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Spatial effects on competition between the larval parasitoids *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) and *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae) parasitising the Mediterranean flour moth, *Ephestia kuehniella* Zeller

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Abstract

The Mediterranean flour moth *Ephestia kuehniella* is a suitable host for the hymenopteran parasitoids *Habrobracon hebetor* and *Venturia canescens*. The two larval parasitoids differ in the following biological traits: *H. hebetor* is an idiobiontic, gregarious ectoparasitoid, and *V. canescens* is a koinobiontic, solitary endoparasitoid. Previous laboratory data obtained in small-scale experiments (Petri-dish) showed that moth larvae parasitised by *V. canescens* were paralysed by *H. hebetor*, i.e. the latter parasitoid out-competed *V. canescens* completely. However, field observations in Central European bakeries and mills showed that both species occur simultaneously. Moreover, *V. canescens* seems to be more common than *H. hebetor* in these stored-product environments harbouring populations of *E. kuehniella*.

These observations led to the hypothesis that spatial scale affects the competition between the two parasitoids. Keeping the host-parasitoids ratios constant, experimental chambers with three different volumes were tested, with a maximum volume of 8 m^3. Hosts were exposed in four patches per room. In trials conducted in small boxes, the increase of parasitism by increasing female *H. hebetor* was confirmed. It was shown that the presence of *V. canescens* did not affect parasitism by *H. hebetor* under these conditions. With increasing number of female *H. hebetor* in the box, the number of emerging adult *E. kuehniella* decreased. The additional presence of *V. canescens* drastically reduced the number of *E. kuehniella* emerging in all trials. Spatial effects on competition were detected by comparison of experimental boxes and cages with different volumes. With increasing volume, the percentage of *V. canescens* emerging was constant, and the number of *H. hebetor* progeny was decreasing. Patch exploitation was decreasing in *H. hebetor* with increasing volume, but not in *V. canescens*. The data suggest that *V. canescens* is more competitive in large rooms where *H. hebetor* fails to completely find and exploit host patches.

Key words: Biological control, stored products, idiobiont, koinobiont, host-finding.

Introduction

The two larval parasitoids *Venturia canescens* (Gravenhorst) and *Habrobracon hebetor* (Say) share a number of pyralid lepidopteran hosts, e.

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g. the Mediterranean flour moth *Ephestia kuehniella,* the Indianmeal moth *Plodia interpunctella,* and the tropical warehouse moth *E. cautella*. *H. hebetor* is an idiobiont, gregarious ectoparasitoid, i.e. the moth larva is paralysed prior to oviposition, and several parasitoid larvae develop on one host larva (Hase, 1922). *V. canescens* is a koinobiont, solitary endoparasitoid, i. e. the moth larva is not paralysed prior to oviposition, and only one parasitoid larva develops within one host larva, which is killed shortly before pupation (Hase, 1937).

In small-scale laboratory experiments, Press et al. (1977) showed that *H. hebetor* completely out-competes *V. canescens* in case of simultaneous occurrence. Due to paralysation of *V. canescens*-parasitised hosts by *H. hebetor,* the larva of *V. canescens* ceases development and is consumed by the *H. hebetor*-larva together with the moth larva. On the other hand, *V. canescens* is not able to develop within *H. hebetor*-parasitised moth larvae. However, in mills, warehouses and retail stores, both parasitoid species were found to naturally occur simultaneously (Prozell and Schöller, 1998; Lukas, 2002). Moreover, in cases where laboratory-reared *H. hebetor* were released for biological control, *V. canescens* continued to occur naturally (Prozell and Schöller, unpubl. data). One potential factor explaining this difference between laboratory- and field observations is the different spatial size of the habitats. Effective long-range host-finding is known for both parasitoid species (Parra et al., 1996; Desouhant et al., 2005), but its effect on competition was not studied yet. The aim of the present study was to compare the effect of the size of different experimental rooms on competition between *H. hebetor* and *V. canescens.*

Materials and methods

Insect rearing

Ephestia kuehniella was reared in 3l-vials by adding 500 moth eggs to 500 ml wheat grains. On top of the wheat, a disc consisting of rolled corrugated cardboard (diameter approx 100 mm, thickness approx. 15 mm) was placed to provide hiding space for pupation. *Venturia canescens* was reared at 20 ± 1 °C and 60 to 70 % r.h. in constant darkness on last-instar larvae of *E. kuehniella* at a ratio of 70 larvae : five parasitoids, *V. canescens* was honey-fed and had a maximum age of 24 h at the beginning of each experiment. *H. hebetor* was reared at 26 ± 1 °C and 60 to 70 % r.h. in constant darkness on last-instar larvae of *E. kuehniella* at a ratio of ten larvae : two female parasitoids, *H. hebetor* was honey-fed and had a maximum age of 24 h. *Venturia canescens* and *H. hebetor used* for the experimental trials, had a maximum age of 6 h.

