

Native parasitoids and their potential to control the invasive leafminer, *Cameraria ohridella* DESCH. & DIM. (Lep.: Gracillariidae)

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Abstract

In spite of the fact that since the end of the eighties, the horse chestnut leafminer, *Cameraria ohridella*, has established itself throughout Europe, native predators such as ants and birds are not attuned to this neozoic species. In contrast, several parasitic wasp species already started to exploit the invasive horse chestnut leafminer, but until now parasitisation rates are quite low, mainly because of asynchrony in the lifecycles of parasitoids and host. Only the removal of leaf litter, in which pupae hibernate, is at the moment a strategy to reduce the infestation level in the next year. Unfortunately, not only hibernating horse chestnut leafminers but also parasitoids are removed, and important resources for biocontrol are unused. In the current study, we investigated the potential efficiency of the horse chestnut leafminer parasitoid complex extracted from leaf litter in defined environments. Parasitoids were released at different densities to investigate density dependence in parasitisation rates.

Although seven different species were released in our experiments, only *Pnigalio agraulis* turned out to be responsible for biocontrol of *C. ohridella*. We recorded parasitisation rates of up to 35%. Overall, parasitisation rates were independent of the leafminer density but increased fourfold if ten times more parasitoid individuals were released. Unfortunately, none of the parasitoid species could be established in the experimental units in the long run. Results are compared to other parasitoid-leafminer systems, and promotion of horse chestnut leafminer parasitoids to support natural selection and biological control of the horse chestnut leafminer is discussed.

Keywords: *Cameraria ohridella*, horse chestnut leafminer, biological control, parasitoid complex, Eulophidae, *Pnigalio agraulis*, *Aesculus hippocastanum*

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Introduction

The horse chestnut leafminer, *Cameraria ohridella* Deschka & Dimic (Lepidoptera: Gracillariidae), was first found in Macedonia in 1984 (Deschka & Dimić, 1986) and has now established itself as a part of almost all of Central European fauna (Butin & Führer, 1994; Heitland *et al.*, 1999; Kindl *et al.*, 2002). Although the trees are not severely damaged (Salleo

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et al., 2003), the infestation of horse chestnut trees creates an undesirable image for the public. This is due to leaf wilting and the early loss of leaves in summer as a result of high infestation rates. The infestation situation has developed heterogeneously in recent years and varied strongly between years and locations. The problem will continue in the future (Heitland *et al.*, 2003).

Although the horse chestnut leafminer can be found everywhere in Europe since the end of the nineties, native predators such as ants (Radeghieri, 2004) and birds (Kehrli & Bacher, 2002; Grabenweger *et al.*, 2005b) already exploit *C. ohridella* but adapt slowly to this neozotic species. However, the most important natural enemies of leafminers are parasitic Hymenoptera (Askew & Shaw, 1974). The composition of the parasitoid complex of *C. ohridella* has been examined in several studies (Grabenweger & Lethmayer, 1999; Hellrigl, 2001; Freise *et al.*, 2002). At the moment, 37 species of ecto- and endoparasitoids are known to exploit *C. ohridella* larvae as hosts (Grabenweger, 2003a). The dominant parasitoids are often the same in different regions and the species composition is quite typical for a leafminer in Europe (Grabenweger, 2003a). However, until now parasitisation rates are generally low (Grabenweger & Lethmayer, 1999), ranging mostly from 0.5 to 5.0% (Freise & Heitland, 2003). The low parasitisation rates can be partly explained by the asynchrony in the life cycles of native parasitoid species and the horse chestnut leafminer (Grabenweger, 2004). Most parasitoids that have overwintered in horse chestnut leaf litter emerge before the host, i.e. moth of the horse chestnut leafminers (Grabenweger, 2004). Consequently, it is likely that most adult parasitoids are dead before suitable host larval stages are available on the horse chestnut trees.

In contrast, the parasitoid complex of other native leafminer species is adapted to the life cycle of its host species and parasitisation rates of 50% or more have been frequently recorded. These high parasitisation rates limit the population development of the leafminers and prevent them from reaching pest status (Askew & Shaw, 1979; Maier, 1984; Mey, 1993). Since it is likely that the adaptation process of native parasitoid species to the horse chestnut leafminer will take decades, augmentative biological control methods are of major interest. Two major problems have to be solved to facilitate an inundative release of parasitoids. First, simple biotechnological methods have to be developed for the parasitoid extraction and the retention of leafminers from horse chestnut leaf litter. Second, the parasitoids have to be released at a time when hosts suitable for parasitisation are present. The first investigations to solve these problems have been done by a Swiss working group (Kehrli, 2004; Kehrli *et al.*, 2005). They collected leaf litter and stored it in a mass rearing device. This device consists of a container filled with leaf litter and openings covered with gauze with a defined mesh size to allow smaller parasitoid species to pass and retain the larger moth individuals. Moreover, to optimise the release schedule, devices were stored in cold storage houses. Overall, the results were promising, but due to several problems only a minor impact on the horse chestnut leafminer could be detected in the field. Although this technique is a good starting point, further development is necessary to meet concerns about applicability in urban greens, where huge amounts of infested horse chestnut leaves are collected each year.

