Parasitism of single or combined pyralid populations by *Venturia canescens* and *Habrobracon hebetor* in laboratory and storeroom conditions

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AUTHOR CONTRIBUTIONS

CC, JR and EL conceived, designed and performed research, EL analyzed data, CC and JR wrote the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST.

The authors declare that they have no conflict of interest.

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KEY MESSAGE

- *Plodia interpunctella* (PI) and *Ephestia kuehniella* (EK) are stored product pests that are parasitized by *Venturia canescens* (VC) and *Habrobracon hebetor* (HH).
- VC, HH increased mortality by 40% of PI, EK or PI+EK in laboratory and small storeroom experiments.
- Reproduction of HH was similar on both hosts, while reproduction of VC was higher on EK than on PI.
- Good biocontrol potential of EK, and of EK+PI when HH and VC were combined. .

ABSTRACT

Venturia canescens and *Habrobracon hebetor* are cosmopolitan parasitoids found in large numbers in food processing facilities in north-eastern Spain, as well as in many other countries. These parasitoids attack larvae of pyralid moths that are important pests of stored products and food industries worldwide. In this study, we evaluated the performance of these two parasitoids when offered single or combined populations of *Plodia interpunctella* and *Ephestia kuehniella,* since these can occur together in storehouses. We tested the parasitoid's performance in small cages under laboratory conditions and small experimental storerooms (≈ 30 m^3). In the laboratory, the two parasitoids were able to reduce pyralid populations by more than 37% over a 48-h period (40-44% for *E. kuehniella,* 37-41% of *P. interpunctella* and 53-55% of both hosts when offered together). Similar results were obtained in small storerooms after 10 days: a greater than 35% reduction in pyralid populations also was obtained when host species were offered either singly (*E. kuehniella* or *P. interpunctella*) or in combination (*E. kuehniella* + *P. interpunctella*) (35-57% for *E. kuehniella,* 40-54% of *P. interpunctella* and 41-46% of both hosts when offered together). Parasitism was consistently good from June through November (mean temperatures from 18 to 28 ºC and 9.5 to 15 h of daylight). Therefore, both parasitoids single or in combination, can be efficient biological control agents of these two pyralid moths when infesting stored food facilities.

KEYWORDS: *Ephestia kuehniella*; *Plodia interpunctella*; Lepidoptera, Pyralidae; larval parasitoids.

1. INTRODUCTION

The Mediterranean flour moth, *Ephestia kuehniella* Zeller, and the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera, Pyralidae), are important pests in storage facilities worldwide (Belda and Riudavets 2013). *Plodia interpunctella* feeds on a broad range of commodities, such as cereal products, nuts, almonds, seeds, cocoa beans, chocolate, dried fruits, tobacco, and pet-food. However, *E. kuehniella* is more restricted in diet and rarely infests stored products other than flour (Cox and Bell 1991; Sedlacek et al. 1996). These two insects sometimes occur simultaneously as mixed populations at some locations, whereas one species might predominate at other locations (Belda et al. 2011; Prozell and Schöller 1997). These insects can develop in the dust accumulated in corners and crevices of mills and other food storage or processing facilities that are difficult to access for cleaning. They can then disperse from these areas and contaminate food products. Although sanitation is fundamental to managing these moths, insecticides are still mainly used for their control. Conventional pesticides and fumigants sometimes exhibit reduced effectiveness due to insect resistance (Attia et al. 1979; Huang et al. 2004). Also, pesticide residues in food products and the environment are matters of concern, so alternative control methods that can minimize or eliminate residue problems are desirable. There are good promising alternatives to conventional pesticides and fumigants, as the use of botanical pesticides that are less toxic to the environment, the application of heat treatments or of controlled atmospheres (Campolo et al. 2013; Isman 2007; Riudavets et al. 2014; Wong-Corral et al. 2013). We will focus on the possibilities of biological control as a control strategy for pests that are located in the facilities were food products are stored.

Habrobracon hebetor (Say) (Hymenoptera, Braconidae) and *Venturia canescens* (Gravenhorst) (Hymenoptera, Ichneumonidae) are two cosmopolitan parasitoids that attack larvae of several species of Lepidoptera, including *P. interpunctella* and *E. kuehniella* (Paust et al. 2008). These parasitoids can be found occurring naturally in facilities as bakeries and mills and commodities as stored figs (Athanassiou and Saitanis 2006; Johnson et al. 2000; Prozell and Schöller 1997, 1998; Sedlacek et al. 1998; Stejskal et al. 2006). In Spain, these parasitoids have been found in dried fruit storehouses and milling companies, where they often aggregate near windows or light sources during spring and summer (Belda and Riudavets 2013). Commercial use has been made of *H. hebetor* in combination with *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae) to control *E. kuehniella* and *P. interpunctella* in organic bakeries and mills in Germany and Austria (Prozell and Scholler 2003), and *H. hebetor* has been combined with mating disruption for control of *P. interpunctella* in a chocolate factory in Italy (Trematerra et al. 2017).

Habrobracon hebetor and *V. canescens* have different life history traits. *Habrobracon hebetor* is a gregarious idiobiont ectoparasitoid; the female paralyses a host larva before laying some eggs on it, preventing any further development of the host. After hatching, several young parasitoid larvae feed on the same host until pupation (Eliopoulos and Stathas 2008). *Venturia canescens* is a solitary koinobiont endoparasitoid; the female lays only one egg inside the host larva, and it continues to feed and develop after parasitism. It is also a thelytokous species, so all individuals are female (Eliopoulos 2006). Both parasitoids prefer the last instar larvae of their hosts, a stage in which the larvae wander in search of a place to pupate (Darwish et al. 2003; Hagstrum and Smittle 1977; Sait et al. 1997). In general, the host in which parasitoids are reared may affect the efficacy of parasitoid release. No differences in demographic parameters were found when *H. hebetor* developed on *E. kuehniella* versus on *P. interpunctella* (Eliopoulos and Stathas 2008), and *V. canescens* develop similarly on third to fifth instar larvae of both *E. kuehniella* and *P. interpunctella,* (Eliopoulos and Stathas 2003, 2005; Harvey et al. 1994). However, the effect of the rearing host on the performance of *H. hebetor* and *V. canescens* it is not well-known

Small-scale laboratory experiments have shown that *H. hebetor* outcompetes *V. canescens* since larvae parasitized by *V. canescens* are subsequently paralyzed by *H. hebetor,* which impedes further development of *V. canescens* larvae (Press et al. 1977). There are also spatial scale effects on parasitoid competition; patch exploitation by *H. hebetor* decreased with increasing volume of experimental cages, but exploitation by *V. canescens* did not (Paust et al. 2008). *Habrobracon hebetor* seems to follow large host groups and has a more limited dispersion than *V. canescens,* whereas the latter is more abundant when host populations are low (Paust et al. 2008). These differences in behaviour may facilitate their coexistence in the same storage facility.

