

# Mass-emergence devices: a biocontrol technique for conservation and augmentation of parasitoids

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## Abstract

Natural enemies frequently prevent the build-up of large pest populations. The conservation and augmentation of antagonists can be an effective biocontrol strategy to increase their impact. We suggest the use of pest-infested plant material as a source for conservation or augmentation of parasitoids. Plant material containing the host and its parasitoids can be collected and stored in large closed devices from which the generally smaller parasitoids can emerge through a selective tissue filter of appropriate mesh size. We describe the construction and field test of mass-emergence devices to conserve or augment populations of indigenous parasitoids of the invasive horse chestnut leafminer, *Cameraria ohridella* (Lepidoptera, Gracillariidae). At a mesh size of 600  $\mu\text{m}$ , 78% of the parasitoids passed through the tissue filter, whereas only 1.1–2.5% of the leafminers overcame the barrier. Mass-emergence devices significantly increased percent parasitism of *C. ohridella* at one of two field sites tested. The observed parasitism rates at both sites were about 2.5-times higher than in the previous two years and at neighbouring sites in the same year. Moreover, percent parasitism during the third generation of *C. ohridella* was among the highest ever observed in Central Europe. Mass-emergence devices may thus contribute to an integrated control strategy against *C. ohridella*. In general, mass-emergence devices are a promising biocontrol approach to manage insect pests with a dormant stage in or on plant residues and should be considered as a supplementary technique in cases where the use of insecticides is restricted.

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## 1. Introduction

The lack of natural enemies is one of the main reasons for the build-up of large pest population densities (DeBach and Rosen, 1991). The reasons why natural enemies are not able to catch up with pest population densities are manifold and reach from causes extrinsic to the enemy (e.g., poor synchronisation, top-down control by their own enemies) to intrinsic factors such as the Allee effect. Biocontrol theory predicts that such natural

enemies may still be effective control agents, provided the ratio of the number of prey per natural enemy can be lowered (Berryman, 1999). This is usually attempted by conservation measures or augmentative mass releases of reared biocontrol agents (Bellows and Fisher, 1999).

In this paper, we suggest the conservation of resident parasitoids or the augmentation of relocated control agents by a biological control approach largely ignored to date. We propose to use pest-infested plant material as a source of parasitoids. Plant material containing both the host and its parasitoids is collected and stored in large closed devices from which insects can emerge through an opening. At the heart of such a mass-emergence device is a selective opening that only allows the

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exit of parasitoids, which are in general smaller than their hosts (Quicke, 1997), but retains the larger hosts. Such a selective opening can be obtained by a tissue filter of appropriate mesh size (e.g., Bonsall and Hassell, 1998; Taylor, 1929). Our approach is inspired by the so-called “phytomyzarium” (Klein and Kroschel, 2002; Klyueva and Pamukchi, 1979), in which a similar principle is applied to separate an agromyzid fly, used to control weeds of the genus *Orobanche* (Orobanchaceae), from its parasitoids. However, the use of filtering devices to separate hosts from parasitoids has also been suggested for other pest systems in the past (e.g., Hulme et al., 1987).

In a number of cropping systems pest-infested plant material is routinely removed and/or destroyed to reduce pest densities (Hulme et al., 1987; MacHardy et al., 2000; <http://woodypest.ifas.ufl.edu/leafmine.htm>). However, the disposal of this plant material does not only reduce pest populations, it also often removes parasitoids feeding on the pest inside plants, thereby disrupting the build-up of parasitoid populations sufficient to control the pest. Alternatively, plant material infested by insect pests and their parasitoids could be stored in mass-emergence devices with selective openings. These devices could be used to reduce pest densities by removal of infested plant material while at the same time conserving parasitoid populations in the field, thereby enhancing the impact of parasitoids on their hosts, and reducing damage caused by the pest. The possibility to employ two complementary control techniques, direct pest reduction and conservation of natural enemies, in a single approach may be a promising control strategy in many pest systems. This approach may be especially suitable to control leafminers, which are important pests in fruit crops, urban environments and glasshouses (Bel-lows and Fisher, 1999), but should also be considered to control other pests that have dormant stages in or on plant residues and pests of stored products.

We developed and tested this biological control approach in the horse chestnut leafminer, *Cameraria ohridella* Deschka and Dimic (Lepidoptera, Gracillariidae). In Europe, *C. ohridella* is a recently introduced, highly invasive pest of unknown origin that spread over large parts of Europe during the last 15 years. It frequently defoliates white flowering horse chestnut trees (*Aesculus hippocastanum* L., Hippocastanaceae) already in summer (reviewed in Backhaus et al., 2002; Heitland et al., 1999; <http://www.cameraria.de>). Aesthetical concerns and the lost filter function of defoliated trees are considered as the main consequences of damage in cities (Thalman et al., 2003); a hypothetical replacement of horse chestnut trees in Berlin alone would cost an estimated 300 million euro (Balder and Jäckel, 2003). The pest is thus mostly an urban problem managed at the scale of cities.