The aims of our greenhouse and field experiments were to quantify the potential efficiency of the parasitoid complex

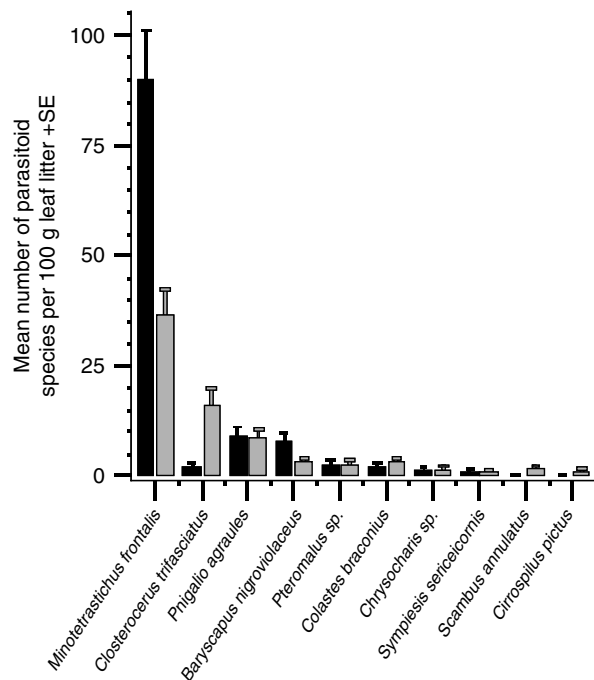


Fig. 1. Parasitoid complex emerging from 100 g of horse chestnut leaf litter infested with the horse chestnut leafminer *C. ohridella*. Leaf litter was collected in the city of Braunschweig in 2004; $n = 10$ (■, female; □, male).

emerging from leaf litter of the previous year under controlled conditions. Therefore, parasitoid species were released at different densities to investigate (i) their contribution to biological control, (ii) the impact of parasitoids on leafminer population development, and (iii) the establishment of species by a single release at a time when suitable host developmental stages are present on trees.

Material and methods

Insect breeding

Since *C. ohridella* and all parasitoid species overwinter inside the mine, i.e. in the fallen horse chestnut leaf litter, all insects used in the experiments were reared from leaf litter collected in the city of Braunschweig in autumn 2004. Leaf litter was stored in a cold room until experimental use. Prior to the experiments, the number of insects emerging from 100 g of dry leaf litter was determined with photoelectors in a climatic chamber (22°C, 80% RH, 16:8 L:D). The results show that approximately 250 *C. ohridella* adults and 50 parasitoid individuals emerged from 100 g of dry leaf litter. The parasitoid complex was dominated by the species *Minotetrastichus frontalis*, *Closterocerus trifasciatus*, and *Pnigallo agraulis* (fig. 1). Since leaf litter quality changes during cold storage (unpublished data), the exact parasitoid density and species composition was monitored again under experimental conditions.

Greenhouse experiments

The effect of host density on parasitisation rates was tested in the greenhouse in 32 gauze tents (1.8 × 2.0 m; 2 m high)

covering single 1–2-m-high potted six-year-old white-flowering horse chestnut trees (*Aesculus hippocastanum*) infested with *C. ohridella* larvae. Four different treatments, each replicated eight times, were tested: (i) few parasitoids – low leafminer density; (ii) few parasitoids – high leafminer density; (iii) many parasitoids – low leafminer density; and (iv) many parasitoids – high leafminer density.

To obtain the different leafminer infestation levels, 10 g (i.e. 25 adult moth (low leafminer density)) and 50 g (i.e. 125 adult moth (high leafminer density)) of leaf litter was used to infest 4–5 horse chestnut trees. At the presence of the first visible mines on the leaves, single trees were placed in the gauze tents for different treatments. Horse chestnut leaf litter was also used to introduce the parasitoid species complex. Therefore, 35 g of leaf litter (approx. 17.5 parasitoid individuals) were introduced in the few, and 350 g of leaf litter (approx. 175 parasitoid individuals) were introduced in the many parasitoids treatments. The leaf litter was stored in a climate chamber (22°C, 80% RH, 16:8 L:D) for six days prior to its introduction in the experimental units in order to accelerate emergence of parasitoids.