There is abundant information on the performance of these parasitoids when provided one host species in confined laboratory conditions or on their occurrence together in the colonization of different stored facilities and commodities, as previously stated. We were interested in assessing their efficacy when mixed host populations were present, which is very common in food industry facilities, and at intermediate spatial scales, such as in small experimental storerooms. We formulated the following hypothesis: 1) Each parasitoid will perform better on the rearing host than on the alternative when offered alone, and better on the rearing host than the alternative when both hosts are offered; 2) For a given parasitoid:host ratio, we hypothesized *H. hebetor* efficacy would decrease in larger storerooms compared to smaller cages, but that the efficacy of *V. canescens* would not. This decrease was predicted to be greater in the presence of mixed host species compared to a single species; and 3) Assuming the previous hypotheses are supported, then the simultaneous release of both parasitoids should improve parasitism efficacy since each species will perform better on its rearing host.

Our aim was to predict the performance of these two parasitoids when released in commercial facilities for the control of single or combined populations of the two pyralid moths. Therefore, we performed trials at two different spatial scales (lab microcosm and storeroom), in which we offered either single or mixed populations of *E. kuehniella* and *P. interpunctella* to females of *H. hebetor* and *V. canescens.* Furthermore, we tested mixed populations of the two parasitoids in storerooms.

2. MATERIALS AND METHODS

Colonies of *E. kuehniella, P. interpunctella, V. canescens,* and *H. hebetor* were started with adults collected from stored-product facilities and mills in north-eastern Spain. They were reared in controlled conditions at 25 ± 1ºC, 65 ± 5% relative humidity (RH) under a photoperiod of 16:8 h (L:D). The moths were reared in l-L glass jars with 250 g of a mixture of white wheat flour and 7% yeast. Two-hundred-fifty eggs were added to each jar. After 3–4 weeks, larvae were obtained for the bioassays and the rearing of the parasitoids. *Habrobracon hebetor* was reared in ventilated glass jars containing third and fourth instar larvae of *P. interpunctella*, and *V. canescens* was reared in ventilated glass jars containing third and fourth instar larvae of *E. kuehniella*. To increase egg loads, adults were provided honey impregnated on absorbent paper.

2.1. Laboratory experiment.

Experiments were conducted under controlled conditions of 28 ± 2ºC, 70 ± 5% RH under a photoperiod of 16:8 h (L:D). Parasitoid females (1- to 7-days-old) were preconditioned for 24 h before the experiment by starving without a host while allowing them access to water and honey. *Habrobracon hebetor* females also were kept with males, in the proportion of one male to every two females. Arenas consisted of ventilated cages (23 × 16 × 8 cm) containing 18 g of flour and 20 hosts of third- to fourth-instar pyralid larvae. Cages for *V. canescens* also contained a moisture and feeding source consisting of a tube (10 × 1 cm) with a 10% honey solution and a cotton plug. Cages for *H. hebetor* contained a strip of 5 × 1-cm filter paper with honey. In each cage, four females of one parasitoid species were released for 48 h. After removing the female parasitoid, cages were kept at 28ºC until the emergence of the adult hosts and adult parasitoids.

For each parasitoid species, three treatments were carried out: two single-species treatments with 20 larvae of *E. kuehniella* or 20 larvae of *P. interpunctella* and one mixed species treatment, with 10 larvae of *E. kuehniella* plus 10 larvae of *P. interpunctella*. Three control treatments (two single and the mixed host combinations, but without parasitoids) were also carried out. Ten replicates per treatment were done, including the controls.

2.2. Small storeroom experiment

The small storeroom experiment was performed from June to November of 2016 in an empty facility that simulated a small storehouse. This facility had four rooms of 25 to 32 m^3, each one with a window of 1.20 × 1.50 m, a door, and a table in the centre as the unique furniture. A roller blind attenuated light from the window. Climatic conditions were those naturally occurring in the rooms and temperatures were raised by the heating system only in the last month (November). During the experimental period, mean temperatures ranged between 18 and 28ºC, mean RH between 59 to 75%, and the period of light between 9 h 30 min and 15 h 9 min (Table 1, supplementary material). The four rooms had similar temperature and humidity conditions.

In each room, there were eight host patches, and each patch consisted of ten third and fourth instar pyralid larvae inside a funnel trap (18 cm high × 15 cm diameter) containing 20 g of a mix of flour plus bran and a corrugated piece of paperboard as a hiding place. Host patches were placed in every corner of the room as follows: four traps in the ground corners and four traps hanged up in the ceiling corners. A tube with a cotton plug containing 20 ml of a 20% honey solution was also hung on one wall of the room for parasitoid feeding. The experiment started when 16 parasitoid females were released in the centre of each room (a parasitoid: host ratio of 0.2) and ended ten days later when traps were collected. Afterwards, rooms were carefully inspected to collect any surviving parasitoid and were kept empty for three days before start the next replicate. Traps were incubated at 25ºC until the emergence of adult hosts or adult parasitoids. Every room had one treatment, and the treatments were alternated from room to room during replication to avoid any bias due to possible differences in room conditions.

For each parasitoid species and the combination of both parasitoids (½ *H. hebetor* and ½ *V. canescens*), three host treatments were conducted: two single treatments with 10 larvae per trap of *E. kuehniella* or with 10 larvae per trap of *P. interpunctella,* and one mixed treatment with five larvae of *E. kuehniella* plus five larvae of *P. interpunctella* per trap. A control treatment, consisting of a trap with the same host combination but closed with a lid to avoid parasitization was also placed in the centre of each room. Four replicates (considering each room as a replicate) were done per host and parasitoid treatment.

2.3. Data analysis

The variables evaluated in the laboratory experiment included the number of larval hosts that completed development from both moth species and their sex ratios, percentage host mortality, number of F_1 progeny of *V. canescens* and *H. hebetor*, and the sex ratio of *H. hebetor*; biocontrol potential, that is the proportion of parasitoid females produced per surviving host in relation to the proportion of parasitoid females released (0.2 per host larvae) was also evaluated. The variables evaluated in the storeroom experiment were the number of larval hosts that completed development from each moth species, percentage host mortality, the number of *V. canescens* and *H. hebetor* adults produced, and number and location within the rooms of parasitized host patches. The proportion of parasitoid females produced per larval host that completed development (biocontrol potential) was calculated using the *H. hebetor* sex ratio determined in the laboratory experiment (0.27 with *E. kuehniella*, 0.27 with *P. interpunctella*, and 0.43 with *E. kuehniella* plus *P. interpunctella*). Comparisons among treatments were made using a one-way analysis of variance (ANOVA) when data complied with the requirements of parametric tests (homoscedasticity of variances); when significant, this test was followed by pairwise Tukey test. When data did not comply with the requirements of parametric tests, the Kruskal-Wallis analysis of variance, a non-parametric equivalent of ANOVA, was used to compare the treatments; when significant, this test was followed by pairwise Mann-Witney *U*-tests. The *p*-values were corrected for multiple comparisons using the Bonferroni technique. Percentage host mortality was calculated and corrected for control mortality as done by Abbott (1925):

(mortality in treatments – mortality in controls) / (100 – mortality in controls) × 100)

After arcsin transformation, the analysis of variance was used to compare treatments. Biocontrol potential was evaluated with a Student *t*-test ($P<0.05$) and the proportion of host patches in each corner of the room was compared by a Chi-square test. The JMP statistical package was used for all analysis (JMP, 8.0.1, 2009).

