

# Biocontrol of *Cameraria ohridella* by insectivorous birds in different landscape contexts

Stefanie Mösch · Elisabeth J. Eilers · Martin Hommes

Received: 15 March 2017 / Accepted: 28 November 2017 / Published online: 9 December 2017  
© The Author(s) 2017. This article is an open access publication

**Abstract** The horse chestnut leaf miner (HCLM) *Cameraria ohridella* Deschka and Dimic (Lepidoptera, Gracillariidae) is a novel but significant pest in Europe. Current control measures are either inefficient or environmentally harmful. Tits (*Parus* spp.) open the mines and prey on HCLM, but the biocontrol efficiency of this behaviour has not yet been quantified. We installed bird nesting-boxes in a biennial field study on four sites close to Brunswick (Germany). On the same sites, we counted HCLM pupae, larvae, opened and closed mines, and parasitised larvae and pupae in leaves collected from horse chestnut (*Aesculus hippocastanum* L., Hippocastanaceae) trees with

and without bird exclusion. In both years, the HCLM number and the proportion of closed mines were higher in bird exclusion trees, particularly on sites with high abundance of tits. Hence, we suggest including the facilitation of birds, particularly tits, in future HCLM biocontrol strategies.

**Keywords** Lepidoptera: Gracillariidae · Insectivorous birds · Predation · Invasive pest insect · *Aesculus hippocastanum* L.

---

Handling Editor: Marta Montserrat.

---

Stefanie Mösch and Elisabeth J. Eilers are authors with equal contributions.

---

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s10526-017-9857-1>) contains supplementary material, which is available to authorized users.

---

S. Mösch · E. J. Eilers · M. Hommes (✉)  
Institute for Plant Protection in Horticulture and Forests,  
Julius Kühn Institut, Messeweg 10-11, 38104 Brunswick,  
Germany  
e-mail: martin.hommes@julius-kuehn.de

E. J. Eilers  
Department of Chemical Ecology, Universität Bielefeld,  
Universitätsstraße 25 UHG W2-119, 33615 Bielefeld,  
Germany

## Introduction

For many species of pest insects, vertebrate predators are known as effective antagonists in addition to parasitoids (Buckner 1966; Kirk et al. 1996; Eilers and Klein 2009). Nevertheless, relatively few studies quantify the effectiveness of birds as predators to reduce agricultural or forestry pests, particularly for leaf mining insects (Kirk et al. 1996; Mols and Visser 2002).

The common horse chestnut (*Aesculus hippocastanum* L., Hippocastanaceae) is a popular tree in Europe, and has been planted in large numbers particularly in parks, alleys and other spaces of urban green. Since the horse chestnut leaf miner (HCLM) *Cameraria ohridella* Deschka and Dimic (Lepidoptera, Gracillariidae) was introduced to Europe in 1984, European chestnuts are constantly under

massive attack by the pest (Grabenweger 2004; Thalmann et al. 2003). In Germany, the HCLM was first reported to be found in Bavaria in 1993 (Butin and Führer 1994). Depending on the local climate 2–4 generations occur annually in Germany (Grabenweger 2003). The last generation overwinters as pupae in fallen leaves. In the spring, the female HCLM places the eggs on the upper surface of the leaves. During their development HCLM larvae are feeding their way through the leaf parenchyma, thereby reducing the photosynthetically active leaf tissue. Larval feeding results ultimately in browning and early senescence of leaves. Moreover, the trees are weakened systematically, resulting for instance in a reduced quality of produced seeds (Thalmann et al. 2003).

Leaf miners, particularly lepidopterans but also dipterans, coleopterans and hymenopterans are spending a great proportion of their life within the realms of one leaf and are thus protected from natural enemies or most chemical influences (i.e. non systemic pesticides). Hence, effective pest management strategies are sparse. In order to minimise the pest pressure of the first generation in spring, the most effective strategy is to collect and remove leaves in autumn (Straw and Bellett-Travers 2004). A similar sanitation practice including collection and shredding of leaves has also been proposed as an effective control strategy against the spotted tentiform leafminer in apple orchards (Vincent et al. 2004). Various parasitoids, mostly eulophid wasps (Hymenoptera, Eulophidae), are specialised on leaf miners (Hespenheide 1991). Approximately 20 species of parasitoids occurring in Germany are known to use the HCLM and other miners as hosts (Grabenweger 2003; Jäckel et al. 2008). However, the effectiveness of native parasitoids in combating HCLM is considered low. The reported parasitisation rates have been below or around five percent (Jäckel et al. 2008).

Insectivorous birds are known to remove leaf-chewing herbivores from trees and thus increase the plant biomass production, for instance in oak (Marquis and Whelan 1994) or willow (Sipura 1999). Together with ants (Faeth 1980), birds (Hespenheide 1991) are the most important predators of mining insects. For instance, it was shown that tits are able to reduce lepidopteran insect pests significantly, thereby securing higher horticultural yields (Mols and Visser 2002). Also, tits were shown to be able to open mines of the holly leaf miner *Phytomyza ilicis* Curtis (Diptera:

Agromyzidae) and mines of two different mining moths (Lepidoptera, Gracillariidae) occurring in alder leaves (Heads and Lawton 1983a, b). The mines of HCLM can be opened by blue tits (*Cyanistes caeruleus* Linnaeus, 1758) and great tits (*Parus major* Linnaeus, 1758) (Grabenweger et al. 2005). The authors also frequently observed the opening of mines by these two species.

