufficiently similar to consider them as related samples. For methods 2, 3 and 4 (2008), we also analysed separately parasitism by the five most common parasitoids of *C. ohridella* in the region, i.e. *M. frontalis*, *P. agraules*, *C. nephereus*, *C. trifasciatus* and *C. braconius* (Girardoz et al. 2006; C. Péré, unpublished data), based on emerged adult parasitoids. For method 1 (both years) and method 4 (2006), the proportion of parasitoids that died before emergence was too high to carry out such analysis. The diversity 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 and 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 methods 2, 3 and 4 (data from 2008 only), which had provided the highest number of parasitoid species. Data from methods 2 and 3, collected at the same sites in 2007, were combined. For the calculation of the index on data of method 4, only sites providing parasitoids were included in the analysis (i.e. 15 sites with *C. ohridella* and 22 sites without *C. ohridella*).

The data of method 4 performed in 2006 and 2008, which included the highest number of sites and individuals of *O. fagi*, were also analysed using a binary logistic regression. The dependant variable examined was parasitism (individual parasitized or not) and the predictors were the host density (number of mines per 1,000 leaves) of *O. fagi* at each site and the presence/absence of *C. ohridella*.

Results

Total mortality, parasitism and predation

The total mortality, overall parasitism, parasitism by parasitoids shared with *C. ohridella* and predation rates of *O. fagi* are presented for all four methods in Table 2. Parasitism was above 30% for methods 2 and 3, but lower than 20% in the two other methods. Predation varied between 14 and 33% and, total mortality between 46 and 91%. The highest total mortality was observed in method 2. Mortality, parasitism and predation rates were not significantly different in the presence and in the absence of horse-chestnut infested by *C. ohridella*, for any of the methods (Table 2). The binary logistic regression model predicting the parasitism rates of *O. fagi* revealed a significant positive association between parasitism and the population density of *O. fagi* and, between parasitism and the presence of *C. ohridella* in 2008, but not in 2006 (Table 3).

Table 2 Comparison of the proportion of total mortality, overall parasitism, parasitism by parasitoid species shared with *C. ohridella* and predation of *O. fagi*, in presence (Pres.) and absence (Abs.) of *C. ohridella*, for the four methods

Method	Year	Number of sites (sample count[a])		Mortality (%)				Overall parasitism (%)				Parasitism[b] (%) (parasitoid species shared)				Predation (%)			
		Pres.	Abs.	Pres.	Abs.	*U*	*P*	Pres.	Abs.	*U*	*P*	Pres.	Abs.	*U*	*P*	Pres.	Abs.	*U*	*P*
1-Exposure of saplings	2006	5 (212)	5 (244)	58.49	51.23	9	0.47	11.79	9.02	13	1.00								
1-Exposure of saplings	2008	6 (1079)	6 (1080)	61.44	58.61	18	1.00	14.09	17.22	13	0.42					16.22	14.17	9	0.15
2-Tagged samples	2007	5 (497)	5 (536)	85.53	91.42	12	0.91	30.78	33.40	9	0.46	16.30	17.35	10	0.60	21.13	25.19	11	0.75
3-Grab samples	2007	7 (704)	7 (697)	73.01	81.64	14	0.18	32.53	30.56	23	0.85	15.77	10.62	26	0.90	26.84	32.99	16	0.28
4-Mass sampling	2006	18 (162)	26 (811)	52.47	45.99	210	0.57	8.02	6.17	222	0.75								
4-Mass sampling	2008	25 (900)	28 (3484)	83.78	84.64	293	0.30	13.44	6.92	341	0.88	5.78	2.70	338	0.81				

[a] Sample count refers to the total number of individuals of *O. fagi* on which the percentages were calculated

[b] Parasitism of *O. fagi* by parasitoid species shared with *C. ohridella* is based on parasitoids reared to the adult stage only. Since, in all methods, some parasitoids died before adult emergence, the presented parasitism rates are underestimated