3 RESULTS

3.1. Laboratory experiment

3.1.1. Mortality of host population – Mortality of the pyralid moths in the control treatment was low, ranging from 7.00 ± 2.49% for *P. interpunctella* and 5.50 ± 1.57% for *E. kuehniella*, singly, and 4.00 ± 1.25% for mixed *P. interpunctella* + *E. kuehniella*. The total mortality of *E. kuehniella*, *P. interpunctella* or the combination was significantly higher in treatments with *V. canescens* or *H. hebetor* than in controls without parasitoids (Table 2, supplementary material). Therefore, both parasitoids were able to increase the mortality of moths. This mortality of hosts, which has been corrected using the control treatment, was greater than 40% and was similar for the two parasitoids with the two hosts, either when larval moths were offered as single species or in combination (F = 0.94, df = 5, 54 P = 0.460) (Figure 1, supplementary material).

3.1.2. Reproduction of parasitoids – The number of F_1 *V. canescens* that emerged from *P. interpunctella* larvae was significantly lower than of *H. hebetor*, while there were no significant differences among the other treatments (Kruskal-Wallis test: χ^2 = 12, 2, df = 5, P = 0.032) (Figure 1). Significantly, more males of *H. hebetor* emerged when single species of host larvae were offered while similar numbers of males and females emerged when combined larvae were offered (Table 2) (Figure 2, supplementary material).

3.1.3. Biocontrol potential – At the end of the trial, in the two treatments with *V. canescens*, significantly more female parasitoids were produced per surviving host than those introduced (one female per five host larvae): in single species of host with *E. kuehniella* and in mixed host populations. However, no significant differences were observed for the other treatments (Table 3, supplementary material) (Figure 2).

3.2. Small storeroom experiment

3.2.1. Mortality of hosts and production of F_1 parasitoids – In the control treatments, the mortality of pyralid moths was low, with percentages of mortality of 1.67 ± 1.231% for *P. interpunctella*, 12.5 ± 2.384% for *E. kuehniella* and 12.5 ± 4.885% for mixed *P. interpunctella* + *E. kuehniella* (4.2 ± 2.507% for *E. kuehniella* and 8.3 ± 4.228% for *P. interpunctella*). When *H. hebetor* was released, its impact on host mortality differed significantly among treatments. In the *P. interpunctella* treatment, the parasitoid significantly increased the mortality of hosts compared to the corresponding controls, whereas no differences were observed in the *E. kuehniella* or the mixed treatment. *Venturia canescens* significantly increased the mortality of hosts compared to the corresponding controls. When both parasitoids were released in combination, there was also a significant increase in mortality from the treatments considered compared to the control (Table 4, supplementary material). This increase in host mortality (normalized to the mortality in the control treatment) was greater than 30% and similar for the two parasitoids with the two hosts, either when offered singly or in combination ($F = 0.88$, $df = 8, 27$, $P = 0.543$) (Figure 3, supplementary material).

Both parasitoids were able to locate host patches in the storerooms and reproduce on the two host species offered. In the case of *H. hebetor*, no significant differences were observed in the number of adults produced when *E. kuehniella, P. interpunctella* or the combination of both hosts was offered (Figure 3A). However, *V. canescens* produced significantly more adults on the *E. kuehniella* population than on *P. interpunctella*, with an intermediate number of adults produced in the combined treatment (Figure 3B). When both parasitoids were released simultaneously, similar results were obtained on their reproduction than when released separately: no significant differences in the number of *H. hebetor* produced in the different host combinations offered but more *V. canescens* emerged in the treatment with *E. kuehniella* or *E. kuehniella* + *P. interpunctella* than in the treatment with *P. interpunctella* (Table 4, supplementary material) (Figure 3C).

3.2.2. Parasitoid distribution – When examining the distribution of the two parasitoids in the small storerooms, a similar number of larvae in the traps with *E. kuehniella*, with *P. interpunctella* or in the combined treatment with both hosts were parasitized by *H. hebetor*. *Venturia canescens* parasitized more larvae in traps involving *E. kuehniella* than *P. interpunctella*. When both parasitoids were released simultaneously, a similar number of larvae in traps were parasitized in all host treatments offered (Table 4, supplementary material). When examining the specific location of these traps in the eight corners of the storerooms, all positions received some parasitism by the two parasitoids in all treatments tested, indicating that female parasitoids were able to disperse in all directions of the storeroom (Figure 4A–F). Light coming from the window did not affect host location by the parasitoids; both parasitoids similarly parasitized hosts in the traps. Furthermore, both parasitoids similarly parasitized host traps located on the ground or the ceiling (Table 5, supplementary material). Therefore, both parasitoids were able to locate host patches situated in any position of the storeroom without showing any preference for a specific position.

3.3.3. Biocontrol potential – As mentioned, the initial host/parasitoid ratio was 0.2 (one female parasitoid for five host larvae). At the end of the trial, significantly more female parasitoids were produced per surviving host in two treatments with the combination of the two parasitoids *V. canescens* + *H. hebetor*: with *E. kuehniella* and in mixed host populations. No significant differences were observed for the other treatments (Table 6, supplementary material) (Figure 5). Therefore, biocontrol would only be expected to be successful with *H. hebetor* + *V. canescens* and two host combinations.

4. DISCUSSION

Both parasitoids similarly reduced the number of adult moths emerged at the two spatial scales tested, independently of the species and combination offered.

Host mortality observed in the present study is supported by the demographic data of the two parasitoids when reared with the two hosts. The r_m values of *H. hebetor* and *V. canescens* are significantly higher than those of their hosts at different host densities: they are 2.2–3.5- and 1.6–2.7-times higher than that of *E. kuehniella* and *P. interpunctella* respectively at 25ºC (Eliopoulos 2006; Eliopoulos and Stathas 2008). Lower mortality of both pyralid populations than those observed in the present study was obtained by Adarkwah and Schöller (2012) when releasing *H. hebetor* and *V. canescens* in 30-kg wheat jars infested with *P. interpunctella* or *E. kuehniella*. In their system, female parasitoids had to search for the host in a depth of 30 cm of grain. In contrast, in our study, moth larvae were easily available, only slightly buried in a thin layer of flour, simulating their presence in the dust accumulated in corners and crevices of mills. Parasitoid effectiveness decreases as soon as moth larvae have the chance to hide in the food substrate (Akinkurolere et al. 2009; Sait et al. 1997).