It has been proposed that vertebrate predators of insect herbivores may also kill insect parasitoids (i.e., intraguild predation) or reduce their efficiency due to competition for the same prey (Battisti et al. 2000; Martin et al. 2013). Birds have a remarkable olfactory system and may use olfactory cues in addition to visual and other cues for finding prey (Cunningham et al. 2009; Nevitt et al. 2008). For insect feeding birds it has been found that herbivore induced plant volatiles (HIPVs) play a major role in prey detection (Amo et al. 2013; Mäntylä et al. 2008). Thus, it is likely that insectivorous birds prey more vigorously on actively feeding HCLM, which induce the production of HIPVs by their host plant, rather than on paralysed HCLM. Furthermore, HCLM infesting parasitoid wasps are too small to fall into the range of prey for blue tits or great tits (Cowie and Hinsley 1988; García-Navas et al. 2012).

The main aim of this study is to quantify the impact of birds, especially tits, as predators on the population density of HCLM in different landscape contexts. Our first hypothesis is that birds, facilitated by nest boxes, reduce chestnut HCLM infestation in urban as well as rural, agriculturally shaped environments and thus contribute markedly to pest control. Secondly, we hypothesise that the antagonistic impact of birds exceeds the impact of other natural enemies, i.e. parasitoids. Thirdly, we assume that, due to their low abundance and small size, parasitoids preying on HCLM are a negligible food source for HCLM-feeding birds and thus hypothesise that a HCLM parasitisation is not correlated to HCLM bird predation.

## Materials and methods

### General setup of field experiments

We conducted the experiments in 2014 and 2015 on four different field sites around Brunswick: a meadow located at the city margin (CM), an agricultural field (AF), and two meadows adjacent to forests (MF). The

locations (Julius-Kühn Institute CM, Hötzum AF and Wohld MF) are 10 km apart each way, respectively. In autumn/winter 2004, we planted chestnut trees of 150 cm height in 34 groups of four trees each. Initially, we used the trees for investigations of mating disruption before conducting the here-mentioned experiments several years later. The four trees of each group built a square with approx. 100 cm side length (referring to the trunks of each tree), surrounded by four wooden poles (height = approx. 270 cm, spacing = approx. 190 cm), which had been buried in the ground. The trees were approx. 200–260 cm high during the experimental period. The trees were cropped annually. As a result, the size and biomass was approximately constant in both study years and flowering or seed production was not observed. By the use of bird-exclusion nets (1 × w = 800 × 800 cm), which we fixed on the respective wooden poles (Fig. 1), we covered half of the tree groups (i.e. 17). The nets (Rantai ABN, Schachtrupp KG) consisted of high-density polyethylene (HDPE), had a mesh size of 5 × 7 mm (excluding birds but not insects) and weighted 33 g/m<sup>2</sup>.

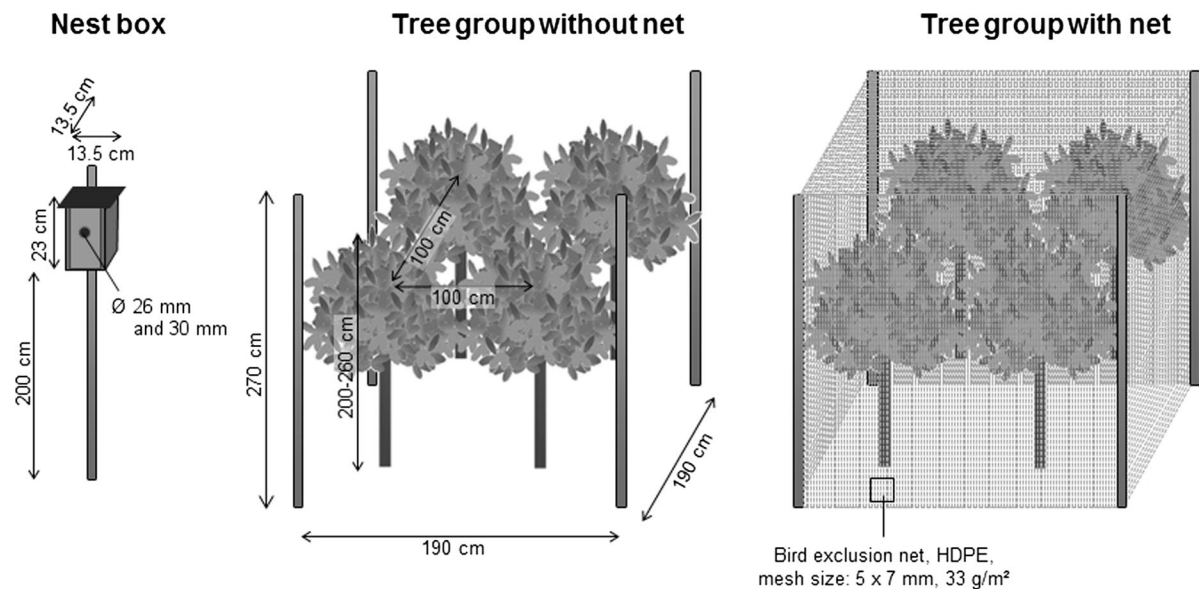
### Nest boxes

We adapted the number of installed nest boxes to the number of preexisting potential nesting locations for

birds (number of installed boxes: CM = 2, AF = 8, MF1 = 8, MF2 = 5). For the sites AF, MF1 and MF2 we chose custom made wooden nest boxes, which were particularly suitable for blue tits and great tits (h × l × w = 23 × 13.5 × 13.5 cm, entrance holes  $\varnothing$  = 26 mm or 30 mm, respectively) and for all four sites, we purchased additional nest boxes (Typ 1B  $\varnothing$  = 26 mm, made of wood-concrete, Schwegler, Schondorf, Germany). We installed the custom made nest boxes in close vicinity (about 1–2 m) to the tree groups (2–4 per site) and fixed them on a wooden pole at a height of about 200 cm (Fig. 1). In addition, we installed the purchased nest boxes in trees at a distance of several meters to the tree groups (2–4 per site). Every four weeks from March until July, we checked the boxes for breeding success and bird species. We counted the number of eggs and young birds. A breed was only considered successful if the majority of birds abandoned the box at the end of the season. We cleaned the boxes in winter.