Table 3 Parameter estimates for the logistic regression model predicting the parasitism of *O. fagi* from the factors presence of *C. ohridella* and host density of *O. fagi*, using data from method 4 (mass sampling) in 2006 and 2008

Year	Parameter	*B*	SE	Wald	df	*P*	Exp (*B*)
2006	Host density of *O. fagi*	0.004	0.00	3.420	1	0.064	1.004
	Presence of *C. ohridella*	−0.102	0.71	0.021	1	0.885	0.903
	Host density of *O. fagi* × presence of *C. ohridella*	0.028	0.03	0.848	1	0.357	1.028
	Constant	−3.142	0.28	123.051	1	<0.001	0.043
2008	Host density of *O. fagi*	0.002	0.001	7.015	1	0.008	1.002
	Presence of *C. ohridella*	1.028	0.278	13.659	1	<0.001	2.796
	Host density of *O. fagi* × presence of *C. ohridella*	0.000	0.002	0.046	1	0.829	1.000
	Constant	−3.038	0.182	277.918	1	<0.001	0.048

Parasitoid complex

Overall, 14 parasitoid species belonging to three taxonomic families were reared from *O. fagi* (Table 4). More parasitoids were obtained from samples collected from natural populations (methods 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 *O. fagi*

A total of 14 parasitoid species was collected from *O. fagi* in the four methods. More parasitoid species were collected from natural populations of *O. fagi* than from mines on the potted *F. sylvativa* saplings that were exposed to parasitism in the field. The absence of *Eubazus minutus* and *Triaspis* sp. in the mines on saplings while these two parasitoids were commonly reared from mines of the natural populations can be explained by the fact that these two braconids are egg-larval parasitoids of beetles (Shaw and Huddleston 1991) and, although eggs were still present on saplings on the day of exposure, they may have been too old to attract parasitoid females, which probably prefer freshly laid eggs. The complete absence of *M. frontalis* in mines on saplings is less understandable, particularly since it was frequently found in the three other methods of sampling and it is the most abundant parasitoid of *C. ohridella* in Switzerland. Saplings were small and it is possible that *M. frontalis*, being a parasitoid of arboreal leaf-miners (Askew and Shaw 1974), does not forage for mines situated close to the ground.

For practical reasons, the four methods could not be carried out in the same year, which prevents a thorough comparison of the efficiency of the methods in assessing mortality

Table 4 Percentage contribution of each parasitoid species of *O. fagi* towards the parasitoid community recorded in the four methods, in presence (Pres.) and absence (Abs.) of *C. ohridella*

Superfamily Family: subfamily	Parasitoid species	1-Exposure of saplings				2-Tagged samples		3-Grab samples		4-Mass sampling				Total	
		2006		2008		2007		2007		2006		2008			
		Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.	Pres.	Abs.
Chalcidoidea															
Eulophidae: Entedoninae	***Chrysocharis nephereus***			2.25		15.69	18.97	24.26	28.50		3.23	21.60	23.90	15.80	17.99
	Closterocerus trifasciatus						1.72							0.00	0.39
	Pediobius saulius						0.86						0.53	0.00	0.39
Eulophidae: Eulophinae	***Pnigalio agraules***			7.87		0.98	1.72	1.48	1.14			6.82	0.53*	3.61	0.77
	Pnigalio soemius	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							0.74						0.23	0.00
	Cirrospilus diallus											2.27	2.65	0.45	0.97
	Cirrospilus pictus												0.53	0.00	0.19
Eulophidae: Tetrastichinae	***Minotetrastichus frontalis***					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*					0.98		0.74				16.00	14.81	3.61	5.42
Ichneumonoidea															
Braconidae: Brachistinae	*Eubazus minutus*					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***	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

In bold are the five main parasitoids of *C. ohridella* in Switzerland. In each method, significant differences in the proportion of each parasitoid species in presence and absence of *C. ohridella* are indicated by an asterisk (Chi-square test, $P < 0.05$)

factors. However, the tagged cohort samples and the grab samples (methods 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.