Currently, the removal of leaf litter, in which pupae of *C. ohridella* overwinter, is the only effective and ecologi-

cally reasonable short-term control measure available (Deschka, 1993; Gilbert et al., 2003; Kehrli and Bacher, 2003). Horse chestnut leaf litter presently is routinely removed and disposed of in many European cities in order to control *C. ohridella*. However, the removal of leaf litter does not only reduce leafminer populations, but also populations of parasitoids hibernating in the mines of their host. Therefore, the use of mass-emergence devices seems to be a promising complementary measure to conserve parasitoids adapted to *C. ohridella* and thereby prevent the annual disruption of the build-up of populations of parasitoids newly recruited on the horse chestnut leafminer. However, not only the removal of newly recruited parasitoids, but also the poor synchronisation of parasitoids, which emerge about one month earlier than their hosts in spring, was hypothesized as a reason for the low impact of parasitoids on *C. ohridella* (Grabenweger, 2004). Even after 15 years of leafminer infestation in Central Europe typical parasitism rates are between 0 and 10%, and mostly below 5% (Heitland et al., 1999). These rates are very low compared to other native and exotic leafminers (Askew and Shaw, 1979; Hawkins, 1993; Maier, 1984).

In this study, we develop a generic approach on how to construct and test mass-emergence devices, which selectively permit parasitoids to emerge out of pest-infested plant material. The first step in the development of a mass-emergence device is the identification of a suitable mesh size that allows passage of the smaller parasitoids, but retains the pest. In a second step, a prototype is constructed, its efficiency in separating parasitoids, and hosts examined and its design refined. Finally, the effect of exposed mass-emergence devices on parasitism rates of the pest is evaluated in the field.

## 2. Materials and methods

### 2.1. Optimal mesh size

The purpose of this laboratory investigation was the evaluation of the maximum mesh size that allows passage of the smaller parasitoids, but retains the larger moths. In early 2001, black 35 L plastic bags were each filled with 50 horse chestnut leaves infested by *C. ohridella*. The opening of each bag led to a separator, which consisted of a slightly conical transparent plastic cylinder (11 cm diameter, 15.5 cm height) that was furnished with a funnel at the bottom (Fig. 1A). The top of the cylinder was covered with tissue filter (Sefar petex, Sefar AG, CH-Heiden) of one of the following specifications: 710  $\mu\text{m}$ /54% (= 710  $\mu\text{m}$  mesh size and 54% open area), 590  $\mu\text{m}$ /40%, 540  $\mu\text{m}$ /59%, 500  $\mu\text{m}$ /48%, or 500  $\mu\text{m}$ /39%. Preliminary investigations revealed that adults of *C. ohridella* are able to pass through a tissue filter of 800  $\mu\text{m}$  mesh size, but were retained by a mesh size of

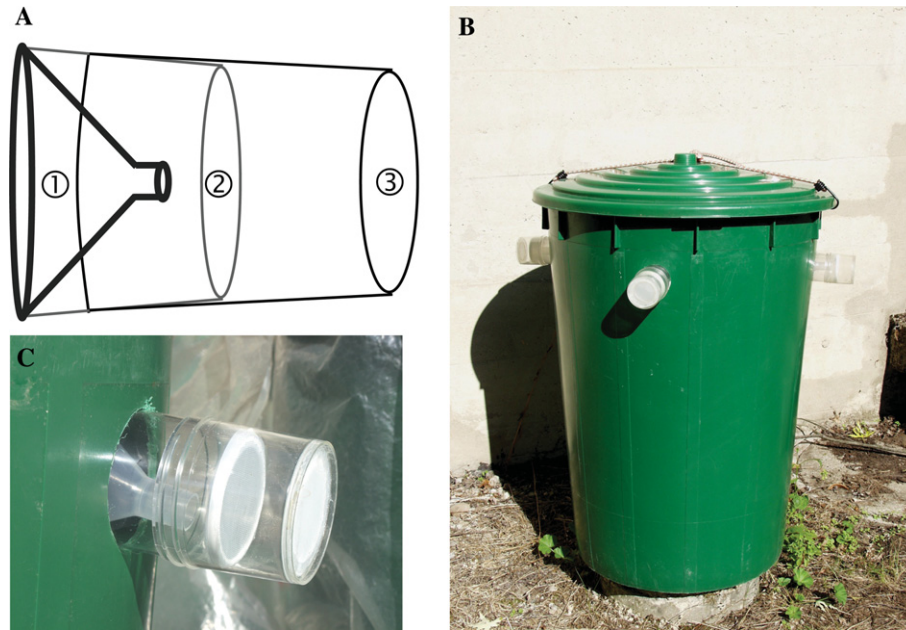


Fig. 1. Construction of the separator (A): ① funnel preventing insects from going back into the elector, ② tissue filter, which operates as a selective barrier, ③ gossamer allowing aeration and locks the system. Pictures of the mass-emergence device (B) and the separator (C).