### Leaf collection and examination

Mid of September of both years, we collected in each case one leaf from the lower, mid and upper third of the canopy of each tree (i.e. three leaves per tree and 12 leaves per group). We stored the leaves in airtight bags sorted by tree at 4 °C and approx. 90% RH in a



**Fig. 1** Experimental setup: Groups of trees with four horse chestnut trees each, with (left) and without (centre) bird exclusion nets. Custom made wooden nest boxes were installed in close proximity to the tree groups

climate storage room. Afterwards, we investigated the leaves with a binocular in the lab. We monitored the HCLM population in Brunswick in close vicinity to the CM site (Julius Kühn Institute) by using two pheromone funnel traps in both years and found each time three HCLM generations. We distinguished small ( $\varnothing < 5$  mm) and large ( $\varnothing \geq 5$  mm) mines, further categorised in closed or opened mines. The reason for the distinction in small and large mines was that the larvae in the small mines are caused by early instars which may be uninteresting for birds due to their lower nutritional value compared to later instars inhabiting larger mines. We defined closed mines as such with intact leaf surface and the larva or pupae still present in the mine. The surface of opened mines was damaged either by the exit of HCLM, opening by birds or other causes. In most cases, the opening by a bird may be distinguished from exit holes caused by emerging HCLM by the fact that it is much larger. However, the surface above the mine is often so porous that it can quickly enlarge by minor physical damage, even after the moth has emerged. Therefore, an exact distinction was not possible. Furthermore, we counted parasitised and intact HCLM larvae and pupae which meant we investigated the larvae and pupae for traces of parasitisation and/or the presence of parasitoids within the HCLM larvae or pupae.

### Statistics

We used the software “R”, version 3.2.3, (R Core Team 2015) for statistical data analysis. We analysed the sum of parasitised HCLM larvae and pupae, and the sum of HCLM larvae and pupae by generalised linear mixed-effects models (GLMM) with poisson distribution and log link function (R packages: ‘lme4’ and ‘MASS’). In order to test for differences among the treatment groups with and without bird exclusion nets and for differences among the sites, we split the data into two subsets for both years and tested the predicting variables ‘net’ (presence or absence of a bird exclusion net) and ‘site’, together with the respective interaction term (net:site). In order to avoid pseudoreplication, we used a nested design with the random factors ‘leaf’ nested in ‘tree’ and ‘tree group’ nested in ‘site’. As a substitute method for conventional post-hoc testing, we applied further GLMMs with poisson distribution and log link function on subsets for the different sites and years and tested the

predicting variable ‘net’ (presence or absence of a bird exclusion net). Again, pseudoreplication was avoided by using a nested design with the random factors ‘leaf’ nested in ‘tree’ and ‘tree’ nested in ‘tree group’. As data for the sum of larvae and pupae per tree were not normally distributed, we compared them pairwise within one field site using Mann and Whitney  $U$  tests. We compared proportional data (i.e., percentage of opened mines) by using  $\chi^2$  tests. In order to evaluate if the opening of mines by birds was density-dependent, we also tested the correlation of opened mines without bird exclusion to the number of larvae and pupae without birds using Pearson’s product-moment correlation. Additionally, we tested the mean number of parasitised larvae and pupae per tree without net for correlation with the mean number of opened HCLM mines on trees without net, which served as a measure of potential bird predation of insects. For this data we applied Pearson’s product-moment correlation (data 2014) and Spearman’s rank correlation (data 2015), as the data from 2014 was normally distributed but the data from 2015 was not.

## Results

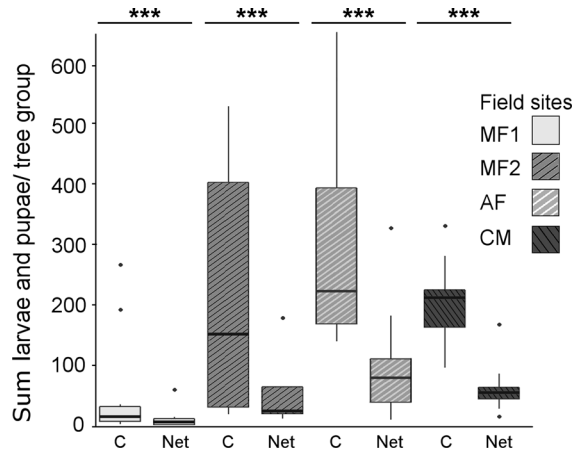
### Breeding birds

The highest numbers of breeding tits in nest boxes were recorded at the meadows adjacent to forests (MF1 four broods in 2014 and two in 2015; MF2 four broods each in 2014 and 2015, respectively). Additionally, we found seven broods of Eurasian tree sparrow (*Passer montanus*) in the nest boxes in 2014 and 2015 at the site MF1. At the agricultural field (AF), eight broods of sparrows and one with tits were recorded in 2014 and at least eleven successful broods of sparrows but none of tits were observed in the nest boxes for the following year (2015) but tits could be observed in the surroundings. Surprisingly, no broods were captured in the nest boxes at the city margin (CM).