The fact that *V. canescens* reproduced better on *E. kuehniella* than on *P. interpunctella* could be due to their adaptation to the rearing host. Females of *V. canescens* released in this study came from a long-term colony reared on *E. kuehniella*, and switching from the rearing host to a new host could have negatively affected the reproduction of the parasitoid (Jones et al. 2015). Nevertheless, *V. canescens* prefers *E. kuehniella* as a host since the female is attracted to volatile compounds emanating from their larvae's silk, whether the parasitoid was reared with *P. interpunctella* or *E. kuehniella* (Belda and Riudavets 2012). On the other hand, *P. interpunctella* larvae are smaller than *E. kuehniella* larvae, and host size is a relevant parameter for the optimal reproduction of *V. canescens*: larger individuals are produced on larger larvae (Eliopoulos and Stathas 2005; Eliopoulos 2006; Hemerik and Harvey 1999). This host preference for *E. kuehniella* is also seen in the performance of *V. canescens* when mixed hosts were offered: reproduction was lower than in the rearing host (one single treatment) when in storerooms. It does not seem that the change of host significantly affected the performance of female *H. hebetor* since there were no differences between hosts in the offspring production. It has been shown that *E. kuehniella* is a high-quality host for *H. hebetor* (Saadat et al. 2014) and the negative effect of the change of host could have been compensated by the better quality of nourishment furnished by *E. kuehniella* as a host.

The scaling up in volume from a small cage (2.94 cm^3) to a room (20–32 m^3) did not affect the efficacy of both parasitoids. It was not a problem for *H. hebetor* to similarly locate and parasitize host patches, and the same occurred with the performance of *V. canescens* in the present study. This results contrast with those of Paust et al. (2008) that found a reduction in parasitism of *H. hebetor* and *V. canescens* when scaling experimental arenas from 6 cm^3 and 18 cm^3 to 8 m^3 during one week of interaction with *E. kuehniella* larvae.

Both parasitoids were able to locate patches in all positions inside the storerooms; parasitized hosts were found in the eight corners were patches were allocated and for the three host combinations tested. Therefore, host spatial distribution inside a storehouse does not seem to be a limiting factor for host finding by any of the two parasitoids tested in the present study. The presence of a natural light source did not affect the distribution of the parasitized patches by any of the two parasitoids; there were a similar number of parasitized patches located on the window wall as on the opposite wall. The light inside the rooms was very much attenuated by a roller blind in the window, which made the light in the room faint, simulating the atmosphere in a commercial storehouse. These conditions were optimal for *V. canescens* since the wasp seems to avoid sunny areas when dispersing in field conditions (Desouhant et al. 2003). Our results indicate that these parasitoids could be effective in most conditions occurring in storehouses in this region during most of the year. The availability of a source of sugars was a key element in the performance of parasitoids in the storeroom experiment. When parasitoids had access to this source of food, they live much longer than those that had no access or with limited access, as shown for *V. canescens* (Desouhant et al. 2005). The same authors mentioned that females in granaries and mills might leave the buildings to find food and have been reported to return to them with food droplets in their mandibles.

The simultaneous release of the two parasitoids did not improve parasitism efficacy as we predicted. Although we did not detect any improvement when the two parasitoids were released at the same time in any of the host combinations offered, also no interference between the two parasitoid species was observed. During the longer interaction time of the small storeroom experiment, the treatment with *E. kuehniella* and that with the combined hosts produced significantly more females of both parasitoids when both species were released simultaneously. This indicates that the biocontrol potential (production of females) in the long term is promising when combining both parasitoids in the presence of a mixed population of pyralids or just *E. kuehniella*. This result agrees with the available literature reporting the natural co-occurrence of these parasitoids in many types of storehouses and in many geographical regions.

In conclusion, *H. hebetor* and *V. canescens* perform well when they are released alone or in combination, and when the hosts present in the premises were *E. kuehniella*, *P. interpunctella* or a combination of these. They are promising biological agents for the control of pyralid moths that contaminates food storage facilities.

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FIGURE CAPTIONS

Fig. 1 Number (mean ± SE) F_1 progeny of *H. hebetor* (HH) and *V. canescens* (VC) produced in cages in which 20 host larvae from the two pyralid moths (*E. kuehniella* [EK] or *P. interpunctella* [PI]) were offered to four females of the parasitoids for 48 h in single or mixed combinations. Significant differences ($P<0.05$) among treatment means are indicated by different letters.

Fig. 2 Number of females of *H. hebetor* (HH) or *V. canescens* (VC) produced per surviving host in the laboratory experiment (*E. kuehniella* [EK], *P. interpunctella* [PI] or the combination of both hosts [EK-PI]) in each of the treatments considered. Red line indicates the proportion of female parasitoids released per host offered. Significant differences ($p<0.05$) from the 0.2 proportion is indicated by an asterisk.

Fig. 3. Number (mean ±SE) of adults of *H. hebetor* (graph A), of *V. canescens* (graph B) or of both parasitoids (graph C) produced per room on the different treatments considered: *E. kuehniella* (EK), *P. interpunctella* (PI) or both pyralids combined (EK-PI). 80 host larvae were offered in 8 patches per room to 16 female parasitoids during the 10-day period (n = 4 rooms).

Fig. 4. Percentage of traps containing host larvae parasitized by *H. hebetor* or by *V. canescens* according to their location in the eight corners of a room, four on the window wall *vs* four on the opposite wall (graphs A, B and C), and four on the ground *vs* four on the ceiling (graphs D, E and F).

Fig. 5 Number of females of *H. hebetor* (HH) or *V. canescens* (VC) produced per surviving host in the small storeroom experiment (*E. kuehniella* [EK], *P. interpunctella* [PI] or the combination of both hosts [EK-PI]) in each of the treatments considered. Red line indicates the proportion of female parasitoids released per host offered. Significant differences ($p<0.05$) from the 0.2 proportion is indicated by an asterisk.

SUPPLEMANTARY MATERIAL

TABLE CAPTIONS

Table 1. Maximum, minimum and mean temperature and relative humidity in one storeroom during the period of this experiment. The number of hours of light of the experimental period is also indicated.

Table 2. Analysis of variance (Kruskall Wallis test) followed by pairwise Mann-Witney *U*-tests of the number of larval hosts that completed development from both moth species in the presence or absence (control treatment) of the parasitoids *H. hebetor* and *V. canescens*. The *p*-values were corrected for multiple comparisons using the Bonferroni technique. Chi-square test of the proportion of males of the F_1 progeny of *H. hebetor,* in the laboratory experiment.