500  $\mu\text{m}$ . The first cylinder was stacked into a second transparent cylinder. The top of the second cylinder was covered by gauze that did not allow passage of insects, but kept the system well aerated. A drop of honey was placed on the gauze to attract emerging insects.

Insects that emerged from the leaves in the dark bag were attracted to the light at the opening of the black bag, passed through the funnel and were caught in the first cylinder. The funnel prevented insects from going back into the litter bag. Insects that successfully passed through the tissue filter were trapped in the second cylinder. The number of moths and parasitoids that were retained by the different mesh sizes, and the sex of all *C. ohridella* that overcame the filters were recorded.

## 2.2. Construction and test of mass-emergence devices

In autumn 2001, we constructed mass-emergence devices (Fig. 1B) that consisted of closed 200 L plastic tubs (55 cm diameter, 80 cm height). The tubs had four round openings of 6.5 cm diameter near the top. In each opening we stuck a separator of the same type as described above (Fig. 1C), only the diameter was smaller (6.5 cm) and the tissue filter had a mesh size of 600  $\mu\text{m}$  and 51% open area. Insects that passed the tissue filter of the first cylinder were trapped in the second.

We tested if the amount of leaf litter placed in the tubs (1, 2, and 4 kg dry horse-chestnut leaf litter per device) and leafminer-infestation level (low versus high) had an effect on the efficiency of the mass-emergence devices in a completely randomised factorial design. Full combination of the two factors resulted in six treatments, which were replicated five times each. Mass-emer-

gence devices were randomly assigned to each of the treatment replicates and arranged in the garden of the Zoological Institute. In late November 2001, dry *C. ohridella*-infested horse chestnut leaf litter with infestation levels of about 15% (“low”) and 70% (“high”) leaf area damaged was collected from two sites in Bern and either 1, 2, or 4 kg of the each source of leaf litter was placed into the tubs.

From 9 March to 22 May 2002, separators were checked weekly and the number of parasitoids and adult moths that passed through the 600- $\mu\text{m}$ -tissue filter was determined. At the end of the experiment the number of parasitoids and moths, which were not able to overcome the barrier and were caught in the first cylinder, was recorded.

The total number of parasitoids that passed through the 600- $\mu\text{m}$ -tissue filter was treated as the response variable, whereas amount of leaf litter and infestation level were treated as nominal factors. The experiment was analysed by two-way ANOVA. The three amounts of leaf litter were pairwise compared by Fisher’s LSD means separation test. Furthermore, we assessed the influence of the amount of leaf litter and infestation level (as a dummy variable) on the total number of parasitoids that overcame the 600- $\mu\text{m}$ -tissue filter by multiple linear regression. Model assumptions were checked by visual inspection of the residual distribution for every statistical test conducted.

## 2.3. Refinement of mass-emergence devices

We hypothesised that at a certain amount of leaf litter the foliage is so compressed inside the mass-emergence

devices that emerging parasitoids are prevented from reaching the openings. To test this, we set up an experiment to assess the influence of the amount of leaf litter placed in the mass-emergence devices on the number of emerging parasitoids. Furthermore, we identified the parasitoid species emerging from *C. ohridella*-infested horse chestnut leaf litter and assessed their abilities to overcome the 600- $\mu\text{m}$ -tissue filter.

The same mass-emergence devices as described above were filled either with 1, 3, 5, 7, or 9 kg dry leaf litter in each of two replicates. Litter in the 9 kg treatments had to be moderately compressed to fit into the mass-emergence devices. Treatments were randomly assigned to one of ten mass-emergence devices. In autumn 2002, dry *C. ohridella*-infested horse chestnut leaf litter with an infestation level of about 50% damaged leaf area was collected at the same sites in Bern as in the year before and the appropriate amount of leaf litter was placed in the mass-emergence devices described above. The devices were set up in the garden of the Zoological Institute.

From 7 March to 22 May 2003, separators were checked weekly. We recorded the number of parasitoids and *C. ohridella* that passed through the 600- $\mu\text{m}$ -tissue filter and identified the parasitoids. At the end of the trial, parasitoids and moths which were not able to overcome the tissue filter and were caught in the first cylinder were identified and counted.

To investigate the influence of the amount of leaf litter in the devices on the ability of parasitoids to reach the openings we analysed the experiment using a backward third-order polynomial regression to enable us to detect non-linear effects. The total number of parasitoids that overcame the 600- $\mu\text{m}$ -tissue filter was treated as the dependent variable with amount of leaf litter as the independent variable. Model assumptions were checked by visual inspection of the residual distribution.