### HCLM larvae and pupae

All experimental trees were infested with HCLM. Among all treatments, the mean number of HCLM larvae and pupae counted on three leaves per tree  $\pm$  SD was  $127.19 \pm 119.43$  (min = 1, max = 476). Up to 285 mines were found in one leaf and up to 582 mines in the

three leaves collected per tree (mean per leaf  $\pm$  s.d. =  $29.62 \pm 38.64$ ; mean per three leaves of one tree  $\pm$  SD =  $88.78 \pm 105.3$ ). The distribution of mines among the leaves investigated per tree was very irregular: the standard deviation of mines per leaf within one tree group and year exceeded the mean of mines per leaf within one tree group and year by up to 238%. The overall HCLM infestation was approx. 50% higher in the second year of our experiments (mean<sub>2014</sub>  $\pm$  SD =  $101.25 \pm 90.36$ , mean<sub>2015</sub>  $\pm$  SD =  $153.13 \pm 137.9$ , see supplementary information table S1). The number of collected HCLM (larvae and pupae) was in both years significantly influenced by the factor site (Table 1). At sites with naturally high bird abundance, particularly CM, the number of collected HCLM (larvae and pupae) was higher at the tree groups with net, compared to control tree groups (Fig. 2, supplementary information table S1). At the agricultural field AF, the difference was less apparent. The MF sites showed a low overall abundance of HCLM. When comparing the data for 2014 and 2015 for the four field sites (supplementary information table S1), the sum of larvae and pupae in the three collected leaves per tree was in all cases lower on control tree groups (without bird exclusion nets) compared to trees with bird exclusion (mean<sub>C</sub>  $\pm$  SD =  $118.2 \pm 75.53$ ; mean<sub>net</sub>  $\pm$  SD =  $403.68 \pm 232.29$ ).



**Fig. 2** Sum of counted HCLM larvae and pupae in three leaves collected per tree on tree groups with bird exclusion nets (“Net”) and control tree groups without nets (“C”) for the field sites: meadow adjacent to forest (MF1 + MF2), agricultural field (AF) and city margin (CM). The boxplots show the median (line), upper and lower quartiles (box surrounding line), range to the most extreme data point which is no more than 1.5 times the interquartile range from the box (whiskers), and outliers (points). The sum of HCLM larvae and pupae within the three leaves per tree in 2014 and 2015 was compared pairwise per site (with vs. without net) applying Mann and Whitney *U* tests, significance: \*\*\**P* < 0.001

**Table 1** Results of generalised linear mixed-effects models (GLMM) for the sum of parasitised HCLM larvae and pupae, and the sum of HCLM larvae and pupae for both years 2014 and 2015

Response variable	Year	Fixed factor	df	Chi <sup>2</sup>	<i>P</i> value
Larvae and pupae	2014	Net	1	11.23	0.0008***
		Site	3	48.2	< 0.0001***
		Net × site	3	0.98	0.807 <sup>n.s.</sup>
	2015	Net	1	19.31	< 0.0001***
		Site	3	12.09	0.0071**
		Net × site	3	2.2	0.532 <sup>n.s.</sup>
Parasitised larvae and pupae	2014	Net	1	17.25	< 0.0001***
		Site	3	50.66	< 0.0001***
		Net × site	3	3.09	0.379 <sup>n.s.</sup>
	2015	Net	1	15.52	< 0.0001***
		Site	3	15.5	0.001**
		Net × site	3	0.16	0.984 <sup>n.s.</sup>

Models were tested for the predicting variables ‘net’ (presence or absence of a bird exclusion net), ‘site’, and the interaction term, respectively. A nested design with the random factors ‘leaf’ nested in ‘tree’ and ‘tree group’ nested in ‘site’ was applied. Number of observations = 408; number of nested groups: ‘tree group’ nested in ‘site’ = 34; ‘leaves’ nested in ‘trees’ = 12. Significance levels: \*\*\**P* < 0.001, \*\**P* < 0.01, *P* > 0.05 = not significant, n.s.

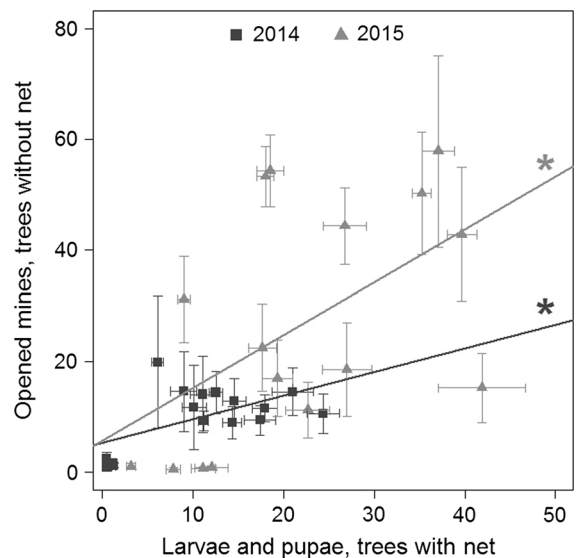


## HCLM mines

At all field sites and in both years (2014 + 2015), the proportion of opened large HCLM mines (Fig. 3a) was higher on control trees without bird exclusion nets compared to trees with bird exclusion (mean<sub>C</sub> ± SD = 70.3 ± 19.1%; mean<sub>net</sub> ± SD = 44.1 ± 15.8%). For small mines (Fig. 3b), the proportion of opened mines was in six out of eight cases (four sites, two years) higher in collected leaves of control tree groups compared to tree groups with bird exclusion (mean<sub>C</sub> ± SD = 45.6 ± 28.8%; mean<sub>net</sub> ± SD = 32.7 ± 26.7%). We found more large than small mines (of all recorded mines, approx. 10% were categorised “small”). The opening of mines on trees without bird exclusion was correlated to the density of HCLM larvae and pupae in the adjacent tree groups with bird exclusion (Fig. 4).