Experimental work

Small plastic boxes (length 32.7 cm, width 21.8 cm, high 9 cm) with a volume of ca. 6,415 cm³, covered with a plastic lid were the smallest unit tested. The medium-sized boxes (length 98.4 cm, width 38.5 cm, high 49.0 cm) with a volume of 18,5631.6 cm³ or ca. 180 l were made from glass, covered with a metal plate. The large cubes (length 200 cm, width 200 cm, high 200 cm) with a volume of 8 m³ were a wooden frame covered with nylon gauze with a mesh-width of 0.7 mm. The moth larvae were exposed in small cages covered with plastic-gauze (mesh-width 0.7 mm) described in Wohlgemuth & Hennlich (1985) which allowed the wasp to parasitise but which prevented escape of the larvae. Each patch consisted of half a tea-spoon of wheat and ten larvae of *E. kuehniella*. Within each of the experimental rooms, four cages, i. e. discrete patches, were exposed. The parasitoids were released in the center of the room.

In the experiments in the small boxes, 2, 4, 8 or 20 female *H. hebetor* were released, i. e. the parasitoid: host-ratio was 1:20, 1:10, 1:5 and 1:2, respectively. Half the number of males were released, respectively. In the experiments on spatial effects, four *V. canescens* were added in each trial. The experimental design is summarized in Table 1. Each trial was replicated three times, the experiment was stopped after one week. The untreated control consisted of the small cages stored in boxes without parasitoids. The number of dead, parasitised, and active larvae as well as pupae of *E. kuehniella* were recorded. Larvae of *H. hebetor*, *V. canescens* and *E. kuehniella* were allowed to emerge, and the number of emerged adults was recorded.

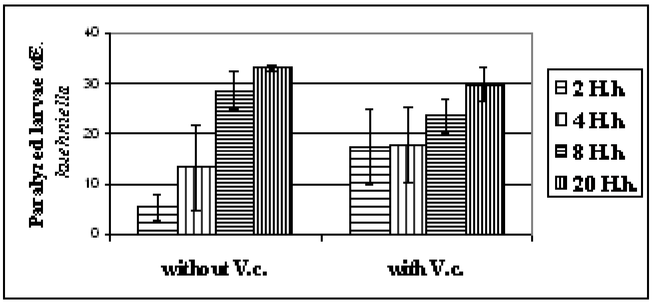

Figure 1. Mean number ± SEM of *E. kuehniella*-larvae paralysed by *H. hebetor* depending on the number of female *H. hebetor*, and the presence of *V. canescens* (n=3).

Results

Experiments in a small box

With increasing number of female *H. hebetor* in the box, the number of paralysed moth larvae increased. No effect of the presence of *V. canescens* on the number of moth larvae paralysed by *H. hebetor* was detected (Figure 1).

With increasing number of female *H. hebetor* in the box, the number of emerging adult *E. kuehniella* decreased. The additional presence of *V. canescens* drastically reduced the number of adult *E. kuehniella* emerging in all trials (Figure 2).

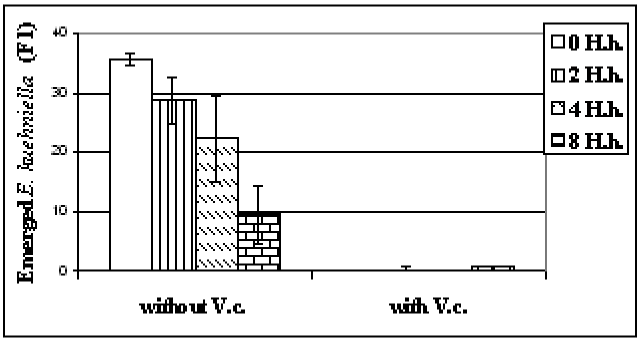

Figure 2. Mean number ± SEM of *E. kuehniella* adults emerging depending on the number of female *H. hebetor*, and the presence of *V. canescens* (n=3).

Table 1. Design of the experiments on spatial effects.