#### 2.4. Field test of mass-emergence devices

The aim of the field test was to determine whether the exposed mass-emergence devices are able to increase parasitism rates of *C. ohridella* under natural conditions. Furthermore, we investigated if a late release of parasitoids increases parasitism rates more than an early release. Field tests of the mass-emergence devices were conducted in a completely randomised block design. The only factor tested was the date of parasitoid release (early, late, and a no release control). The experiment was conducted at two sites (St. Galler Ring and Eglisee) in the city of Basel, Switzerland. At each site more than 500 *A. hippocastanum* trees grew along the streets and leaf litter was removed by city gardeners in autumn 2002. At both sites we selected five blocks of about 30 trees each. In each block we selected three trees, which were separated from each other by 6–8 untreated trees and were located about 60 m apart. These three trees

were randomly assigned to one of the three parasitoid release treatments.

At the end of February 2003, before the emergence of the leafminers and parasitoids, dry *C. ohridella*-infested horse chestnut leaf litter was collected at two sites in Bern and stored in a climate chamber at 5 °C. Trials at St. Galler Ring were conducted with leaf litter from one source, whereas trials at Eglisee were set up with a mixture of leaf litter from both sites. The mass-emergence devices were of the same dimensions as described above, but with the second cylinder omitted to allow release of the parasitoids. Devices were filled with 10 kg of leaf litter and hung in the crown of *A. hippocastanum* trees by placing them on a large branch and fixing them with two belts to the trunk. On 20 March 2003, the 10 mass-emergence devices of the early treatment were hung in the trees. The late treatment was set up on 23 May 2003, when *C. ohridella* mines of early larval instars were present on the experimental trees. Control trees were not treated at all.

To investigate the effect of mass-emergence devices we assessed defoliation by *C. ohridella* and parasitism rates of the pest at the end of the first, second, and third generation when the majority of leafminers of the respective generation was in the pupal stage. The percentage of defoliation by *C. ohridella* was estimated visually (Gilbert and Grégoire, 2003) on 17 June, 24 July, and 4 September 2003. At the same dates, we also collected randomly 30 leaves from the lower crown of each experimental tree. Two hundred intact mines from the first generation and 100 mines from each of the second and third *C. ohridella* generations were dissected for each tree. For each mine it was determined whether leafminers had already emerged or were still present in the mine, and if present they were classified as alive, dead or parasitized. The percent parasitism of *C. ohridella* per tree was calculated as the total number of parasitized leafminers divided by the total number of alive, parasitized, and emerged leafminers, allowing a direct comparison of the different treatments.

Percent parasitism and the arcsine-transformed percentages of defoliation were treated as response variables whereas block, leafminer generation, and date of parasitoid release were treated as nominal factors. The two sites were analysed separately by three-way ANOVAs. Means of the three parasitoid release treatments were pairwise compared by Fisher's LSD post hoc tests. Model assumptions were checked for every statistical test conducted.

### 3. Results

#### 3.1. Optimal mesh size

The most common parasitoids of *C. ohridella*, *Minostrastichus frontalis* Nees, *Closterocerus trifasciatus*

Table 1

Number of parasitoids and moths that passed through or were retained by the tested tissue filters in the laboratory

Insects	Mesh type 500 µm/39%		Mesh type 500 µm/48%		Mesh type 540 µm/59%		Mesh type 590 µm/40%		Mesh type 710 µm/54%	
	Passed	Retained	Passed	Retained	Passed	Retained	Passed	Retained	Passed	Retained
<i>M. frontalis</i>	45 (78%)	13 (22%)	12 (60%)	8 (40%)	12 (48%)	13 (52%)	18 (55%)	15 (45%)	38 (61%)	24 (39%)
<i>C. trifasciatus</i>	9 (69%)	4 (31%)	0 (0%)	1 (100%)	2 (40%)	3 (60%)	3 (100%)	0 (0%)	4 (100%)	0 (0%)
<i>P. agraulis</i>	0 (0%)	9 (100%)	0 (0%)	7 (100%)	0 (0%)	8 (100%)	5 (45%)	6 (55%)	6 (50%)	6 (50%)
<i>Chrysocharis</i> sp.	0 (0%)	4 (100%)	0 (0%)	1 (100%)	1 (20%)	4 (80%)	0	0	2 (50%)	2 (50%)
Ichneumonidae	0	0	0	0	0	0	0	0	1 (33%)	2 (67%)
<i>C. ohridella</i>	1 (0.3%)	322 (99.7%)	0 (0%)	164 (100%)	0 (0%)	628 (100%)	0 (0%)	234 (100%)	7 (1.2%)	562 (98.8%)

Mesh types are specified by the mesh size and the proportion of open area. The percentage of individuals that either passed or were retained is listed in the brackets.