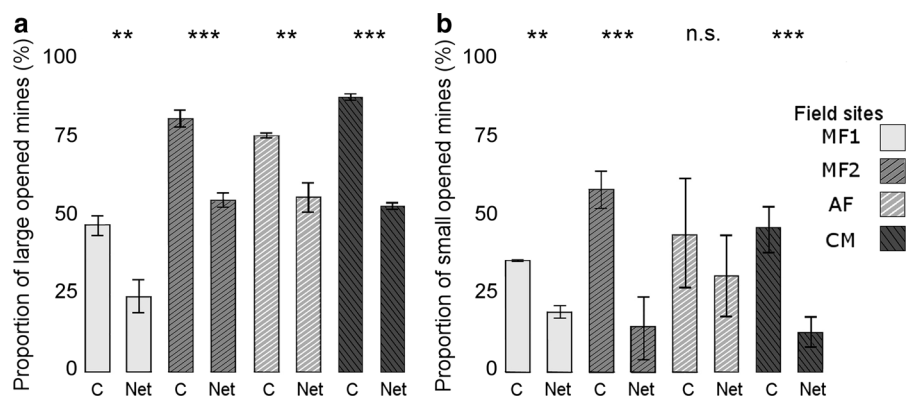
## Parasitoids

Among all treatments, the mean number of parasitised HCLM larvae and pupae in the three collected leaves per tree (± SD) was 13.88 ± 16.59 (min = 0, max = 476), equaling 13.46 ± 22.4% of present HCLM larvae and pupae. In line with the higher abundance of HCLM larvae and pupae, the abundance of parasitised HCLM larvae and pupae was approx. 65% higher in 2015 compared to 2014 (mean<sub>2014</sub> ± SD = 10.5 ± 12, mean<sub>2015</sub> ± SD = 17.25 ± 19.6). In 2015 the number of opened mines, i.e. the measure



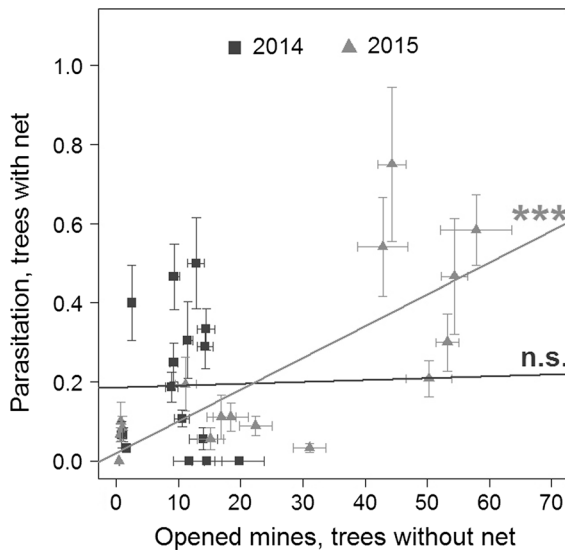
**Fig. 4** Mean number of opened HCLM mines per tree (sum of large and small mines) on trees without bird exclusion nets, plotted against the mean number of HCLM larvae and pupae per tree, which hatched on trees with bird exclusion nets. Error bars indicate SE in both directions. Pearson correlation (2014 data):  $t = 2.563$ ,  $d.f. = 14$ ,  $P$  value = 0.022,  $cor = 0.565$ , linear equation:  $y = 0.427x + 5.21$ ; Pearson correlation (2015 data):  $t = 2.364$ ,  $d.f. = 14$ ,  $P$  value = 0.033,  $cor = 0.534$ , linear equation:  $y = 0.953x + 5.623$ . Significant correlations are marked with asterisks: \* $P$  value < 0.05

of bird activity, was within the three collected leaves on control trees (without net) significantly higher with higher abundance of parasitised larvae and pupae (Fig. 5). The presence of bird exclusion nets had a



**Fig. 3** Average percentage of opened large (a) and small (b) chestnut leafminer mines (large = diameter > 5 mm) on chestnut leaves collected from the field sites in 2014 and 2015 ( $n = 34$  tree groups with 12 collected leaves per group and year). The total number of counted opened and intact mines corresponds to 100%, respectively. Error bars indicate SE. Data

are shown for the three types of sites: meadow adjacent to forest (MF1 + MF2), agricultural field (AF) and city margin (CM). The proportions were pairwise compared (with net vs. without net), using  $\chi^2$  tests (\*\*\* $P < 0.001$ , \*\* $P < 0.01$ ,  $P > 0.05$  = not significant, n.s.)



**Fig. 5** Mean number of parasitised HCLM larvae and pupae within the three collected leaves per tree which hatched on the same trees (with bird exclusion nets), plotted against the mean number of opened HCLM mines within the three collected leaves per tree (sum of large and small mines) on trees without bird exclusion nets (as a measure of potential bird predation). Error bars indicate SE in both directions. Pearson correlation (2014 data):  $t = 0.055$ ,  $d.f. = 14$ ,  $P$  value = 0.957,  $cor = 0.137$ , linear equation:  $y = 0.001x + 0.187$ . Spearman's rank correlation (2015 data):  $P$  value < 0.001,  $d.f. = 14$ ,  $\rho = 0.752$ , linear equation:  $y = 0.008x + 0.02$ . Significant correlations are marked with asterisks: \*\*\* $P$  value < 0.001,  $P$  value > 0.05 = not significant, n.s.

significant impact on the number of captured parasitoids from HCLM larvae and pupae in both years (Table 1), which were higher within the collected leaves of trees with net (supplementary information table S1). However, the effect was only significant on the sites AF and CM (Table 2).