The emergence pattern of parasitoids from the leaf litter and species composition was monitored with three photoelectors in the same greenhouse, each containing 25 g of leaf litter. The emerging parasitoids were collected and determined every second day. The main emergence period of the parasitoids was ten days after the introduction of leaf litter in the experimental units. At that time, the age distribution and natural mortality of horse chestnut larvae was quantified in two additional gauze tents, which contained either three horse chestnut trees of the low or high leafminer density treatments.

All experimental units were randomly distributed in two greenhouses of 10 × 24 m in size. During the experiment, temperature in both greenhouses was monitored with data loggers. The experiment was terminated 22 days after the introduction of leaf litter containing parasitoids and all leaves were collected. At this time, we expected that parasitized leafminers would be easy to detect and that the chances for successful breeding would be high. To estimate parasitisation rates, samples of six infested leaves, i.e. two with low, intermediate and high mine density, were taken from three replicates per treatment and dissected. Upon dissection, the number of living and dead *C. ohridella* larvae, as well as pupal or larval stages of parasitoids were counted and the parasitisation rate (parasitized hosts × 100) per (hosts alive + hosts parasitized + hosts dead) was calculated (Freise, 2001). Dead larvae were taken into account, because the dissection period lasted three weeks. Superparasitism was counted as a single parasitisation event. Parasitoid larvae, pupae and *C. ohridella* larvae containing endoparasitoids were transferred into small plastic vials equipped with a piece of moist filter paper. All vials were checked on a daily basis for emerging parasitoids. Adult parasitoids were identified and counted.

Field experiments

Experiments took place at three different study sites on the terrain of the Federal Biological Research Centre for Agriculture and Forestry in Braunschweig, Hötzum and Essehof, Germany. At each site several planted groups of five eight-year-old white-flowering horse chestnut trees (*Aesculus hippocastanum*, 1.9 ± 0.2 m high) were enclosed by

a 3-m-high gauze tent covering a base area of 2.0 m × 1.8 m. To protect the trees from heavy rainfall, the roof of all tents was build of transparent plastic tarpaulin. All trees were attached to a watering system. On all study sites, abiotic conditions, such as wind speed and air temperature, were monitored.

To create the same initial number of mines in all experimental units, 8 g of leaf litter, i.e. 20 adult moths, were introduced into each of the 22 gauze tents. Approximately four weeks after the introduction of leaf litter with *C. ohridella* pupae, mines were counted on all leaves. The mine density in each experimental unit, i.e. on five trees, was 693.0 (±49.7 SE). Parasitisation rates and impact of parasitoid species on horse chestnut leafminer population development was estimated in three treatments, i.e. control without parasitoid release, low and high parasitoid release density. All treatments were equally distributed among the different study sites.

Parasitoids were extracted from dry leaf litter in a climate chamber (22°C, 80% RH, 16:8, L:D). Therefore, photoelectors were prepared with either 28 g (low parasitoid density, approx. 14 individuals) or 280 g (high parasitoid density, approx. 140 individuals) of leaf litter. Emerging parasitoids were collected every second day and immediately released in the corresponding treatments. The first parasitoids were released on July the 14th, at a time when late larval developmental stages of *C. ohridella* were already present.

To monitor the temporal pattern of parasitoid emergence under field conditions, additional photoelectors were prepared with 28 g ($n=3$) and 280 g ($n=3$) of leaf litter. Emerging parasitoids were collected 1–2 days and stored in a freezer for later identification and counting of individuals.

In all treatments and replicates, mine densities and parasitisation rates were estimated two times. At the presence of the first larval generation of the horse chestnut leafminer, the number of mines was counted in all experimental units. To determine the parasitisation rate of the first moth generation and the species composition of the parasitoid complex, 10% of all mines equally distributed over five trees in each experimental unit were cut out of the horse chestnut leaves. Single mines were then transferred into small plastic vials, provided with moist filter paper and stored at 22°C and 80% RH in climatic chambers until parasitoid or moth emergence.

At the end of August, i.e. four weeks after the emergence of the first moths generation, the mine density was assessed a second time. Therefore, all mines on each of the five trees per experimental unit were counted. Finally, parasitisation rates of the second moth generation were determined at mid-September by dissecting approximately 100 mines equally distributed over the leaves of the five trees in each experimental unit.

Statistical data analysis

Percentages were arcsine-transformed prior to calculations. Normality of data distribution was verified by a Kolmogorov-Smirnov test. Heterogeneity of variance was tested by a Levene test. Parasitisation rates of the different treatments were compared by a univariate analysis of variance. For data sets with non normal distributions, the Kruskal-Wallis H-test was chosen. For pairwise comparisons, we used Mann-Whitney *U* tests or *t*-tests.

Table 1. Summarised univariate analysis of variance of parasitisation rates at two different parasitoid densities (few, many) and two different leafminer host densities (low, high).