Table 3. Student *t*-test of the biocontrol potential of *H. hebetor* and *V. canescens,* that is the proportion of parasitoid females produced per surviving host in relation to the proportion of parasitoid females released (0.2 per host larvae), in the laboratory experiment.

Table 4. One way analysis of variance followed by Tukey-tests of the number of larval hosts that completed development from both moth species in the presence or absence (control treatment) of the parasitoids *H. hebetor* and *V. canescens,* of the host selection of two parasitoids, and of the number of F_1 progeny produced by the two parasitoids on the two hosts, in the small room experiment.

Table 5. Chi-square test of the proportion of host patches (traps) parasitized by the parasitoids *H. hebetor* and *V. canescens* in each corner of the room. No comparisons were made for *V. canescens* with *P. interpunctella* because only one trap was parasitized.

Table 6. Student *t*-test of the biocontrol potential of *H. hebetor* and *V. canescens* in the small room experiment. The proportion of parasitoid females produced per larval host that completed development was calculated using the *H. hebetor* sex ratio determined in the laboratory experiment (0.27 with *E. kuehniella*, 0.27 with *P. interpunctella*, and 0.43 with *E. kuehniella* plus *P. interpunctella*).

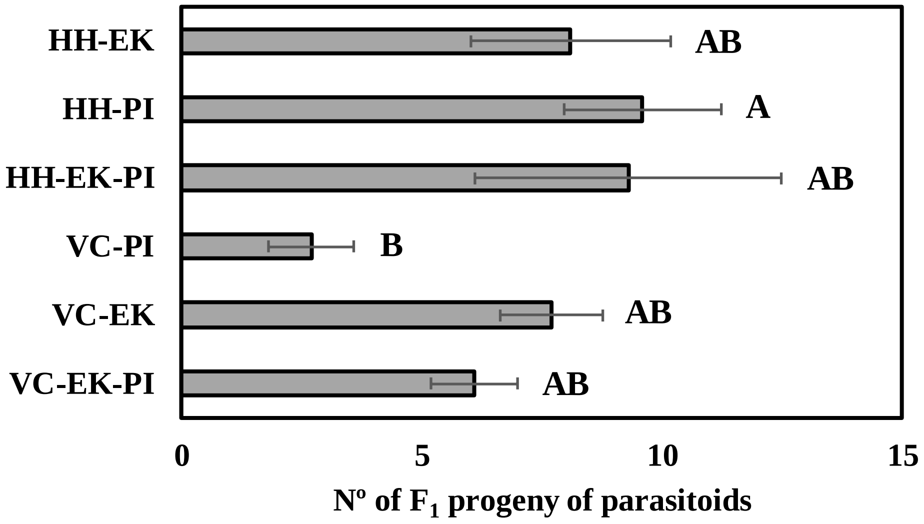
FIGURE CAPTIONS

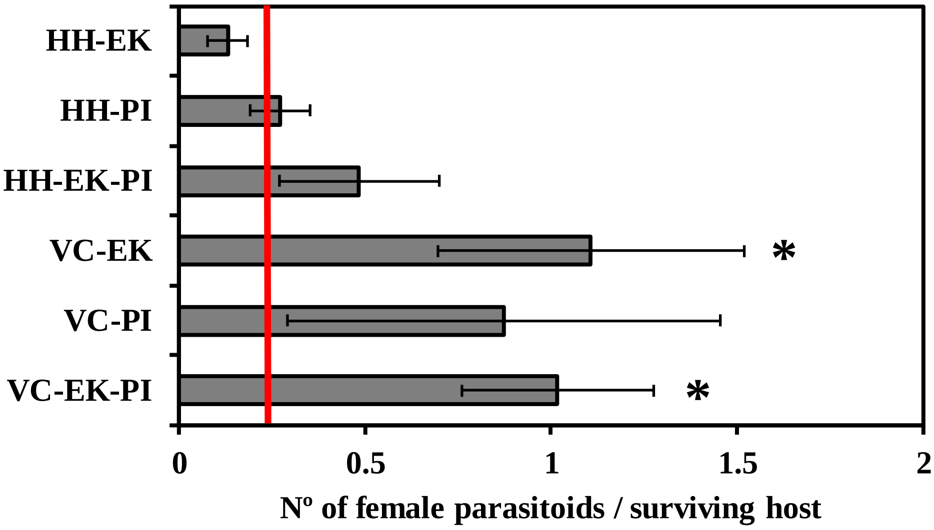
Fig. 1. Mean (± SE) percentage mortality of adults moths (*E. kuehniella* [EK], *P. interpunctella* [PI] or both pyralids combined [EK-PI]), when normalized to the control treatment, in the different treatments considered when the parasitoids *H. hebetor* (HH) and *V. canescens* (VC) were released during a 48-h period. There were no significant differences among treatments (*P*<0.05).