## Discussion

In line with our first hypothesis, our results show that the treatment with bird exclusion nets prevented the birds from opening the HCLM mines, which is reflected by a reduced density of HCLM larvae and pupae on control trees (without net). The efficiency of HCLM control by birds in our experiments was estimated approx. 40% opening of large mines and removal of larvae or pupae, and 30% of small mines. Although not all open mines may be attributed to birds but may also result from hatching adult HCLM, the

abundance of HCLM larvae and pupae was reduced to even approx. 75% when comparing the abundances among all sites in collected leaves from tree groups with and without bird exclusion. Vertebrates and particularly birds have also been shown in other study systems to be effective biocontrol agents for insect pests in trees (Eilers and Klein 2009). The impact of nest boxes was not as prominent as expected. Particularly at the city margin (CM) site, the nest boxes were not accepted and thus no brood was observed. This is, however, likely attributed to a combination of two reasons: First, sufficient natural nesting opportunities for small hole-nesting birds were available in adjacent private gardens, and second, our choice for the position the two nest boxes at this site was inauspicious. In personal observations, we confirmed a high number of tits and sparrows at the city margin site.

As sparrows have to our knowledge never been described to open the HCLM mines and we also did not observe such behaviour, we assume that they are unable to open the mines due to their blunt beaks. Therefore, sparrows were in this study not considered to play a role in HCLM control. In addition to bird predation, parasitism, host feeding, predation by arthropods, plant defence reactions, leaf senescence, intra-specific competition and inter-specific competition with a fungal disease have been proposed as the main reasons for HCLM larval and pupal mortality (Girardoz et al. 2007).

Our second hypothesis regarding the efficiency of birds compared to parasitoids in HCLM control is partly supported by our findings. For this hypothesis, the size of mines was not considered, as it is difficult to attach importance of the different HCLM instars to the damage that is caused to the tree at the end of the life cycle and to evaluate the importance of the survival of different instars for the later size of the entire HCLM population. The total number of parasitised larvae and pupae in the three collected leaves per tree at the different sites ranged from < 0.1 (MF2, 2014) to approx. 14 (AF, 2015) per tree (the summarised numbers per tree group are listed in supplementary information table S1), indicating that some of the sites showed very low parasitism rates. Furthermore, the abundance of parasitoids also differed strongly between the two study years. However, it has to be considered that we only determined the abundance of parasitised HCLM larvae and pupae in leaves that were collected in autumn. The actual parasitoid

**Table 2** Results of generalised linear mixed-effects models (GLMM) for the sum of parasitised HCLM larvae and pupae, and the sum of HCLM larvae and pupae for the four sites: city margin (CM), agricultural field (AF), and meadows adjacent to forests (MF1, MF2)

Response variable	Site	Fixed factor	df	Chi <sup>2</sup>	P value
Larvae and pupae	MF1	Net, 2014	1	4.77	0.029*
		Net, 2015	1	2.02	0.1556 <sup>n.s.</sup>
	MF2	Net, 2014	1	1.25	0.264 <sup>n.s.</sup>
		Net, 2015	1	3.91	0.048*
	AF	Net, 2014	1	4.05	0.0441*
		Net, 2015	1	6.6	0.0102*
CM	Net, 2014	1	7.92	0.0049**	
	Net, 2015	1	16.57	< 0.0001***	
Parasitised larvae and pupae	MF1	Net, 2014	1	1.57	0.2097 <sup>n.s.</sup>
		Net, 2015	1	2.6	0.107 <sup>n.s.</sup>
	MF2	Net, 2014	1	0.00	1 <sup>n.s.</sup>
		Net, 2015	1	2.49	0.1144 <sup>n.s.</sup>
	AF	Net, 2014	1	13.79	0.0002***
		Net, 2015	1	8.33	0.0039**
	CM	Net, 2014	1	10.04	0.0015**
		Net, 2015	1	4.58	0.0323*

The data for both years (2014, 2015) were considered individually by producing data subsets. The predicting variable was ‘net’ (presence or absence of a bird exclusion net). A nested design with the random factors ‘leaf’ nested in ‘tree’ was applied and ‘tree group’ was included as an additional random factor. Number of observations:  $n_{MF1} = 240$ ,  $n_{MF2} = 144$ ,  $n_{AF} = 192$ ,  $n_{CM} = 240$ ; nested groups: ‘leaves’ nested in ‘trees’ = 12

Significance levels: \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$ ,  $P > 0.05$  = not significant, n.s.

abundances were thus likely underestimated. Furthermore, from our data we cannot conclude if the parasitisation rate is related to the size of the mines, i.e. if smaller or larger mines are preferred by parasitoids.

While birds in the size of tits are moving within a radius of several hundred meters around their nest to collect prey (Siriwardena 2010; Tremblay et al. 2005), parasitoids like the chalcid wasp *Trichogramma* species are only migrating several centimetres up to a few meters (Suverkropp et al. 2009). Due to the high body mass and the quick flying ability, birds are sophisticated navigators and able to capture large distances (Bullock et al. 2002). In addition to visual cues, they recognise their prey via HIPV from infested trees (Amo et al. 2013; Mäntylä et al. 2008). In contrast it has been found that locomotion of parasitoids occurs mostly passive and non-directional, as it depends on wind conditions (Noldus et al. 1991). Thus, for parasitoids, directed locomotion may only be possible within a small special range. As a consequence, birds may achieve significantly enhanced

efficiency levels as natural enemies of mining insects compared to parasitic wasps, particularly in large plants like trees. However, we did neither test the foraging range and orientational ability of HCLM parasitoids, nor did we find any studies on this topic. Hence, data specific to the efficiency of HCLM parasitoids but also predators is still sparse.

