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Parasitism by *Venturia canescens* and *Habrobracon hebetor* on mono- and heterospecific populations of pyralid moths located in laboratory and experimental store houses

Cristina Castañé[1], Jordi Riudavets[1], Eric Lucas[2]
[1]IRTA, Ctra Cabrils Km 2, 08348-Cabrils (Barcelona), Spain; [2]Département des Sciences Biologiques, Université du Québec à Montréal (UQAM), CP 8888, succursale Centre-Ville Montréal (Québec, Canada), H3C 3P8
e-mail: cristina.castane@irta.es

Abstract: *Plodia interpunctella* and *Ephestia kuehniella* are important pests of stored products that are both parasitized by *Venturia canescens* and *Habrobracon hebetor*. These two cosmopolitan larval parasitoids are found in large numbers in food processing facilities in north-eastern Spain. In laboratory, we evaluated their performance when mono and heterospecific populations of 3rd-4th larval stages of the two moths were offered. We also examined the dispersion capability of females when larvae of the two moths were offered also alone or in combination. For this purpose hosts were offered in the eight corners of experimental rooms of aprox. ≈ 30 m^3 during three days; the experimental rooms had a window for considering the influence of natural light illumination on their dispersion pattern. Both parasitoids reduced the emergence of both moth species by half in the laboratory, in comparison with the controls, either when offered separately or in combination. Reproduction of *H. hebetor* was also similar in presence of both hosts, while *V. canescens* had a significantly higher reproduction on *E. kuehniella* than on *P. interpunctella* larvae. In experimental rooms both parasitoids were able to similarly parasitize larvae located in any of the eight corners, independently of the window situation. However, both parasitoids were more efficient parasitizing *E. kuehniella* than *P. interpunctella* larvae.

Key words: *P. interpunctella*, *E. kuehniella*, heterospecific hosts, spatial dispersion, parasitoids

Résumé: *Plodia interpunctella* et *Ephestia kuehniella*, importants ravageurs des denrées entreposées sont tous deux attaquées par *Venturia canescens* et *Habrobracon hebetor*. Ces deux parasitoïdes larvaires cosmopolites sont retrouvés en grand nombre dans les structures agroalimentaires du nord-est de l'Espagne. En laboratoire, nous avons évalué leurs performances face à des populations mono et hétérospécifiques de 3ème et 4ème stades larvaires des deux pyrales. Nous avons également étudié la capacité de dispersion des femelles en présence de populations mono et hétérospécifiques de pyrale. Pour ce faire, les hôtes étaient placés dans les huit coins de chambres expérimentales ≈ 30 m^3 durant trois jours, puis les larves étaient conservées à 28 degrés jusqu'à émergence afin d'évaluer le taux de parasitisme. Les chambres expérimentales avaient toutes une fenêtre afin d'estimer l'effet de la lumière naturelle sur leur patron de dispersion. Les deux parasitoïdes ont réduit de moitié le taux d'émergence des pyrales adultes en laboratoire aussi bien dans les populations hétéro que monospécifiques d'hôtes. La reproduction de *H. hebetor* était similaire en présence des deux hôtes, tandis que *V. canescens* avait une reproduction significativement plus élevée sur les larves d'*E. kuehniella* que sur celles de *P. interpunctella*. En chambre expérimentale, les deux parasitoïdes étaient capables de parasiter les larves dans tous les coins de la chambre, et ce indépendamment de la position de la fenêtre. Néanmoins, les deux parasitoïdes étaient plus efficaces pour parasiter *E. kuehniella* que *P. interpunctella*.

Mots-clefs: *P. interpunctella*, *E. kuehniella*, population hétérospécifique, dispersion spatiale, parasitoïdes

Introduction

The Mediterranean flour moth *Ephestia kuehniella* Zell. and the Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera, Pyralidae) are important pests of stored facilities worldwide. They may occur simultaneously, in mixed populations, in some premises while one or the other species may predominate in others. They can develop in the dust accumulated in corners and crevices of mills and other food facilities where it is difficult to reach for cleaning and also it is difficult to reach with conventional pesticides. From these areas they can disperse and contaminate a wide range of food products. To keep an exquisite sanitation of the facilities is fundamental to reduce populations of these moths but still it is not enough and insecticides need to be applied for their control. However, conventional pesticides and fumigants have loose effectiveness due to resistance problems (i.e. Attia *et al*., 1979) and they are also a matter of concern for their residues in food products and in the environment. In this situation it is necessary to develop alternative control methods that are effective and that can overcome the residues problem.

Habrobracon hebetor Say (Hymenoptera, Braconidae) and *Venturia canescens* Gravenhorst (Hymenoptera, Ichneumonidae) are two cosmopolitan parasitoids that have been found spontaneuosly in several facilities in Spain, specially agregated in areas near windows or light sources (Belda & Riudavets, 2013). These are two larval parasitoids that have different life history traits. *H. hebetor* is an idiobiont and gregarious ectoparasitoid: the female paralyse the host larvae prior to lay a number of eggs on top of it, preventing any further development of the host after initial parasitization. After hatching, several young larvae feed on the same host, until they pupate (Eliopoulos & Stathas, 2008). *V. canescens* is a koinobiont solitary endoparasitoid: the female lay only one egg inside the host and the larvae continuous its development after being parasitized. This is a thelytokous species, and only females are produced (Eliopoulos, 2006).