Source of variation	df	Mean square	F-value	P-value
Corrected model	3	596.63	2.704	0.116
Intercept	1	5964.27	27.04	0.001
Leafminer density	1	0.251	0.001	0.974
Parasitoid density	1	1782.52	8.08	0.022
Leafminer density × parasitoid density	1	7.113	0.032	0.862
Error	8	220.61		

Dependent variable was parasitisation rate per experimental unit, i.e. a single horse chestnut tree ($n=3$).

Results

Greenhouse experiment: effects of host density on parasitisation rates

Mean daily temperature in both greenhouses during the experiments was approximately 17°C (1. greenhouse: 17.1°C ± 0.2 SE; 2. greenhouse: 17.3°C ± 0.2 SE). As expected, nine different parasitoid species emerged from the leaf litter introduced into the experimental units. Nevertheless, monitoring of emerging parasitoid species revealed that slightly more parasitoid individuals (86.66 ± 5.99 per 100 g leaf litter) as expected emerged. *Minotetrastichus frontalis* appeared to be the most abundant species with a mean number of 62.8 (±6.4 SE) individuals per 100 g leaf litter. As the second and third most abundant species, *Closterocerus trifasciatus* (10.8 ± 4.8 SE individuals) and *Pnigalio agraulis* (5.2 ± 2.4 SE individuals) were identified. All other species occurred irregularly at very low densities (0.8–2 individuals per 100 g). The emerging period for *P. agraulis* lasted from day 3–6, while *M. frontalis* and *C. trifasciatus* emerged from day 6–13 after introduction into the experimental units.

At the time of the main parasitoid emergence from the leaf litter, 96.8% (±1.3 SE) of *C. ohridella* larvae had reached late larval developmental stages or had pupated (29.7% ± 0.1 SE). Only 3.0% (±1.3 SE) of the individuals were L3 and 0.1% (±0.1 SE) were L2 larvae. Natural mortality of *C. ohridella* larvae was 1.6% (±1.6 SE) on trees with low and 4.9% (±2.4 SE) on trees with high leafminer density.

At an average density of 379 (±43.5 SE) mines per tree, the introduction of 30.33 (±2.10 SE) parasitoids resulted in a parasitisation rate of 7.1% (±7.1 SE). Leaving the parasitoid density constant, a five-fold increase in the host density (1951 ± 186.1 SE) did not influence the parasitisation rate (9.9% ± 9.9 SE). In contrast, four-fold higher parasitisation rates (34.3% ± 10.9 SE) were recorded if ten times more parasitoids (i.e. 300.66 ± 20.97 SE) were released at a host density of 379 mines per tree. With an increasing host density, the parasitisation rate remained nearly constant at 31.9% (±9.8 SE). Analysis of variance showed a significant influence of parasitoid densities (few and many) on the parasitisation rate (table 1), whereas parasitisation rates were not affected by numbers of *C. ohridella* larvae, i.e. 379 and 1951 mines, on the experimental trees (table 1, fig. 2).

Emerging parasitoids were counted and identified to estimate their contribution to the total future parasitisation rate. Not all species could be identified because of fungal infections or diapausing pupae. Nevertheless, 98.94% of the emerging species from parasitoid larvae of dissected

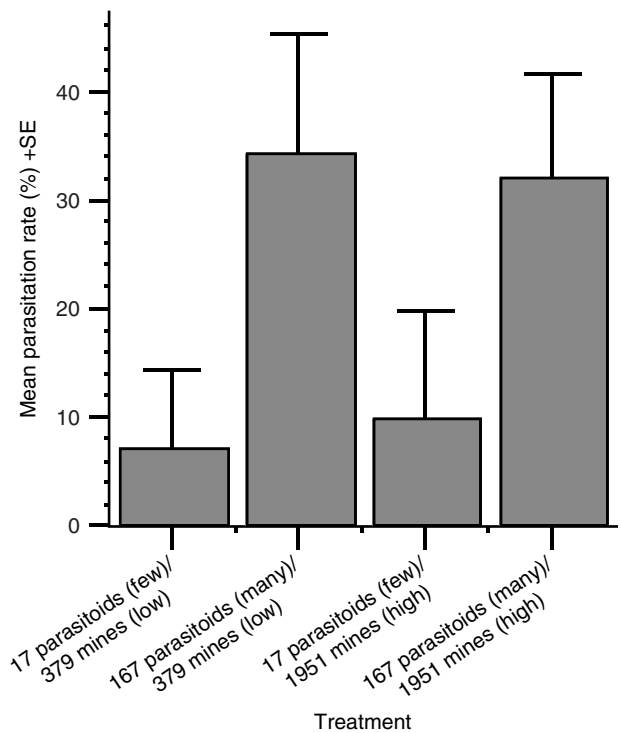


Fig. 2. Mean parasitisation rate in the different treatments in the greenhouse experiment. Parasitisation rates were calculated on the basis of all *C. ohridella* larvae (dead and alive) recorded during dissection of six leaves per tree. See table 1 for significant difference ($n=3$).

leaves, and 98.59% of the emerging species from mined horse chestnut leaves collected at the end of the experiment were identified as the species *P. agraulis*. The remaining species were *M. frontalis* (1.06 and 1.06%), *Closterocerus trifasciatus* (0.001 and 0.21%) and *Colastes braconius* (0.001 and 0.14%).