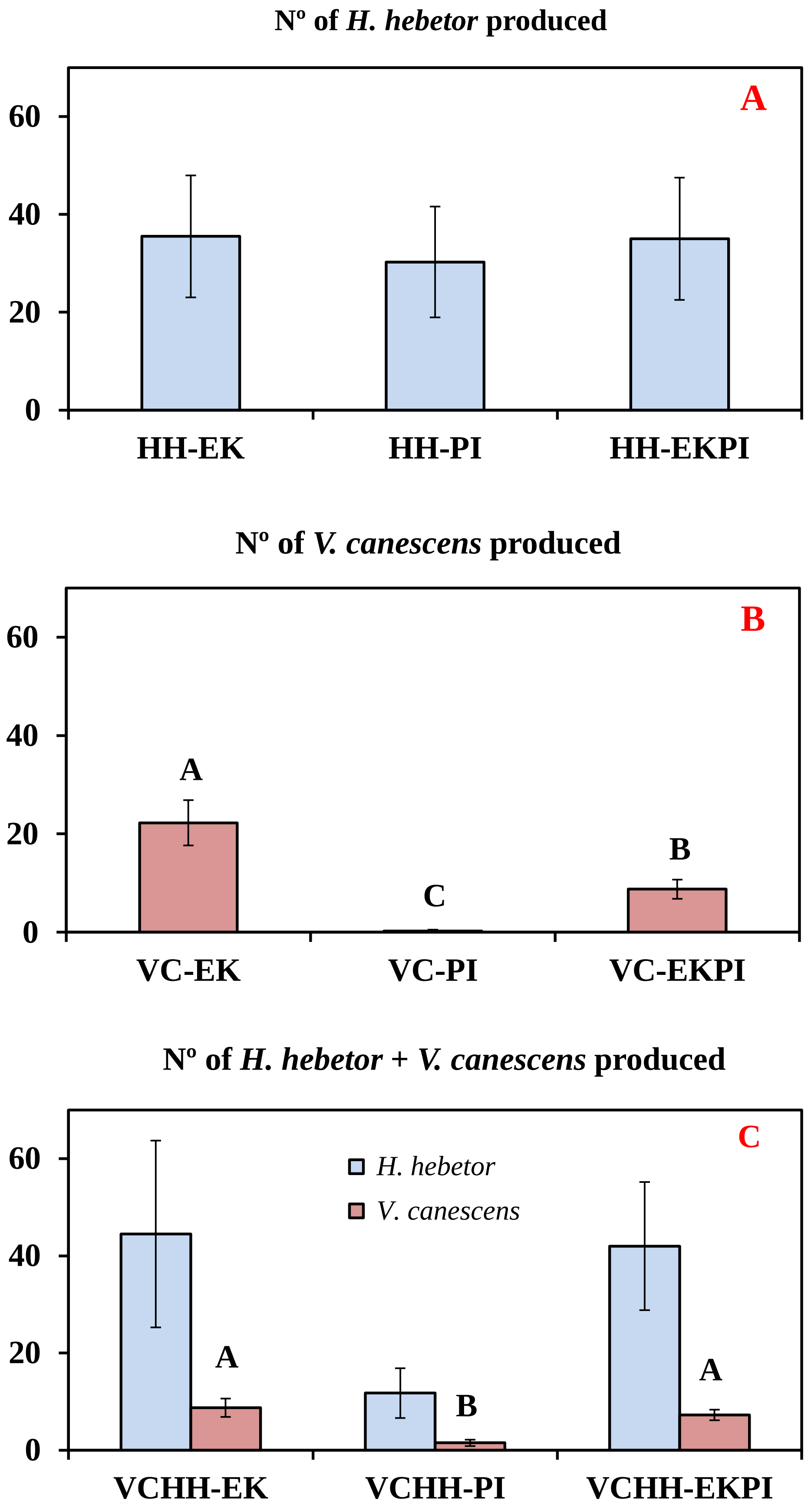
Fig. 2. Percentage of females and males of *H. hebetor* (HH) produced when 20 larvae of *E. kuehniella* (EK)*, P. interpunctella* (PI) or a combination of both (EK-PI) were offered during a 48-h period. Significant differences (*P*<0.05) in the proportion of males are indicated by an asterisk.

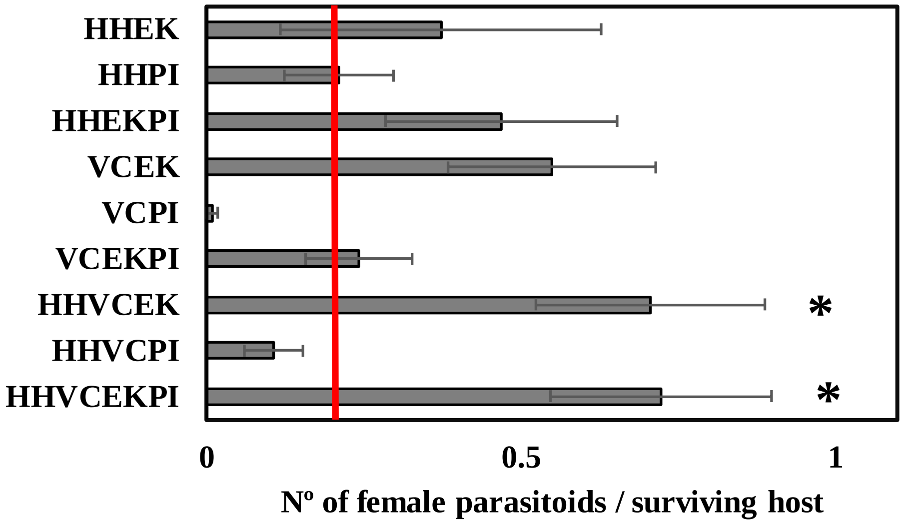
Fig. 3. Mean (± SE) percentage mortality of adults moths (*E. kuehniella* [EK], *P. interpunctella* [PI] or both pyralids combined [EK-PI]), when corrected by mortality in

the control treatment, in the different treatments considered when the parasitoids *H. hebetor* (HH) and *V. canescens* (VC) were released during a 10-day period. There were no significant differences among treatments ($P<0.05$).


HH-EK
HH-PI
HH-EK-PI
VC-PI
VC-EK
VC-EK-PI
AB
A
AB
B
AB
AB
0
5
10
15
Nº of F_1 progeny of parasitoids


HH-EK
HH-PI
HH-EK-PI
VC-EK
VC-PI
VC-EK-PI
*
*
0
0.5
1
1.5
2
Nº of female parasitoids / surviving host


Nº of H. hebetor produced
A
60
40
20
0
HH-EK
HH-PI
HH-EKPI
Nº of V. canescens produced
B
60
40
20
0
A
C
B
VC-EK
VC-PI
VC-EKPI
Nº of H. hebetor + V. canescens produced
C
H. hebetor
V. canescens
60
40
20
0
A
B
A
VCHH-EK
VCHH-PI
VCHH-EKPI


HHEK
HHPI
HHEKPI
VCEK
VCPI
VCEKPI
HHVCEK
HHVCPI
HHVCEKPI
*
*
0
0.5
1
Nº of female parasitoids / surviving host

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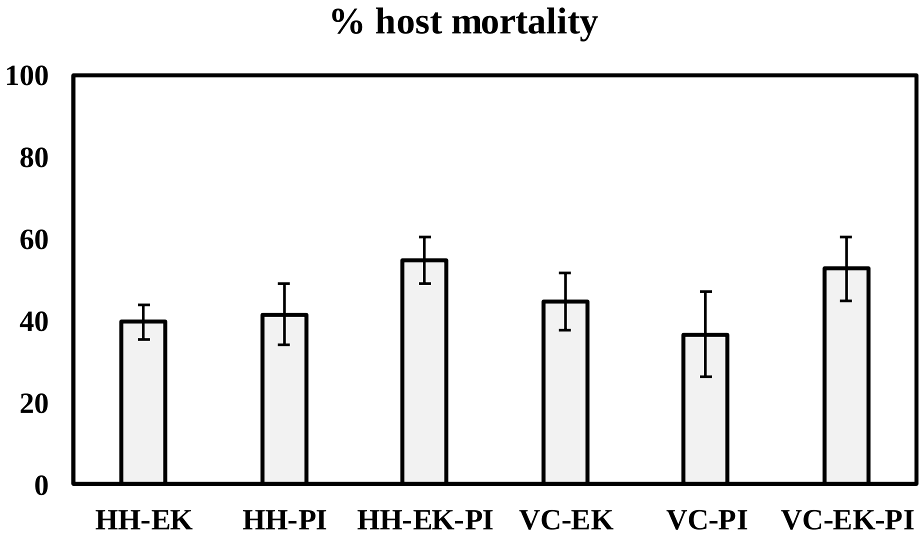