In this study we evaluated the performance and dispersal capability of these two parasitoids when mono and heterospecific combinations of *E. kuehniella* and *P. interpunctella* were offered both in the laboratory and in experimental store rooms.

Material and methods

Insect colonies

Moths (*E. kuehniella*, *P. interpunctella*), and parasitoids (*V. canescens*, *H. hebetor*) colonies were started with individuals collected in stored-product facilities and feed mills of north-eastern Spain. Moths were reared on wheat flour and *H. hebetor* was reared on larvae of *P. interpuctella* while *V. canescens* was reared on larvae of *E. kuehniella* under controlled conditions (28 ± 2 °C, 70 ± 5% RH, and 16:8 h L:D photoperiod).

Laboratory experiment

Performed under the same controlled conditions as the rearing colonies. Prior to the test, female parasitoids were starved without prey for 24 hours. Four females were released in a ventilated cage (8 cm diameter) containing 20 hosts of 3^{rd}-4^{th} instar pyralid larvae, a spoon of flour and a strip of honey solution. After 48 hours females were removed and cages kept at 28 °C until the emergence of the adult hosts and/or adult parasitoids. Three treatments were tested: 20 larvae of *E. kuehniella*, 20 larvae of *P. interpunctella* or 10 larvae of *E. kuehniella* +10 larvae of *P. interpunctella*. Three control treatments were also included with the same host's combinations but without parasitoids. 10 replicates per treatment and parasitoid species were done.

Small storage room experiment

Experiments were done from January to July 2014 in a simulated warehouse with four rooms of ≈ 30 m^3. Each room had a window to evaluate the incidence of natural light on parasitoid dispersal. As in the previous experiment, for each parasitoid species three treatments were tested: *P. interpunctella*, *E. kuehniella* or *P. interpunctella* + *E. kuehniella* 3^{rd}-4^{th} instar larvae. Every treatment was done in one room with eight traps consisting of an open cup with 10 host larvae each and a spoon of flour. These traps were located in the four corners of the ground and the four corners of the ceiling. A control treatment consisting of a closed cup with 10 larvae was also included. Prior to the test, female parasitoids were starved without prey for 24 hours. In each room 16 female parasitoids were released during three days. Afterwards traps were collected and kept at 28 °C until the emergence of the adult hosts and/or adult parasitoids.

Data analysis

Variables were analysed by a one way ANOVA.

Results and discussion

Laboratory experiment

Host emergence was significantly lower (F = 18.24, df = 8, 81, $P < 0.001$) in treatments with the two parasitoids than in the control treatments (Figure 1). This indicated that the two parasitoids were able to reduce the host population either when offered as monospecific or in a heterospecific combination of the two pyralids.

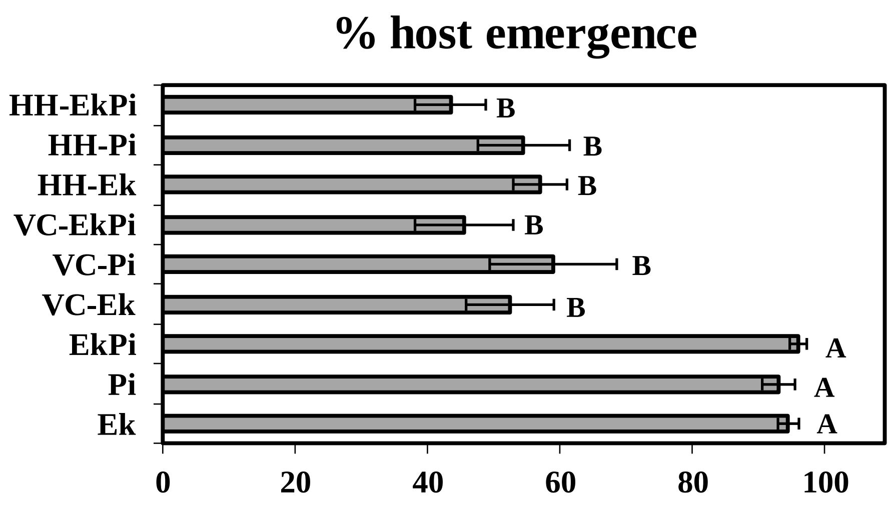

Figure 1. Percentage of host emergence in laboratory conditions in the different treatments considered (20 *E. kuehniella* (Ek), 20 *P. interpunctella* (Pi), 10 *E. kuehniella* + 10 *P. interpunctella* (Ek + Pi)) with the two parasitoids (*H. hebetor* (HH) and *V. canescens* (VC)) and in the controls without any parasitoid.