Apparent competition between *C. ohridella* and *O. fagi*

We had previously observed that *O. fagi* and other leaf miners were less abundant in the surroundings of horse-chestnut infested by *C. ohridella* than at control sites (Péré et al. 2010b). This observation suggested 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 the current study, however, we did not find much evidence for apparent competition between *C. ohridella* and the larval–pupal stages of *O. fagi*. Explanations for these unexpected results can be divided into two categories: (1) natural enemies of *C. ohridella* play a role in the observed difference in population densities of *O. fagi*, but their effect was not revealed in the present study for one or another reason; (2) horse-chestnut trees and *C. ohridella* affect populations of *O. fagi* through another mechanism.

It is possible that our observations and experiment failed to detect a true effect of natural enemies, for two reasons that may have occurred together or independently: a density-dependent host–parasitoid relationship and the exceptionally high population densities of *O. fagi* during the study. Woodcock and 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 suggests a positive relationship between total parasitism rates and host population size. Since populations of *O. fagi* were lower at sites with *C. ohridella* compared to control sites, a positive effect of the presence of *C. ohridella* on the parasitism of *O. fagi* may have been masked by the density-dependent relationship. As a matter of fact, when population density was included as a factor in the logistic regression analysis, this latter revealed a significant effect of the presence of *C. ohridella* on parasitism of *O. fagi*, at least in 2008.

Another explanation is the unfortunate timing of the experiments and observations. These were all made between 2006 and 2008, a period when populations of *O. fagi* were exceptionally high throughout the investigated region. Populations of *O. fagi* are known to fluctuate dramatically from very low densities to outbreak situations (Bale 1984; Day and Watt 1989). It cannot be ruled out that the increased parasitism and predation in presence of *C. ohridella* can be measured only at low *O. fagi* densities. The presence of parasitoids and predators of the invasive moth may locally prevent the increase of *O. fagi* populations when these are low, but local variations in densities of *O. fagi* between sites with and without *C. ohridella* may remain for some years when *O. fagi* populations increase. This could explain why Péré et al. (2010b) found significantly lower densities of *O. fagi* populations in the presence of horse-chestnut infested by *C. ohridella* in a year of low weevil density (2005) and still observed similar differences in 2006 and 2008, when the density of *O. fagi* had increased 20-fold. 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 and predators probably has very little influence on parasitism and predation of the large number of *O. fagi* in the region. To verify this hypothesis, saplings artificially infested with *O. fagi* should be exposed, preferably at different leaf miner densities, when natural populations are in the latency phase. Alternatively, infestations could be created in the field using adults exposed in sleeved cages or field cages. However, it can also be argued that, had apparent competition occurred at low *O. fagi* density, one might have expected that the difference in *O. fagi* densities with the presence and absence of infested horse-chestnut would have become smaller during the general increase in *O. fagi* density, which was not the case (Péré et al. 2010b).

It is also possible that apparent competition does not occur between the two leaf miners and that the presence of *C. ohridella* or horse-chestnut affects *O. fagi* populations through another mechanism. One reason why apparent competition may not occur between the two leaf miners is the, possibly underestimated, local dispersal behaviour of the parasitoids (Antolin and Strong 1987). Parasitoids emerging from *C. ohridella* may quickly spread far from the investigated beech trees before starting to oviposit on *O. fagi* or other leaf miners. The fact that the two parasitoid complexes do not together constitute a closed system but that most of their parasitoids are shared with many other leaf miners is clearly an issue in this study. Another issue is the possible 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 and Luff 1978). Thus, it is also possible that horse-chestnut repels *O. fagi* adults, or that adult weevils are less attracted to an area with mature horse-chestnut than when mature trees are all suitable trees for adult feeding. 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 2 or 3 weeks earlier than *C. ohridella*. The impact of volatiles from horse-chestnut on populations of *O. fagi* should be tested using uninfested horse-chestnut trees as control. Unfortunately, in most of Europe all horse-chestnut trees situated in natural or semi-natural environments, i.e. where both leaf miners may co-occur, are permanently infested by the invasive leaf miner. Maintaining horse-chestnut trees free from *C. ohridella* during several years would require the use of insecticides, which is likely to affect insect populations in general.