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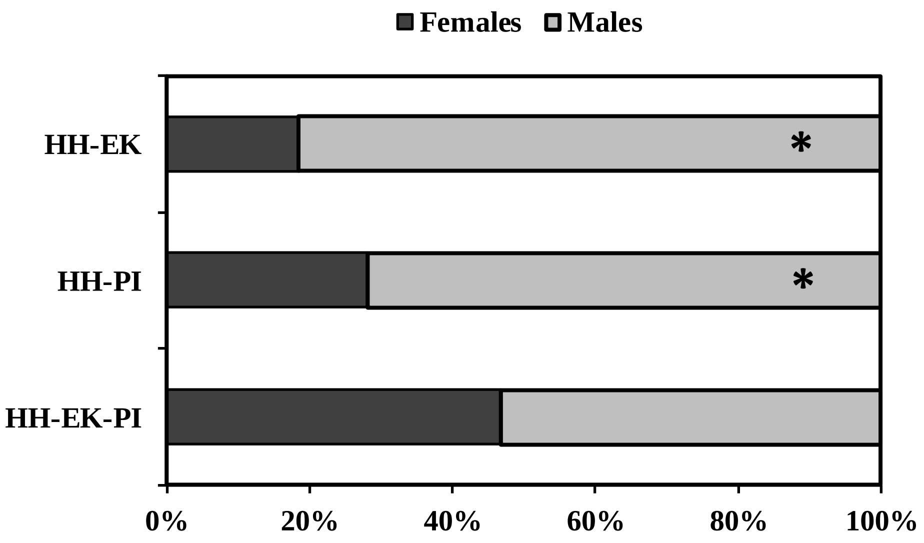

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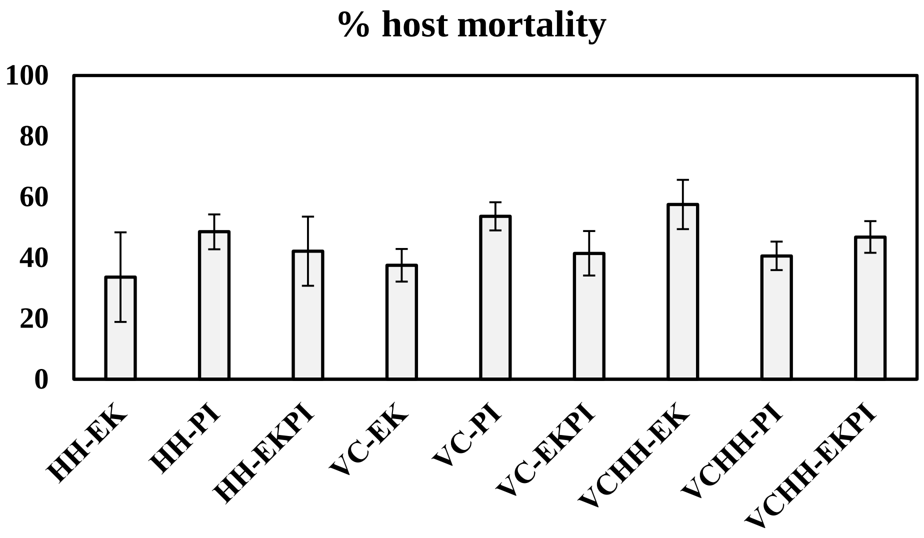