Semi-field experiment: impact of parasitoids on leafminer population development

Similar to the greenhouse experiment, the mean daily temperature was approximately 17°C at all experimental sites (Braunschweig: 17.9°C ± 0.3 SE; Hötzum: 17.3°C ± 0.4 SE; Essehof: 17.14°C ± 0.4 SE).

Seven days after exposure of the leaf litter in the photoelector, the first parasitoids emerged. From then onwards, emerging individuals were released every second day in the experimental units. At that time, 61% (±2.4 SE) of the leafminer population were in the fourth larval developmental stage. The rest of the population was in the L3- (34.1% ± 1.3 SE) and L2-stage (4.2% ± 1.9 SE).

The monitoring of parasitoid emergence in the field revealed that parasitoid density in the leaf litter was lower than expected. On average 110 (±1.51 SE) parasitoids emerged from 280 g and 12 (±1.53 SE) from 28 g of leaf litter and were introduced in the experimental units in the field. Nevertheless, the released parasitoid complex was composed of eight different species. Similar to the greenhouse experiment, *M. frontalis* (67.8%), *P. agraulis*

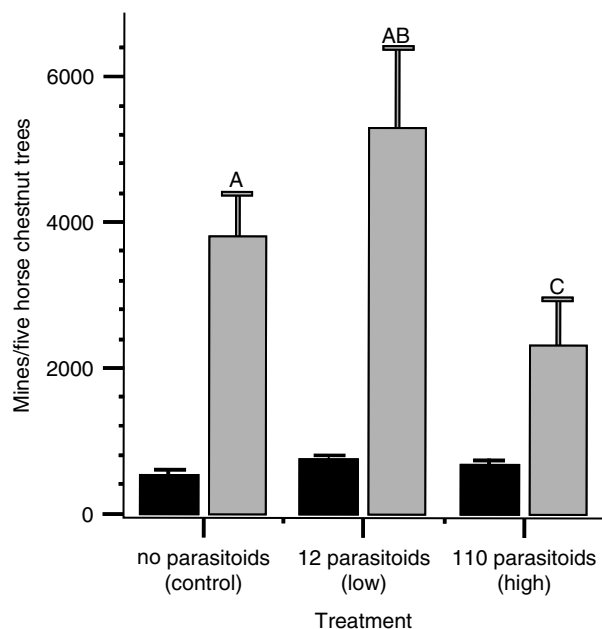


Fig. 3. Mean number of mines per experimental unit, i.e. five horse chestnut trees, during the first and second moth generation for the different treatments in the semi-field experiment. Different letters indicate significant difference among treatments for the second moth generation (U test, $p < 0.05$, $n = 5$) (■, first leafminer generation; ▒, second leafminer generation).

(12.3%), and *C. trifasciatus* (7.38%) were the most abundant species.

Parasitization rate of the first moth generation

On average 693 (± 49.7 SE) mines were distributed on the leaves of the five horse chestnut trees in each of the experimental units (fig. 3). The introduction of 12 parasitoids, i.e. at a parasitoid to mine ratio of 1:57.75, resulted in a mean parasitization rate of 1% (± 0.4 SE). With a ten-fold increase in released parasitoids, i.e. a parasitoid to mine ratio of 1:6.3, the mean parasitization rate increased significantly to 11% (± 3.8 SE). In experimental units without parasitoid release, none of the horse chestnut leafminers were parasitized (fig. 4).

The breeding success of the total number of parasitoid individuals isolated by dissections was 68%. Although it was not possible to rear the remaining 32% of the isolated parasitoid to adulthood, we assume that most of them belong to the species *Pnigalio agraulis* because of similar larval morphology. *P. agraulis* was identified as the dominant species parasitizing the first leafminer generation in the low parasitoid (66.7% \pm 33.3 SE), as well as in the high parasitoid density treatment (92.4% \pm 5.1 SE). Only three other parasitoid species, *Cirrospilus viticola*, *Closterocerus trifasciatus* and *Pteromalus* sp., were identified. In total, 77% of the *P. agraulis* individuals found in all treatments emerged from leafminer larvae and the remaining 23% from pupae.