Although we did not detect an indirect effect between *C. ohridella* and *O. fagi* via the sharing of natural enemies, the fact that population densities of *O. fagi* are lower in the vicinity of horse-chestnuts infested by *C. ohridella* has been clearly shown. Further investigations should consider both the effect of shared natural enemies at low *O. fagi* densities and the effect of horse-chestnut itself, through volatiles or other antagonistic interactions. More generally, we believe that invasive leaf miners provide good models to understand the ecological effects of invaders and in particular, to assess apparent competition between invasive and native species because leaf miners are often parasitized by the same polyphagous parasitoids, and parasitism in leaf miners is easily monitored. Péré et al. (2010b) identified other native leaf miner species that appear to be affected by the presence of horse-chestnut infested by *C. ohridella*, for example *Orchestes quercus* and *Chromatomyia periclymeni* in France or several Lepidoptera in Bulgaria. Apparent competition could be investigated on these species as well.

Acknowledgments We thank Wade Jenner, Alain Roques, Sven Bacher, Mark Shaw and anonymous reviewers for their useful comments on a previous version of this manuscript. We also thank Julia Bilat, Damien Vielle and Carolin Weser for their help in field work, Hannes Baur for the identification of chalcid parasitoids and Urs Schaffner for help in statistical analyses. R. Bell would like to thank Brent Emerson for his support during his M.Sc. He was supported by a studentship from the UK National Environment Research Council. This project was funded by the EU FP6 project ALARM (Assessing LArge scale environmental Risks for biodiversity with tested Methods, GOCE-CT-2003-506675) and grants from the Loterie Romande and the University of Neuchâtel.

References

Antolin MF, Strong DR (1987) Long distance dispersal by a parasitoid (*Anagrus delicatus*, Mymaridae) and its host. Oecologia 73:288–292

Askew RR, Shaw MR (1974) An account of the Chalcidoidea (Hymenoptera) parasitising leaf-mining insects of deciduous trees in Britain. Biol J Linn Soc 6(4):289–335

Bale JS (1981) Seasonal distribution and migratory behaviour of beech leaf mining weevil, *Rhynchaenus fagi* L. Ecol Entomol 6:109–118

Bale JS (1984) Bud burst and success of the beech weevil, *Rhynchaenus fagi*: feeding and oviposition. Ecol Entomol 9:139–148

Bale JS, Luff ML (1978) The food plants and feeding preferences of the beech leaf mining weevil, *Rhynchaenus fagi* L. Ecol Entomol 3:245–249

Bernardo U, Monti MM, Nappo AG et al (2008) Species status of two populations of *Pnigalio soemius* (Hymenoptera: Eulophidae) reared from two different hosts: an integrative approach. Biol Control 46:293–303

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

Carvalheiro LG, Buckley YM, Ventim R et al (2008) Apparent competition can compromise the safety of highly specific biocontrol agents. Ecol Lett 11:690–700

Day KR, Watt AD (1989) Population studies of the beech leaf mining weevil (*Rhynchaenus fagi*) in Ireland and Scotland. Ecol Entomol 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. J Pest Sci 75:152–157

Girardoz S, Kenis M, Quicke DLJ (2006) Recruitment of native parasitoids by an exotic leaf miner, *Cameraria ohridella*: host-parasitoid synchronisation and influence of the environment. Agric For Entomol 8:49–56

Girardoz S, Quicke DLJ, Kenis M (2007a) Factors favouring the development and maintenance of outbreaks in an invasive leaf miner *Cameraria ohridella* (Lepidoptera: Gracillariidae): a life table study. Agric For Entomol 9:141–158

Girardoz S, Tomov R, Eschen R et al (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. Bull Entomol Res 97:445–453