Reproduction of *H. hebetor* per host larvae offered was similar in the two pyralids when presented alone or in combination. But, more males than females were produced in the monospecific host treatments and only in the treatment with both hosts combination the proportion of females produced was similar to that of the males. Reproduction of *V. canescens* per host larvae was lower in *P. interpunctella* than in *E. kuehniella* or in the combination of both hosts. The production of *H. hebetor* females only surpassed the proportion introduced in the combined host treatment. In the case of *V. canescens* the production of females surpassed the proportion introduced in the treatments with combined host and with *E. kuehniella*. Thus, only in these treatments biocontrol is expected to be successful.

Small storage room experiment

Temperatures along the whole study period ranged between 23-28 °C and relative humidity between 30-80%.

Both parasitoids were able to parasitize larvae of the two pyralids, offered alone or in combination, when located in any of the eight corners of the rooms, indicating that they are able to disperse in any direction of the premise. The presence of a source of light, as it was a wall with a window, did not seem to determine host location ability and both parasitoids parasitized with a similar frequency the hosts located in the traps of the window wall and the traps located in the opposite wall. While *H. hebetor* similarly parasitized traps located in the ground than located on the ceiling, *V. canescens* preferentially parasitized traps located in the ground.

In contrast with the results of the lab experiment, neither *H. hebetor* nor *V. canescens* were able to significantly reduce the emergence of the two pyralids, either when offered alone or in combination, in relation with their control treatments ($F = 8.65$, $df = 5, 154$, $P < 0.001$ for *H. hebetor* and $F = 8.90$, $df = 5, 137$, $P < 0.001$ for *V. canescens*) (Figure 2). Although reproduction of both parasitoids was low in all hosts combinations, it was higher on *E. kuehniella* than on *P. interpunctella*, with intermediate reproduction values obtained in the mixed host combination.

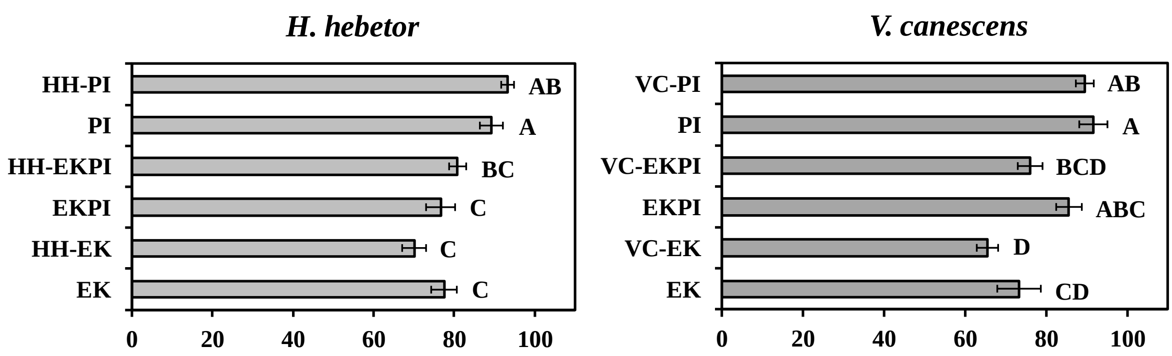

Figure 2. Percentage of host emergence when by *H. hebetor* (HH) or *V. canescens* (VC) were released in small rooms (≈ 30 m^3) in which different treatments were considered: ten 3rd-4th instar larvae of *E. kuehniella* (Ek), *P. interpunctella* (Pi) or *E. kuehniella* + *P. interpunctella* (Ek + Pi) per trap in eight traps per room. Controls without any parasitoid were also included in each room.

As conclusion, these two parasitoids show potential as biological control agents of moths infesting storehouses and premises. Both parasitoids were able to reduce survival of *E. kuehniella* and *P. interpunctella* in small cages in the laboratory and successfully reproduce on them. However, biocontrol effectiveness was specifically predicted for *H. hebetor* with mixed host populations and for *V. canescens* with *E. kuehniella* populations.

Both parasitoids showed a good dispersal capacity in the experimental rooms, attacking hosts in all locations offered. No interference of the light source, the window, could be observed as it was initially presumed. This indicates that attractiveness of the host is higher than that of the light. However, when the same host combinations as those tested in the laboratory were offered in a bigger scale, that is in experimental store rooms, both parasitoids were able to reproduce to a certain extent but they were not successful in significantly reducing host emergence. These results indicate that further studies have to be performed on different host:parasitoid release ratio and on the interaction time of parasitoids and hosts to achieve an acceptable pyralid control. Both parasitoids had a better reproduction on *E. kuehniella* than on *P. interpunctella,* in spite that laboratory colonies of *H. hebetor* used for these experiments were reared on *P. interpunctella.* One possible explanation for these results is the bigger size of *E. kuehniella* 3^{rd}-4^{th} instar larvae in relation to the same instars of *P. interpunctella;* this bigger biomass could facilitate host location and reproduction of the two parasitoids in that moth species.

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