Development of the leafminer population and parasitization rates

The leafminer density increased in the control treatment, i.e. without introduction of parasitoids, from 693 (± 49.7 SE)

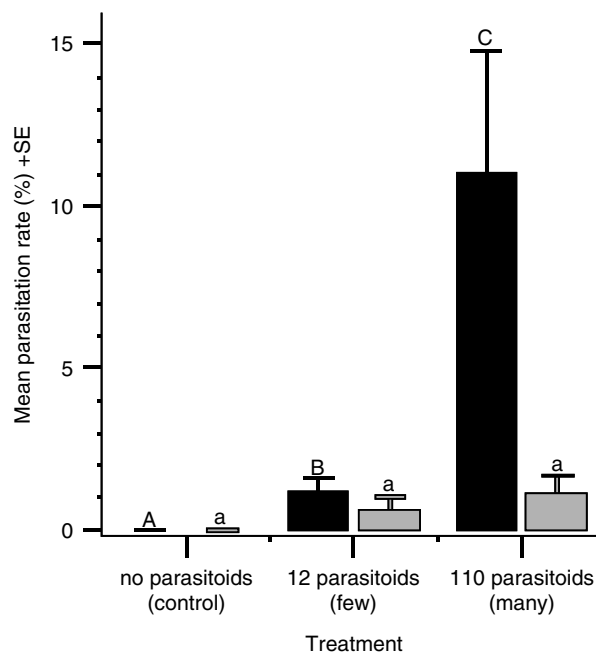


Fig. 4. Mean parasitization rates for the first and second moth generation in the different treatments of the semi-field experiment. Parasitization rates were calculated on the basis of all *C. ohridella* larvae (dead and alive) found during dissection of 10% of all mines (first generation) or 100 mines (second generation) per experimental unit, i.e. five horse chestnut trees. Different letters indicate significant difference within each leafminer generation (U test, $p < 0.05$, $n = 9$) (■, first leafminer generation; ▒, second leafminer generation).

to an average of 3789 (± 593.2 SE) mines per experimental unit in the second leafminer generation (fig. 3). The leafminer population development was similar to the control if 12 parasitoids were released in the experimental units. In contrast, the introduction of 110 parasitoids resulted in a leafminer density in the second generation that was significantly lower compared to the control and the introduction of 12 parasitoids (H-test, Chi-square = 9.3, $df = 2$, $p = 0.010$). Compared to the control, the average mine density was reduced by 39% (± 17.1 SE), i.e. 2309 (± 652.4 SE) mines per experimental unit (fig. 3).

Most of the leafminers were at the L3- (39% \pm 2.6 SE) and L4- (30% \pm 3.7 SE) developmental stages at the time of the parasitization rate estimation. The parasitization rates of the second leafminer generation were, in general, low and without significant differences among the treatments (fig. 4) (ANOVA, $F = 2.459$, $df = 2$, $p = 0.107$). In treatments with an initial release of 12 parasitoids, the parasitization rate recorded for the second leafminer generation was similar to the first leafminer generation (fig. 4) (t -test, $t = 1.213$, $df = 16$, $p = 0.243$). In contrast, in the treatment with an initial release of 110 parasitoids, the parasitization rate decreased ten-fold compared to parasitization rates achieved for the first leafminer generation (fig. 4) (t -test, $t = 2.753$, $df = 16$, $p = 0.014$). We successfully reared 6% of the total amount of parasitoid individuals isolated by dissections in this generation. *P. agraulis* was the only species. It was not possible to rear the remaining 94% of the isolated parasitoids to adulthood. Because of similar larval morphology, it

is likely that most of them also belonged to the species *P. agraulis*.

Discussion

In their natural habitats, phytophagous insects, especially leafminers, are effectively controlled by parasitoids. In many cases, the parasitisation rates exceed a level of 50% (Askew & Shaw, 1979; Mey, 1993). If leafminers invade new host plants in habitats far away from their place of origin, they are most likely able to build up huge population densities because a specialized parasitoid complex is lacking (Cornell & Hawkins, 1993). Different examples show that, after a certain time, parasitoids in the new invaded habitat also exploit the new host species, but mostly play only a limited role in the regulation of the population dynamics of the invader (Stojanović & Marković, 2005; Vercher *et al.*, 2005). Only if specialised parasitoid species follow the invader, as was observed, for example, for *Phyllonorycter platani* in England (Godfray *et al.*, 1995), herbivore population densities are under natural control within a short period of time. If natural enemies do not follow the invader, the introduction of new species of parasitoids from the place of origin of the leafminer is often suggested in classical biological control programs. For example, Garcia-Marí *et al.* (2004) were able to show that the establishment of the eulophid parasitoid *Citrostichus phyllocnistoides* between 1996 and 1999 as a natural enemy of the citrus leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in Spain was successful. In 2000 and 2001, the parasitoid expanded and became the most abundant species in all the citrus orchards and the mean percentage of parasitism increased from 20–25% to nearly 60%.