Many native birds like tits are non-migratory and may thus provide their ecosystem service as natural enemies of mining insects all year long. Parasitic wasps, however, have to establish a sufficient population density first in order to reduce the population density of the leaf miners. Moreover, due to their small size and potentially mainly passive locomotion, parasitoids may be more prone to climatic factors and shifts in prey density than birds.

Regarding our third hypothesis, we cannot exclude that birds compete with other natural enemies for their prey HCLM as suggested for other bird predator and prey species (Battisti et al. 2000; Martin et al. 2013) or that birds may even prey on HCLM parasitoids. The before-mentioned studies found detrimental effects of



insectivorous birds on insect parasitoids (1) for a generalist bird which reduced the number of large parasitoids (two ichneumonid wasps and one peromalid wasp) (Battisti et al. 2000) and (2) for an unknown number of bird species and parasitoids, preying on various lepidopteran pest insects in an agricultural system (Martin et al. 2013). Blue tits and great tits are generalists and their prey is often dominated by lepidopteran larvae (Cowie and Hinsley 1988; García-Navas et al. 2012). But particularly in urban areas, both species also prey on other insects, spiders, and birdseed, if available (Cowie and Hinsley 1988). Foraging natural enemies of HCLM, such as the eulophid wasps *Minotetrastichus frontalis* and *Pnigalio agraulis* (Jäckel et al. 2008), are very unlikely to fall prey for blue or great tits, as they are too small and alternative food sources are likely more attractive to the birds. As a conclusion, due to the relatively low abundance and size of parasitoids (i.e. low nutritional value), predation of foraging parasitoids by tits may be a negligible factor. However, further studies are required to support this hypothesis. In order to trace small or enclosed prey, birds use HIPV (Amo et al. 2013; Mäntylä et al. 2008), which are induced and exclusively emitted by the plant during herbivore attack. The emission of HIPV is thus likely reduced or even suppressed if the HCLM encounter parasitism, because the herbivorous activity is suppressed and the miners become eventually paralysed. Due to the high density of HCLM in all of our observed trees, the tits probably preferred to prey on intact HCLM instead of parasitoid-infested, paralysed HCLM. Again, further experiments are inevitable to support this hypothesis.

The opening of mines as a measure of bird activity and the presence of parasitoids were positively correlated for the field data of 2015 (Fig. 5). On trees with net, the abundance of HCLM larvae and pupae was higher 2015 compared to 2014 (supplementary information table S1) on three out of four field sites. Apparently, the higher number of HCLM in 2015 resulted in a higher number of recorded parasitised HCLM larvae and pupae (supplementary information table S1) and simultaneously in a higher number of opened mines (in 2015, approx. 80% more mines were opened compared to 2014). Hence, the population density of the HCLM determines both the predation by vertebrate and invertebrate antagonists and at times with high bird predatory activity, a reduction of

parasitoid abundance due to competition with birds for prey could not be confirmed.

In this study, we collected leaves in autumn and evaluated them for mines and HCLM individuals. An interesting question would be if birds also use hibernating HCLM during winter months as a source of protein and peck them out of fallen leaves on the ground. If this is the case, the impact of birds on the population density of HCLM would have been even underestimated in this study.

According to our results, the naturally established populations of birds can reduce HCLM infestation already markedly, as we see a significant effect on HCLM density due to bird exclusion also at the city margin site, where our nest boxes were not occupied by birds. Hence, additional experiments are required to test how the abundance of birds close to chestnut trees can actively be influenced for reasons of HCLM biocontrol, if necessary. For instance, the size and the entrance hole of the nest boxes determine which birds are able to breed therein.

In the present study, we found evidence for two tit species acting as effective biocontrol agents against the invasive pest insect horse chestnut leaf miner (HCLM), which is among the most detrimental pests of chestnuts in Europe. The birds are not only preying on large HCLM, but also opening small mines, therefore preventing damage to the trees to a great extent. In accordance, the facilitation of birds, i.e. by the installation of nest boxes, is an IPM approach which may contribute significantly to plant protection. Therefore it is, however, most relevant to consider the size of the nest box entrance hole in order to promote breeding of birds such as blue and great tits, which are able to prey on HCLM. The reproducibility of results in a second field season shows the robustness of the interaction between birds and the pest. These findings have potentially significant implications for urban tree protection against herbivores. However, in order to explain the role of bird facilitation in urban environments and the impact on parasitoids, further experiments are required.

**Acknowledgements** We thank the technical assistants of the JKI Brunswick, particularly Kirsten Strauß for help with field work and laboratory examination of samples. Furthermore, we thank the gardeners of the JKI Brunswick for help with horticultural issues.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