Experimental room	No. larvae *E. kuehniella*	No. *H. hebetor* female/male	No. *V. canescens*
Small box	40	2 / 1	4
Small box	40	4 / 2	4
Small box	40	8 / 4	4
Small box	40	20 / 10	4
Medium-sized box	40	2 / 1	4
Medium-sized box	40	4 / 2	4
Medium-sized box	40	8 / 4	4
Medium-sized box	40	20 / 10	4
Large cube	40	2 / 1	4
Large cube	40	4 / 2	4
Large cube	40	8 / 4	4
Large cube	40	20 / 10	4

The effectiveness of the parasitoid releases was corrected by natural mortality of *E. kuehniella* according to Abbott (1925), with X-Y / X x 100 = percent effectiveness; X = untreated control, percentage living insects; Y = treatment, percentage living insects.

Spatial effect

In the small and medium-sized boxes, no difference in the number of paralysed moth larvae was found for the respective host-parasitoid ratios (Figure 3). Significantly less paralysed moth larvae were recorded in the large cube compared with the small and medium-sized boxes ($p<0.05$). No paralysed larvae were found in the trials with eight *H. hebetor* in the large cube.

If no *H. hebetor* were present, no difference in the number of emerged progeny of *V. canescens* was recorded in the small and medium-sized boxes, respectively. Less *V. canescens* emerged in the large cubes. The presence of *H. hebetor* significantly reduced the number of emerged progeny of *V. canescens*, this reduction was less pronounced in the large cube (Figure 4).

The results of the effect of two different ratios of females of the two parasitoid species are summarized in Table 2. If only *V. canescens* was present, there was no difference between the percentage host-patches parasitised by *V. canescens* in the small boxes and the medium-sized boxes, all patches were found. The number of

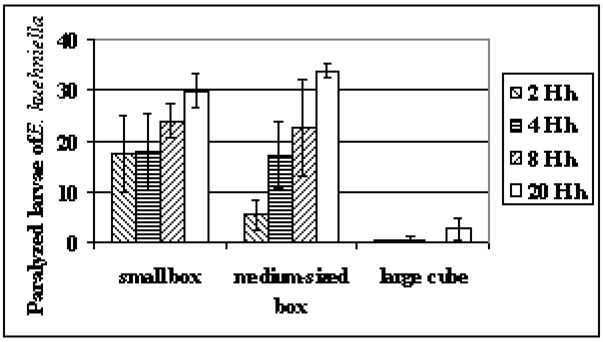

Figure 3. Mean number ± SEM of *E. kuehniella* larvae paralyzed by *H. hebetor* depending on the volume of the experimental room (n=3).

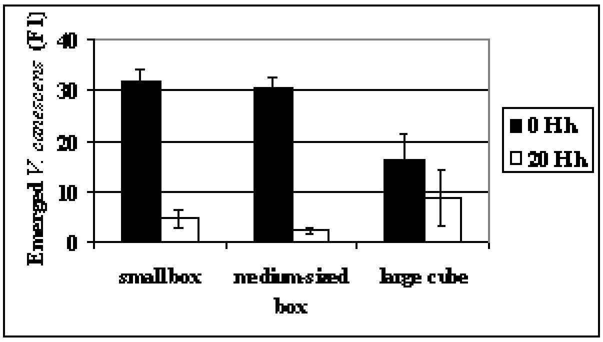

Figure 4. Mean number ± SEM of emerged progeny of *V. canescens* depending on the volume of the experimental room and the presence or absence of *H. hebetor*.

Table 2. Percent host-patches parasitised by *Venturia canescens* and *Habrobracon hebetor*, respectively, depending on the size of the experimental boxes, and the ratio of females of the two parasitoid species.

	Small box	Medium-sized box	Large cube
4 V. canescens* / no *H. hebetor			
Percentage host-patches parasitised by *V. canescens*	100 a	100 a	58.3 b
Mean number of *V. canescens* progeny per host-patch located	7.9	7.7	7.0
4 V. canescens* / 2 *H. hebetor			
Percentage host-patches parasitised by *H. hebetor*	66.7 B	33.3 C	8.3 D
Mean number of *H. hebetor* progeny per host-patch located	6.5	7.0	1.0
4 V. canescens* / 20 *H. hebetor			
Percentage host-patches parasitised by *H. hebetor*	100 A	100 A	25.0 C
Mean number of *H. hebetor* progeny per host-patch located	7.4	8.4	2.7
Percentage host-patches with progeny of *V. canescens*	58.3 b	41.7b	41.7 b
Mean number of *V. canescens* progeny per host-patch (patches with at least one progeny of *V. canescens* only)	2.0	1.4	5.2