Westwood, *Pnigalio agraulis* Walker and *Chrysocharis* sp. (all Hymenoptera: Eulophidae, Grabenweger and Lethmayer, 1999), and a few ichneumonid wasps emerged from the leaves. Specimens of all eulophid wasp species were able to pass through the 590-µm-tissue filter, whereas all *C. ohridella*, except one male, were retained by a mesh size of 590-µm or below (Table 1). Furthermore, six of the seven moths that overcame the tissue filter of 710 µm mesh size, were males.

### 3.2. Construction and test of mass-emergence devices

Most individuals of *C. ohridella* passed through the filter in early May, whereas most of the parasitoids were already caught a month earlier, in early April 2002 (Fig. 2). In total 11,839 parasitoids (= 77.8%) passed through the barrier, while 3375 were retained. By contrast, only 546 moths (= 1.1%) of more than 50,000 passed through the 600-µm-tissue filter. The infestation level ( $F = 18.44$ ,  $df = 1,24$ ,  $P < 0.001$ ) and the amount of leaf litter ( $F = 192.85$ ,  $df = 2,24$ ,  $P < 0.001$ ) had a highly significant impact on the number of parasitoids trapped (Fig. 3), whereas the interaction ( $F = 0.20$ ,  $df = 2,24$ ,  $P = 0.82$ ) was not significant. More insects emerged out of the high-infested leaf litter and the number of parasitoids that passed the 600-µm-tissue filter increased linearly with the

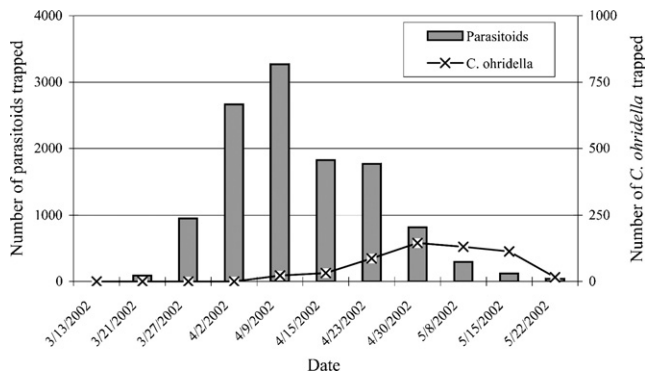


Fig. 2. Total number of parasitoids (left axis) and *C. ohridella* (right axis) that passed through the 600-µm-tissue filter of the 30 mass-emergence devices over the emergence period in 2002. Note the different scales of the y axes. Grey bars indicate the number of emerged parasitoids and crosses the number of *C. ohridella* caught.

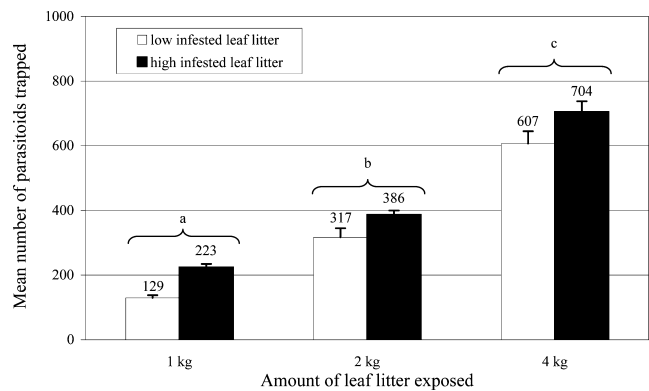


Fig. 3. Impact of infestation level and amount of horse chestnut leaf litter on the mean number of parasitoids (+SE) that passed through the 600-µm-tissue filter of mass-emergence devices over the emergence period in 2002. The three amounts of leaf litter were pairwise compared by Fisher's LSD means separation test; amounts of leaf litter with different letters are significantly different ( $P < 0.001$ ).

amount of exposed leaf litter (Number of parasitoids =  $158.7 * \text{amount of litter} + 86.6 * \text{infestation level} - 19.0$ ,  $n = 30$ ,  $R^2 = 0.94$ ,  $P < 0.001$ ). Up to 800 parasitoids out of 4 kg leaf litter and on average  $172.1 \pm 36.7$  SD parasitoids out of 1 kg horse chestnut leaf litter overcame the filter.

### 3.3. Refinement of mass-emergence devices

Only 2.5% of the 23,900 *C. ohridella* overcame the 600-µm-tissue filter, whereas 78.3% of the 3224 parasitoids that emerged passed through the barrier. Fifty eight percent of the parasitoids were *M. frontalis*, 12% *C. trifasciatus*, 9% *P. agraulis*, 4% *Chrysocharis* sp., and 17% belonged to other wasp species. As in the laboratory trial, the larger *P. agraulis* had difficulty to pass through the 600-µm-tissue filter; only 23% of this species overcame the barrier (Fig. 4). However, about 90% of the emerged *M. frontalis* and *C. trifasciatus* passed through the filter. The total number of parasitoids that overcame the 600-µm-tissue filter increased linearly with the amount of exposed leaf litter; the quadratic and cubic terms were eliminated by the backward regression (Number of parasitoids =  $48.1 * \text{amount of litter} + 19.4$ ,  $n = 10$ ,  $R^2 = 0.94$ ,  $P < 0.001$ , Fig. 5). Thus, we found no indication that parasitoids had