## References

- Amo L, Jansen JJ, van Dam NM, Dicke M, Visser ME (2013) Birds exploit herbivore-induced plant volatiles to locate herbivorous prey. *Ecol Lett* 16:1348–1355
- Battisti A, Bernardi M, Ghirardo C (2000) Predation by the hoopoe (*Upupa epops*) on pupae of *Thaumetopoea pityocampa* and the likely influence on other natural enemies. *BioControl* 45:311–323
- Buckner CH (1966) The role of vertebrate predators in the biological control of forest insects. *Annu Rev Entomol* 11:449–470
- Bullock JM, Kenward RE, Hails RSH (2002) Dispersal ecology: 42nd symposium of the British Ecological Society. Cambridge University Press, Cambridge
- Butin H, Führer E (1994) Die Kastanien-Miniermotte (*Cameraria ohridella* Deschka & Dimic) ein neuer Schädling an *Aesculus hippocastanum*. *Nachrichtenbl Deut Pflanzenschutzd* 46:89–91
- Cowie RJ, Hinsley SA (1988) Feeding ecology of great tits (*Parus major*) and blue tits (*Parus caeruleus*), breeding in suburban gardens. *J Anim Ecol* 57:611–626
- Cunningham SJ, Castro I, Potter MA (2009) The relative importance of olfaction and remote touch in prey detection by North Island brown kiwis. *Anim Behav* 78:899–905
- Eilers EJ, Klein A-M (2009) Landscape context and management effects on an important insect pest and its natural enemies in almond. *Biol Control* 51:388–394
- Faeth SH (1980) Invertebrate predation of leaf-miners at low-densities. *Ecol Entomol* 5:111–114
- García-Navas V, Ferrer ES, Sanz JJ (2012) Prey selectivity and parental feeding rates of Blue Tits *Cyanistes caeruleus* in relation to nestling age. *Bird Study* 59:236–242
- Girardoz S, Tomov R, Eschen R, Quicke DLJ, Kenis M (2007) Two methods of 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
- Grabenweger G (2003) Parasitism of different larval stages of *Cameraria ohridella*. *BioControl* 48:671–684
- Grabenweger G (2004) Poor control of the horse chestnut leafminer, *Cameraria ohridella* (Lepidoptera : Gracillariidae), by native European parasitoids: a synchronisation problem. *Eur. J Entomol* 101:189–192
- Grabenweger G, Kehrli P, Schlick-Steiner B, Steiner F, Stolz M, Bacher S (2005) Predator complex of the horse chestnut leafminer *Cameraria ohridella*: identification and impact assessment. *J Appl Entomol* 129:353–362
- Heads PA, Lawton JH (1983a) Studies on the natural enemy complex of the holly leaf-miner: the effects of scale on the detection of aggregative responses and the implications for biological control. *Oikos* 40:267–276
- Heads PA, Lawton JH (1983b) Tit predation on the holly leaf-miner - the effect of prickly leaves. *Oikos* 41:161–164
- Hespenheide HA (1991) Bionomics of leaf-mining insects. *Ann Rev Entomol* 36:535–560
- Jäckel B, Grabenweger G, Hopp H, Balder H, Koch T, Schmolling S (2008) Zur Leistungsfähigkeit heimischer Parasitoide bei der Kontrolle der Rosskastanienminiermotte, *Cameraria ohridella* (Lep., Gracillariidae). *Mitt Dtsch Ges Allg Angew Ent* 16:261–264
- Kirk DA, Evenden MD, Mineau P (1996) Past and current attempts to evaluate the role of birds as predators of insect pests in temperate agriculture. *Curr Ornithol* 13:175–269
- Mäntylä E, Alessio GA, Blande JD, Heijari J, Holopainen JK, Laaksonen T, Piirtola P, Klemola T (2008) From plants to birds: higher avian predation rates in trees responding to insect herbivory. *PLoS ONE* 3(7):e2832
- Marquis RJ, Whelan CJ (1994) Insectivorous birds increase growth of white oak through consumption of leaf-chewing insects. *Ecology* 75:2007–2014
- Martin EA, Reineking B, Seo B, Steffan-Dewenter I (2013) Natural enemy interactions constrain pest control in complex agricultural landscapes. *Proc Natl Acad Sci USA* 110:5534–5539
- Mols CMM, Visser ME (2002) Great tits can reduce caterpillar damage in apple orchards. *J Appl Ecol* 39:888–899
- Nevitt GA, Losekoot M, Weimerskirch H (2008) Evidence for olfactory search in wandering albatross, *Diomedea exulans*. *Proc Natl Acad Sci USA* 105:4576–4581
- Noldus LPJJ, van Lenteren JC, Lewis WJ (1991) How *Trichogramma* parasitoids use moth sex pheromones as kairomones: orientation behavior in a wind tunnel. *Physiol Entomol* 16:313–327
- R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.r-project.org/>
- Sipura M (1999) Tritrophic interactions: willows, herbivorous insects and insectivorous birds. *Oecologia* 121:537–545
- Siriwardena GM (2010) The importance of spatial and temporal scale for agri-environment scheme delivery. *Ibis* 152:515–529
- Straw NA, Bellett-Travers M (2004) Impact and management of the horse chestnut leaf-miner (*Cameraria ohridella*). *Arboric J* 28:67–83
- Suverkropp BP, Bigler F, van Lenteren JC (2009) Dispersal behaviour of *Trichogramma brassicae* in maize fields. *B Insectol* 62:113–120
- Thalmann C, Freise J, Heitland W, Bacher S (2003) Effects of defoliation by horse chestnut leafminer (*Cameraria ohridella*) on reproduction in *Aesculus hippocastanum*. *Trees-Struct Funct* 17:383–388
- Tremblay I, Thomas D, Blondel J, Perret P, Lambrechts MM (2005) The effect of habitat quality on foraging patterns, provisioning rate and nestling growth in Corsican Blue Tits *Parus caeruleus*. *Ibis* 147:17–24
- Vincent C, Rancourt B, Carisse O (2004) Apple leaf shredding as a non-chemical tool to manage apple scab and spotted tentiform leafminer. *Agric Ecosyst Environ* 104:595–604

**Stefanie Mösch** The focus of the work of Stefanie Mösch is research of harmful organisms in urban green and the development of integrated control strategies.

**Elisabeth J. Eilers** investigates (chemo-)ecological interactions of agriculturally relevant insects with their hosts or antagonists.

**Martin Hommes** work focuses on the development of IPM tools and strategies for pests in horticultural crops. The work was carried out in the Entomology group of the Institute for Plant Protection in Horticulture and Forests at Julius Kühn Institute, Brunswick, Germany.