Girardoz S, Volter L, Tomov R et al (2007c) Variations in parasitism in sympatric populations of three invasive leaf miners. J Appl Entomol 131:603–612

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

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, Avtzis N, Girardoz S et al (2005) Parasitism of *Cameraria ohridella* (Lepidoptera, Gracillariidae) in natural and artificial horse-chestnut stands in the Balkans. Agric For Entomol 7:291–296

Grabenweger G, Hopp H, Jackel B et al (2007) Impact of poor host-parasitoid synchronisation on the parasitism of *Cameraria ohridella* (Lepidoptera: Gracillariidae). Eur J Entomol 104:153–158

Grabenweger G, Kehrli P, Zweimüller I et al (2010) Temporal and spatial variations in the parasitoid complex of the horse chestnut leafminer during its invasion of Europe. Biol Invasion 12(8):2797–2813

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

Hespenheide HA (1991) Bionomics of leaf mining insects. Annu Rev Entomol 36:535–560

Holt RD (1977) Predation, apparent competition, and the structure of prey communities. Theor Popul Biol 12(2):197–229

Holt RD, Kotler BP (1987) Short-term apparent competition. Am Nat 130(3):412–430

Kenis M, Auger-Rozenberg MA, Roques A et al (2009) Ecological effects of invasive alien insects. Biol Invasions 11:21–45

Morris RJ, Müller CB, Godfray HCJ (2001) Field experiments testing for apparent competition between primary parasitoids mediated by secondary parasitoids. J Anim Ecol 70:301–309

Morris RJ, Lewis OT, Godfray HCJ (2004) Experimental evidence for apparent competition in a tropical forest food web. Nature 428:310–313

Mouillot D, Lepretre A (1999) A comparison of species diversity estimators. Res Popul Ecol 41:203–215

Müller CB, Godfray HCJ (1997) Apparent competition between two aphid species. J Anim Ecol 66:57–64

Nielsen BO (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 JS (2002) Interactive catalogue of world Chalcidoidea 2001. Taxapad CD, Vancouver

Péré C, Augustin S, Turlings TCJ et al (2010a) The invasive alien leaf miner, *Cameraria ohridella* and the native maple, *Acer pseudoplatanus*: a fatal attraction? Agric For Entomol 12:151–159

Péré C, Augustin S, Tomov R et al (2010b) Species richness and abundance of native leaf miners are affected by the presence of the invasive horse-chestnut leaf miner. Biol Invasions 12:1011–1021

Prenter J, MacNeil C, Dick JTA et al (2004) Roles of parasites in animal invasions. Trends Ecol Evol 19:385–390

Pullin AS (1985) A simple life table study based on development and mortality in the beech weevil, *Rynchaenus fagi* L. J Biol Educ 19:152–156

Settle WH, Wilson TL (1990) Invasion by the variegated leafhopper and biotic interactions: parasitism, competition, and apparent competition. Ecology 71:1461–1470

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

Tompkins DM, Draycott RAH, Hudson PJ (2000) Field evidence for apparent competition mediated via the shared parasites of two gamebird species. Ecol Lett 3:10–14

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

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

Valade R, Kenis M, Hernandez-Lopez A et al (2009) Mitochondrial and microsatellite DNA markers reveal a Balkanic origin for the highly invasive horse-chestnut leaf miner *Cameraria ohridella* (Lepidoptera, Gracillariidae). Mol Ecol 18:3458–3470

Van Nouhuys S, Hanski I (2000) Apparent competition between parasitoids mediated by a shared hyperparasitoid. Ecol Lett 3:82–84

Woodcock BA, Vanbergen AJ (2008) Parasitism of the beech leaf-miner weevil in a woodland: patch size, edge effects and parasitoid species identity. Insect Conserv Divers 1:180–188

Yu D, Van Achterberg C, Horstmann K (2005) World Ichneumonoidea 2004. Taxonomy, biology, morphology and distribution. Taxapad CD, Vancouver