Table 2

Impact of exposed mass-emergence devices on percent defoliation of *A. hippocastanum* trees and percent parasitism of *C. ohridella* at St. Galler Ring and Eglisee in Basel

Source of variance	df	St. Galler Ring		Eglisee	
		Parasitism	Defoliation	Parasitism	Defoliation
Block	4	0.009	<0.001	0.282	0.053
Date of release (DR)	2	0.048	0.148	0.673	0.122
Generation (GE)	2	<0.001	<0.001	<0.001	<0.001
DR * GE	4	0.277	0.985	0.832	0.931
Error	32				

Values shown are the degrees of freedom (*df*) and associated probabilities of the three-way ANOVA. Percent defoliation was arcsine-transformed prior to analysis.

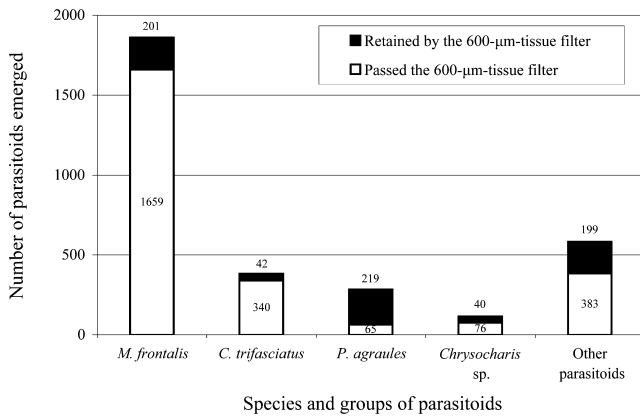


Fig. 4. Total number of parasitoids that passed through or were retained by the 600-µm-tissue filter of the 10 mass-emergence devices over the emergence period in 2003.

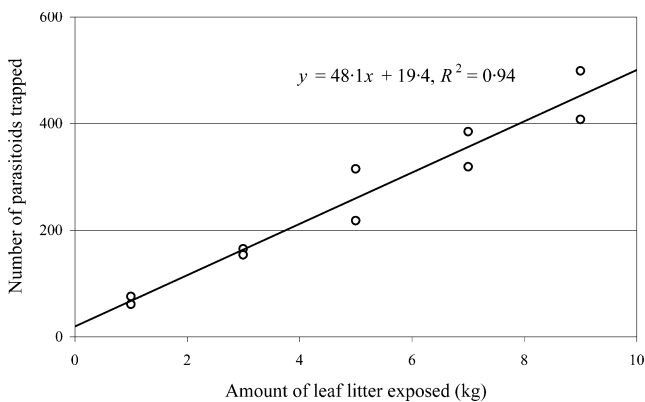


Fig. 5. Impact of the amount of horse chestnut leaf litter exposed on the number of parasitoids that passed through the 600-µm-tissue filter of the 10 mass-emergence devices over the emergence period in 2003. Data were fitted by a linear regression model.

difficulty in emerging from larger amounts of moderately compressed litter. Although the efficiency of the separators was comparable to the year before, only  $55.3 \pm 9.8$  SD parasitoids out of 1 kg horse chestnut leaf litter passed through the filter in spring 2003.

### 3.4. Field test of mass-emergence devices

Parasitoid augmentation significantly increased percent parasitism of *C. ohridella* at St. Galler Ring

(Table 2, Fig. 6A). The early release of parasitoids significantly increased percent parasitism compared to the late release treatment (Fisher's LSD:  $P=0.04$ ) and the no-release control (Fisher's LSD:  $P=0.02$ ). However, mass-emergence devices did not increase percent parasitism of *C. ohridella* compared to the no-release control at Eglisee (Table 2, Fig. 6B). At both sites percent parasitism in the third generation was significantly higher than in the first and second generation (Table 2, Figs. 6A and B). Overall, percent parasitism at St. Galler Ring was  $4.8\% \pm 2.5$  SD in the first,  $4.0\% \pm 3.1$  SD in the second and  $13.1\% \pm 7.8$  SD in the third generation of *C. ohridella*. At Eglisee, percent parasitism was quite similar with  $6.0\% \pm 3.4$  SD in the first,  $4.2\% \pm 2.3$  SD in the second and  $13.7\% \pm 5.7$  SD in the third generation.

The exposed mass-emergence devices did not reduce defoliation of *A. hippocastanum* trees by *C. ohridella* at the two sites (Table 2). The defoliation level increased significantly over the three generations at both sites (Figs. 6C and D).