parasitised larvae per patch ranged between six and ten. Less host-patches were parasitised in the large cube. There was no difference in the mean number of *V. canescens* progeny per host-patch located between the three scales. If two female *H. hebetor* were added to the four *V. canescens*, both the percentage of host-patches parasitised by *H. hebetor* and the mean number of *H. hebetor* progeny per host-patch located was decreasing with increasing size of the experimental boxes. If twenty female *H. hebetor* were added to the four *V. canescens*, all host-patches were parasitised by *H. hebetor* in the small and the medium-sized boxes, and one fourth of the host-patches in the large cube. In this trial, about half of the host-patches was successfully parasitised by *V. canescens* in the small and the medium-sized boxes. However, the percentage host-patches parasitised by *V. canescens* was not affected in the large cube, and more *V. canescens* progeny was produced compared to the small and the medium-sized boxes.

Effectiveness of the parasitoids

In the untreated control, 35.7 *E. kuehniella* emerged, corresponding to 89.17 %. The effectiveness of the parasitoids *H. hebetor* and *V. canescens* corrected by natural mortality is given in Table 3. Effectiveness of *H. hebetor* without *V. canescens* was increasing in the small box with increasing number of females (Table 2). Control of *E. kuehniella* was greater when both parasitoid species were present compared to the presence of *H. hebetor* alone. No difference in effectiveness was detected in all treatments with *V. canescens* in the small – and medium-sized boxes. Maximum effectiveness was 89.17 %, which was achieved both with *V. canescens* and the combination of *V. canescens* and *H. hebetor*. In the large cube, effectiveness decreased to about 50 %, maximum was 69.17 % and minimum 33.35 %.

Discussion

In trials in small boxes, the findings of Press et al. (1977) were confirmed, i. e. at this scale the reproduction of *H. hebetor* is not affected by the presence of *V. canescens* but the reproduction of *V. canescens* is significantly reduced by the presence of *H. hebetor*. However, the extinction of *V. canescens* after release of *H. hebetor* under practical conditions predicted by Press et al. (1977) was not confirmed after up-scaling the experiment. Whereas parasitism of *H. hebetor* was significantly lower in the large cube compared to the small rooms, there was no such effect in *V. canescens*, suggesting a better long-range host finding in this species. Few studies addressed the minimum distance which is necessary for the parasitoids to locate their hosts. In trials of Murr (1930) *H. hebetor* found always larvae of *E. kuehniella* 10.5 cm distant from the release point, but she did not test other distances. In our trials, the maximum distance in the small boxes was 14 cm. Parra et al. (1996) showed the ability of *H. hebetor* to find hosts 65 cm distant to the release point in a wind-tunnel experiment, a distance comparable to our medium-sized boxes. In our large cubes, the minimum distance to the moth larvae was 2 m. The host-finding ability of *V.*

Table 3. Effectiveness of 0, 2, 4, 8 und 20 female *H. hebetor*, respectively, with and without the presence of *V. canescens* in a small box, and with the presence of *V. canescens* in a medium-sized box and a large cube corrected according to Abbott (1925).

Number female *H. hebetor* / treatment	0	2	4	8	20
Effectiveness without *V. canescens* in small box [%]	0	7.5	33.35	65.85	75.85
Effectiveness with *V. canescens* in small box [%]	89.17	88.35	89.17	87.50	89.17
Effectiveness with *V. canescens* in medium-sized box [%]	85.85	89.17	88.35	88.35	89.17
Effectiveness with *V. canescens* in large cube [%]	45.85	69.17	50.00	50.00	33.35

canescens was investigated by Desouhant et al. (2005). They showed *V. canescens* to find hosts 75 cm distant from the release site.

V. canescens was found to be widely distributed in Central Europe in bakeries and mills together with *E. kuehniella* (Prozell and Schöller, 1998; Lukas, 2002). The co-existence of two larval parasitoids of *E. kuehniella* using the same kairomone suggest differences in their respective biology, which might also be of interest for their application in biological control. The higher number of progeny per female in *H. hebetor* could allow this species to establish in aggregations of *E. kuehniella*, whereas *V. canescens* should also be able to persist in small scattered populations of *E. kuehniella* due to its good long-range host finding and thelytokous mode of reproduction. Currently, *H. hebetor* is provided commercially in Europe as release units containing 25 pupae in a cardboard-card, which allows distribution of the wasps at the developmental sites of the moths (Prozell and Schöller, 2003). The finding that *H. hebetor* has a weaker long-range host finding compared to *V. canescens* supports this approach, because the release-units can be distributed within the infested facilities. In cases where parasitism by *H. hebetor* is not complete, the combination of the two parasitoids can improve the biological control of *E. kuehniella*.

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