The invasion of Europe by the horse chestnut leafminer started in 1984 from Macedonia (Deschka & Dimić, 1986), and today the leafminer is an inherent part of the European fauna (Heitland *et al.*, 1999). Classical biological control, i.e. the introduction of natural enemies of the horse chestnut leafminer, was discussed years ago (Kenis, 1997), but until now the area of origin of the leafminer remains unclear. None of the possible options could be completely discarded; but, most likely, *C. ohridella* originated from another host genus in a non-European region (Kenis *et al.*, 2004; Kenis *et al.*, 2005). For this reason, the biocontrol potential and adaptation process of the native parasitoid complex is of major interest from an economic (i.e. to tree nurseries, local communities responsible for leaf litter removal), as well as from an ecological (population dynamics, adaptation of natural enemies) point of view.

Currently, only the removal and decomposition of horse chestnut leaf litter, which contains the overwintering leafminer, can be recommended to reduce horse chestnut leafminer densities in public and private greens. Although this method is quite successful, it affects not only the leafminer density but also the adaptation process of native parasitoids in a dramatic way, since not only horse chestnut leafminer but also the natural enemies, i.e. hymenopteran parasitoids, overwinter in the leaf litter. At the moment, the impact of native parasitoid species on horse chestnut leafminer population development is small, but it is only a matter of time until parasitoids are better adapted and start to limit the population growth of *C. ohridella*. In this sense, the removal of leaf litter is counterproductive, because each year the parasitoid selection process restarts.

Our results show that *Minotetrastichus frontalis* and *Prigalio agraulis* were the dominant species in the leaf litter collected at the end of the season in Braunschweig, Germany. For example, from 100 g dry horse chestnut leaf litter (volume of approx. 50 l) 60 *M. frontalis* and six *P. agraulis* individuals were extracted in addition to several other species. From the literature, we know that *M. frontalis* is a gregarious ectoparasitoid preferring larval developmental stages (Noyes, 2002; Grabenweger, 2003a; Lupi, 2005) and is able to develop as a facultative hyperparasitoid (Freise, 2001; Noyes, 2002). In contrast, *P. agraulis* is a solitary ectoparasitoid attacking larvae and pupae of the horse chestnut leafminer. Both species are polyphagous, attacking, as a minimum, more than 60 different leafminer species (Noyes, 2002). Parasitisation rates in our experiments range between 1 and 35%. Although both parasitoids belong to the most frequently found species in urban and natural stands of infested white horse chestnuts throughout the year (Grabenweger & Lethmayer, 1999; Hellrigl, 2001; Freise & Heitland, 2003; Grabenweger, 2003a), the species *P. agraulis* almost exclusively was parasitizing the horse chestnut leafminer in our greenhouse and semi-field experiments.

The response of *P. agraulis* to increasing host densities, i.e. the functional response, cannot be evaluated conclusively, especially since parasitism rates at low host densities were not covered by our experiments. However, at least at the quite high host densities tested in our experiments, the parasitisation rate is independent of the leafminer density. This was observed for both tested parasitoid densities. Nevertheless, the parasitisation rate increased four-fold if ten times more parasitoid individuals were released. This result was supported by results of our semi-field experiments. In total each parasitoid individual was able to parasitize approximately 30 horse chestnut leafminers, which seems to be the maximum realised fecundity of *P. agraulis*. Nevertheless, it is likely that this parasitoid species frequently contributes to host mortality by host feeding. At the frequently observed high horse chestnut leafminer field densities, the missing functional response disqualifies *P. agraulis* as an efficient antagonist; but, in the future, the parasitoid might be better adapted to either the host and/or high leafminer densities.

Parasitisation rates for the horse chestnut leafminer of 10–20% have been reported at different European locations (Grabenweger & Lethmayer, 1999; Freise *et al.*, 2002; Grabenweger *et al.*, 2005a; Lupi, 2005; Volter & Kenis, 2006). However, Freise & Heitland (2003) point out that it is more likely that the parasitisation rates range between 1 and 5% and attribute the discrepancy to different methodologies in the estimation of parasitisation rates. In a first attempt to investigate the effects of artificially synchronised parasitoids on the biocontrol of the horse chestnut leafminer, Kehrlí *et al.* (2005) used mass-emergence devices and recorded parasitisation rates of up to 17%. However, due to technical problems, the high parasitisation rates could not be attributed to the impact of parasitoid augmentation. Additionally, the authors stated that 77% of *P. agraulis* were retained by the mesh size of 600 µm used in their experiments. In our study, *P. agraulis* turned out to be the dominant parasitoid species parasitizing horse chestnut leafminer larvae. This underlines the importance of this species for the control of *C. ohridella*.