## 4. Discussion

Our study demonstrated that emerging parasitoids can be separated from their host mechanically by their body size alone and that the set up of mass-emergence devices in the field can significantly increase parasitism rates. Following the stepwise development process outlined above it should be possible to build mass-emergence devices specifically adapted to different pest systems. The critical step in the development is the determination of the mesh size of the filter, whereas the design of the container is very flexible, and can take into consideration requirements of the specific system. Below we first discuss the main findings of the study with respect to the control of the horse chestnut leafminer, and second, consider implications of our work for the biocontrol practitioner.

### 4.1. Control of horse chestnut leafminer

In our field experiment, it is likely that we even underestimated the impact of the mass-emergence devices on

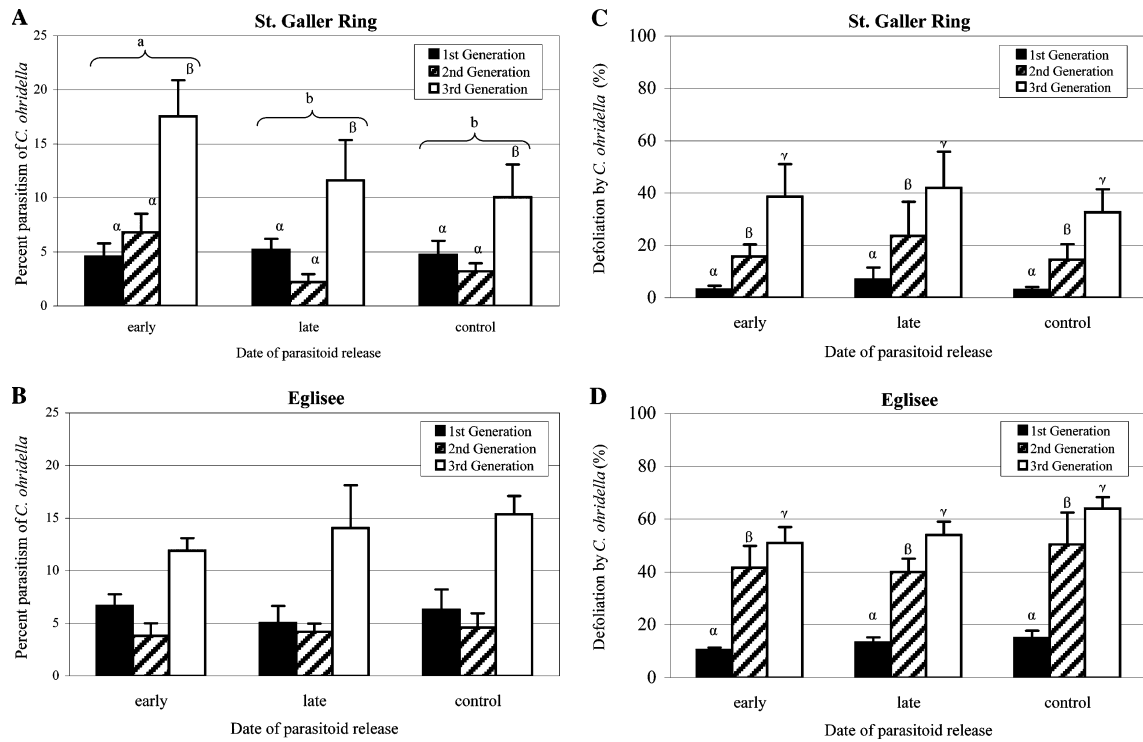


Fig. 6. Impact of exposed mass-emergence devices on (A–B) mean percent parasitism of *C. ohridella* (+SE), and (C–D) mean percent defoliation of *A. hippocastanum* trees (+SE) over the three generations at the two sites in Basel. Dates of parasitoid release with different Latin letters are significantly different ( $P < 0.05$ ) and leafminer generations with different Greek letters are significantly different ( $P < 0.01$ ). Black bars indicate the first, hatched bars the second and white bars the third generation of *C. ohridella*.

parasitism rates, because an unknown proportion of the parasitoids released will have dispersed over the whole site and not only parasitized larvae of *C. ohridella* on experimental trees, but also on control and untreated trees. Parasitoid dispersal would obscure differences in percent parasitism between treatment and control trees, but increase the overall parasitism rates at both sites. In support of this, the percent parasitism of leafminers at both sites was about two to three times higher than at neighbouring sites in Basel over the previous three years (2001: percent parasitism 2, 4, and 2% in the 1st, 2nd, and 3rd generation, respectively; 2002: 2 and 2% in the 1st and 2nd generation, respectively; 2003: 3, 4, and 7% in the 1st, 2nd, and 3rd generation, respectively; unpublished data of S. Girardoz and M. Kenis; data were collected and analysed in a similar way as described here). Moreover, the percent parasitism of 17.6% we measured in the third generation of the early treatment at St. Galler Ring (Fig. 1) is among the highest ever observed for *C. ohridella* in Central Europe (Heitland et al., 1999).