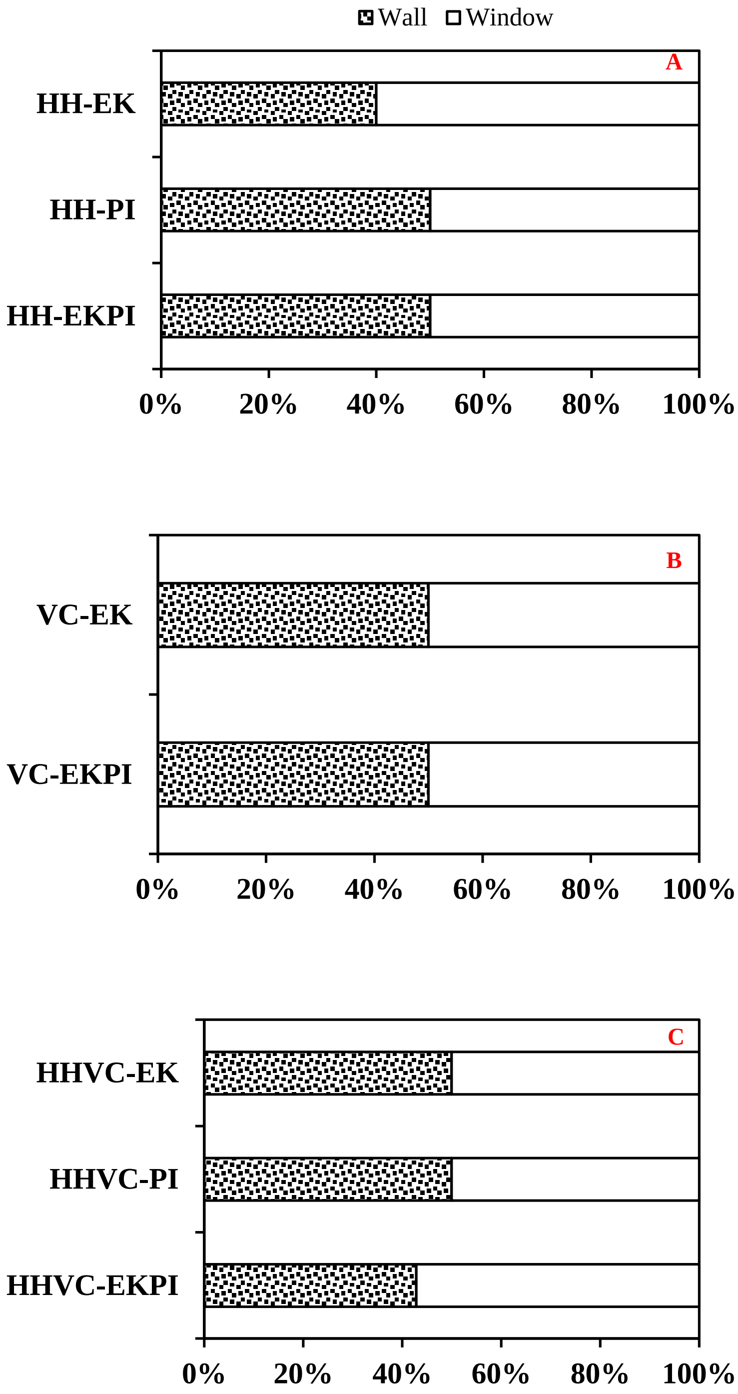
▨ Wall
☐ Window
A
HH-EK
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HH-EKPI
0%
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40%
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VC-EK
VC-EKPI
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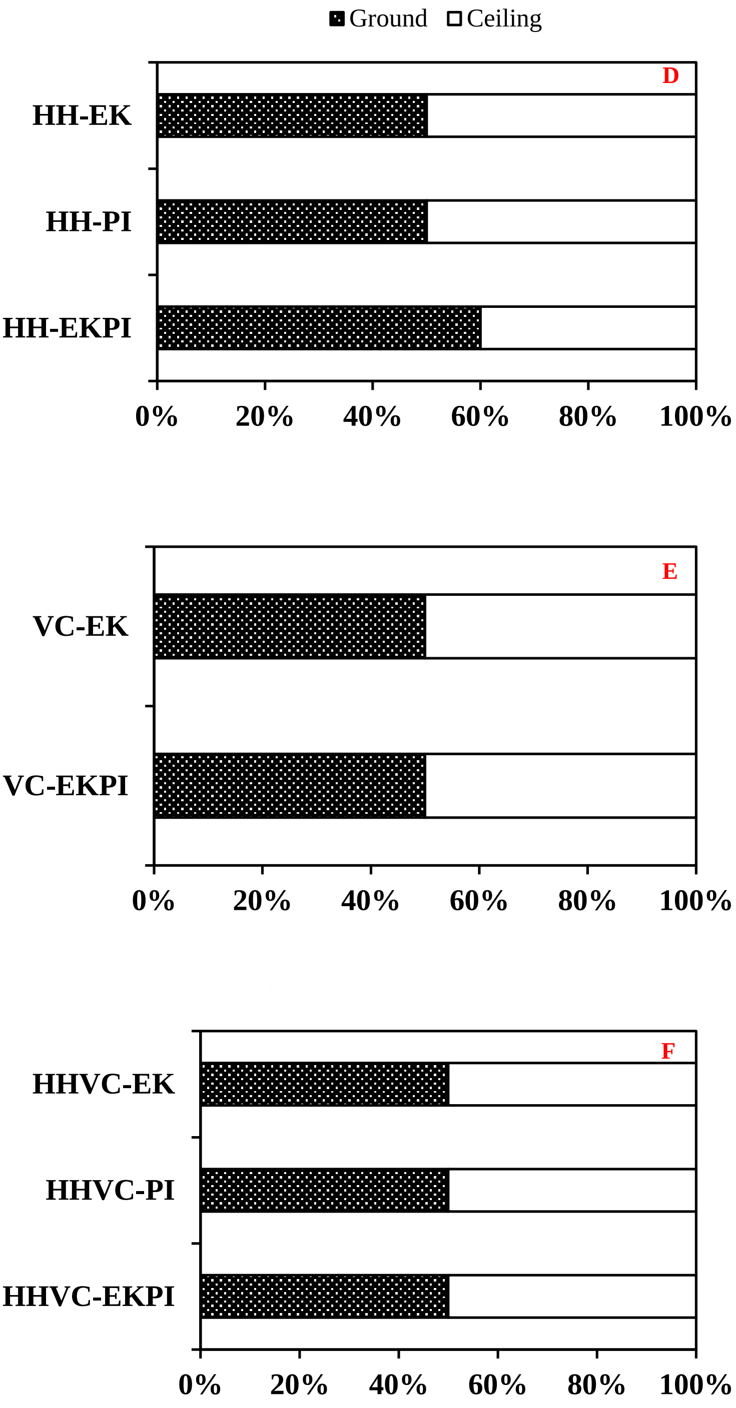
▨ Ground
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HHVC-EK
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	Tº			**HR**			**Hours of light**
	Max	Min	**Mean**	Max	Min	**Mean**	
June	27.9	21.6	**25.64**	66.7	44.9	**59.81**	**14:55-15:09**
July	32.9	24.7	**27.39**	99.9	24.1	**60.25**	**15:09-14:29**
August	30.2	25.2	**27.92**	65.3	49.7	**59.81**	**14:28-13:16**
September	29.2	20.8	**25.01**	66.9	54.6	**61.24**	**13:16-11:54**
October	25.4	16.6	**19.66**	78.6	56	**72.02**	**11:54-10:32**
November	23.7	12.1	**18.75**	78.1	53	**66.05**	**10:30-09:30**

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Host sp.	Host emergence			Males of *H. hebetor*		
	χ^2	df	*P*	χ^2	df	*P*
E. kuehniella	39.15	5	<0.001	34.66	1	<0.001
P. interpunctella	31.97	5	<0.001	19.01	1	<0.001
E. kuehniella + *P. interpunctella*	57.23	8	<0.001	0.39	1	0.531

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Host sp.	*H. hebetor*			*V. canescens*		
	t	df	*P*	*t*	df	*P*
E. kuehniella	1.30	9	0.887	2.19	9	0.023
P. interpunctella	0.89	9	0.194	1.16	9	0.138
E. kuehniella + *P. interpunctella*	1.32	9	0.109	3.17	9	0.006

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Parasitod sp.	Host emergence			Host selection			Parasitoid reproduction		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
H. hebetor	5.53	5, 18	0.003	0.19	2, 9	0.828	0.06	2, 9	0.945
V. canescens	19.08	5, 18	<0.001	28.76	2, 9	<0.001	63.79	2, 9	<0.001
H. hebetor + *V. canescens*	36.77	5, 18	<0.001	1.80	2, 9	0.220	1.75 (Hh) 8.44(Vc)	2, 9	0.227 0.009

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Host sp.	Window wall *vs* opposite wall					
	H. hebetor		*V. canescens*		*H. hebetor* + *V. canescens*	
	χ^2	P	χ^2	P	χ^2	P
E. kuehniella	0.20	0.655	0.04	0.841	0.05	0.827
P. interpunctella	0.25	0.617	-	-	0.33	0.564
E. kuehniella + *P. interpunctella*	0.06	0.808	0.25	0.612	0.05	0.827
	Ground *vs* ceiling					
	H. hebetor		*V. canescens*		*H. hebetor* + *V. canescens*	
	χ^2	P	χ^2	P	χ^2	P
E. kuehniella	0	1	0.04	0.841	0.05	0.827
P. interpunctella	0	1	-	-	0	1
E. kuehniella + *P. interpunctella*	1.47	0.220	0.25	0.25	0	1

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Host sp.	*H. hebetor*			*V. canescens*			*H. hebetor* + *V. canescens*		
	t	df	*P*	*t*	df	*P*	*t*	df	*P*
E. kuehniella	0.68	3	0.273	2.12	3	0.062	2.77	3	0.035
P. interpunctella	0.11	3	-22.20	28.76	3	1	0.20	3	0.092
E. kuehniella + *P. interpunctella*	1.46	3	0.50	1.80	3	0.325	2.98	3	0.029