The maximum parasitisation rates recorded in our study are in the range estimated for other leafminer species. For

example, parasitisation rates of 30–50% were recorded for native birch leafminers *Coleophora serratella* (Hymenoptera: Tenthredinidae) in northern Germany (Pschorn-Walcher, 1980) and 30–67% for the locust leafminer *Phyllonorycter robiniella* (Lepidoptera: Gracillariidae) in Serbia (Stojanović & Marković, 2005). Therefore, parasitisation rates of 35% recorded in our study indicate that the promotion of parasitoids could be a strategy not only to increase parasitisation rates in the field, but also to limit horse chestnut leafminer population development (see discussion below). Moreover, the contribution of parasitoid species other than *P. agraulis* could raise parasitisation rates above 35%, if interspecific competition does not counterbalance the overall efficiency. To assess the overall impact of parasitoid species on horse chestnut leafminer population development more field studies are necessary.

An unexpected result was that the most abundant parasitoid species, i.e. *M. frontalis*, was not contributing to leafminer parasitisation in our experiments. In the field, this species is normally one of the dominant species during the first larval leafminer generation and reaches parasitisation rates comparable to *Pnigalio agraulis* (Grabenweger & Lethmayer, 1999; Freise, 2001; Grabenweger, 2003b; Stojanović & Marković, 2004). Two non-exclusive reasons might contribute to the observed results. (i) The parasitoid species *M. frontalis* is not a facultative hyperparasitoid as described by Freise (2001) and Hellrigl (2001) but is an obligate hyperparasitoid. This would explain the complete failure of *Minotetrastichus frontalis* in our experiments, since parasitised leafminer larvae were not available. (ii) An alternative explanation for the failure of *M. frontalis* could be unfavourable experimental conditions. In particular, carbohydrate- and amino acid-containing food sources, which might be important for egg maturation and survival, were almost entirely missing in our experimental units. More detailed studies are needed to clarify the role of *M. frontalis* in the parasitoid complex.

More important to the public than increased parasitisation rates *per se* is the impact of parasitoids on leafminer population development. Sustainable effects can only be achieved if the reduced number of surviving moth larvae results in lower number of leafminers on the horse chestnut trees in the next generation. The desirable effect should be that horse chestnut trees look healthy in terms of green leaf area and that trees will most likely not show early leaf fall. In our experiments, population increase in the control treatment from the first to the second larval leafminer generation was six-fold. Approximately 700 emerging adults of the first larval generation produced almost 4000 mines on five horse chestnut trees in the second larval generation. A parasitisation rate of 1% was without any effect on leafminer population development, while 11% was enough for leafminer population to be reduced by approximately 40%. Since host feeding is a common phenomenon for leafminer parasitoids (Askew & Shaw, 1979; Casas, 1989; Neale *et al.*, 1995; Bernardo *et al.*, 2006), we have to keep in mind that not only parasitised larvae but also killed larvae contributed to the lower number of emerging adults and, consequently, to the reduced mine density in the second leafminer generation.

Although approximately 11% of the first larval generation of the leafminer was parasitized by *P. agraulis*, only a few horse chestnut leafminer larvae of the second generation were parasitized. Most likely, the asynchrony in the

developmental times of the parasitoid and the host is responsible for this unexpected result. Compared to the horse chestnut leafminer, the parasitoid species *P. agraulis* grows two times faster (personal observation) and, therefore, it is likely that most parasitoid species do not live long enough to parasitize hosts of the following leafminer generation. Even the fact that part of each horse chestnut leafminer generation enters diapause (Dimić *et al.*, 2000; Freise, 2001) and are, therefore, continuously available as hosts, did not lead to the increased or at least constant parasitisation rates in our experiments. Diapausing horse chestnut pupae are less frequently parasitized than other developmental stages, including non-diapausing pupae (Freise, 2001). Most likely, diapausing pupae are protected by a physical defence (Freise & Heitland, 2004), i.e. the silky cocoon and/or behavioural defences, i.e. host wriggling inside the mine (Meyhöfer *et al.*, 1994; Bacher *et al.*, 1996). Finally, it is likely that the effect of host-parasitoid asynchrony on parasitisation rates was enhanced by our semi-natural conditions, which do not allow immigration of parasitoids developing on other leafminer species in the habitat.

Although overlapping horse chestnut generations in the field might guarantee for continuous availability of horse chestnut larvae, the observations made so far indicate that parasitisation only slightly increases from one to the other generation (Kehrli, 2004). To which extent alternative host species in the field are responsible for the overall low parasitisation rates should be investigated in the future.

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