It is not surprising that our rather small-scale augmentation measures did not have an immediate impact on the defoliation level of *A. hippocastanum* trees. Hawkins et al. (1993) noted that parasitism of at least 36% is required for pest control to be effective. For this purpose the number of parasitoids released during our experiment was presumably much too low in relation to the number of horse chestnut trees at the two sites. For

parasitoid populations to build sufficiently to impact the density of *C. ohridella* will probably take longer than a single season. Thus, mass-releases of parasitoids by means of our devices should be continued over several years. Whether natural enemies can control pest populations will ultimately depend on the recruitment of the pest relative to the enhanced activity of its antagonists (Berryman, 1999). Thus, every effort should be made to properly remove horse chestnut leaf litter at sites, where parasitoid conservation or augmentation is carried out (Gilbert et al., 2003; Kehrlı and Bacher, 2003).

In the garden trials, the majority of parasitoids emerged about one month earlier than *C. ohridella* and about two months before the first usable host stages were present (Freise and Heitland, 2001; Grabenweger, 2003), supporting the hypothesis that poor synchronisation of the leafminer and its parasitoids might be one of the main reasons for low parasitism rates of *C. ohridella* (Grabenweger, 2004). However, the late release of parasitoids in our field study, which was an attempt to better synchronize parasitoids with their hosts, did not lead to an expected higher percent parasitism compared to the early release or to the control treatment. Extended storage of parasitoids at low temperatures or irregularities in the temperature control of the storage facility may have been responsible for this failure. Alternatively, there may be no need to synchronize the parasitoids with their host, but further research is needed to clarify this point.

Even when the foliage in the mass-emergence devices was moderately compressed parasitoids seemed to have no difficulty reaching the openings. It appears that even greater amounts of pest-infested leaf litter than used in our study can be placed in single mass-emergence devices without compromising their efficiency. However, parasitoid species of similar body size as their host might have difficulties in passing through the tissue filter, as was the case for *P. agraulis* in our study. Fortunately, *M. frontalis*, by far the most abundant parasitoid of *C. ohridella* in Central Europe (Grabenweger, 2003), had no difficulty in passing through the filter. The few hosts that were able to overcome the mesh size of the filter used in our study were mainly males of *C. ohridella*, which are in general smaller than the females (Freise, 2001). From an ecological point of view, the escape of males of a pest can often be tolerated, because population growth and therefore pest outbreaks are mainly determined by the population size of fertile females. Thus, mass-emergence devices could be used as a component of an integrated control strategy against the horse chestnut leafminer in urban areas of Europe.

#### 4.2. Implications for the biocontrol practitioner

Mass-emergence devices for parasitoids can be used as a biocontrol technique either to conserve or to augment natural enemies. In our case, we used leaves from Bern for mass releases in Basel, and therefore augmented local parasitoid densities. However, one can also use litter from the same site and in this way conserve the local parasitoid community.

A wider use of augmentative releases of parasitoids for biocontrol is often hindered by their high-cost compared to other control methods (Van Driesche and Bellows, 1996). Mass-emergence devices to augment or conserve local parasitoid populations can be a low-cost alternative to the release of conventionally reared natural enemies. The basic principle of this technique, the self-separation of the smaller parasitoids from their larger hosts, both of which emerge out of stored plant material, is widely applicable. The approach may be useful against a range of herbivorous pests provided the following criteria are met:

1. The pest as well as its parasitoids have a dormant stage in or on the plant.
2. The pest-infested plant material can be collected and stored in mass-emergence devices.
3. The parasitoids are smaller than their host.
4. The emergence of parasitoids can be synchronised with the phenology of the pest.

Pest species fulfilling these criteria include other leafminer pests, such as the spotted tentiform leafminer (*Phyllonorycter blancardella* [Fabr.]) in apple orchards

(Pottinger and Le Roux, 1971), or pests of stored seeds (grains, beans), but other pests where conventional control failed or is restricted should also be considered. For example, mass-emergence devices may be of great value in integrated and organic production schemes, where chemical pest control is restricted or even prohibited.

In summary, the use of mass-emergence devices for parasitoids has several advantages. First, mass-emergence devices can be used to conserve the resident enemy populations compared to the conventional disposal of pest-infested plant material. Second, parasitoids released from mass-emergence devices are already pre-adapted to the target host and its natural environment, in contrast to conventionally mass-reared parasitoids, which frequently have a low acceptance of the target organism and a low survival rate in the field after release (Bautista and Harris, 1997; Gandolfi et al., 2003; Lewis et al., 1990). Finally, the devices are relatively cheap, easy to construct and can be applied to a broad range of plant protection and cropping systems. Thus, mass-emergence devices should be considered as an alternative pest control approach and this supplementary technique could be of particular value under conditions where the use of insecticides is restricted.

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