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# A banker box to improve the impact of *Habrobracon hebetor* on stored product insects

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**Abstract:** The treatment of store room walls, ceilings, floors and other structures is necessary as part of the good hygienic procedures recommended for reducing pests problems. Structural pests such as some moth species hide in the floor, corners, cracks and crevices inside machinery and are always difficult to control. With the continuous reduction of the availability of the insecticides, it is relevant to evaluate potential alternative methods of control. *Habrobracon hebetor* is a gregarious ectoparasitoid of pyralid moths that is found in large numbers in several food processing facilities in north-eastern Spain. *H. hebetor* can be a good candidate for biological control of moths that contaminate structures. In this work, we developed a banker box system for rearing *H. hebetor* on *Ephestia kuehniella* larvae and for releasing them progressively in the room. Different host-parasitoid ratios were tested to optimize the efficiency of the rearing box. Also, different apertures in the box were tested in order to allow the exit of adult parasitoids but avoid the larval moths to escape.

**Key words:** *E. kuehniella*, pyralid moths, biological control, parasitoid

### Une boîte-réservoir pour optimiser l'impact de *Habrobracon hebetor* sur les insectes des denrées entreposées

**Résumé:** De bonnes mesures d'hygiène pour lutter contre les insectes ravageurs impliquent le traitement des murs, plafonds, planchers et autres structures. Les ravageurs structuraux, tels que les lépidoptères, se cachent dans les crevasses, interstices de planchers et de plafonds, machineries, failles et autres recoins et sont de ce fait toujours difficiles à contrôler. Avec la réduction continuelle des insecticides disponibles, il est important d'explorer des méthodes alternatives de lutte. *Habrobracon hebetor* est un ectoparasitoïde grégaire de pyrales, et on le retrouve en grand nombre dans plusieurs entrepôts ou fabriques du nord-est de l'Espagne. Il pourrait constituer un bon candidat pour la lutte biologique contre les papillons qui se cachent au sein des structures de production ou d'entreposage. Dans cette étude, nous avons mis sur pied un système de boîte-réservoir pour élever *H. hebetor* sur des larves d'*Ephestia kuehniella* et pour le relâcher graduellement à l'extérieur. Différents ratios d'hôtes-parasitoïdes ont été testés pour évaluer l'efficacité de la boîte-réservoir. De plus, deux tailles d'ouvertures pratiquées dans la boîte-réservoir pour permettre la sortie des parasitoïdes, tout en empêchant la sortie des hôtes, ont été comparées.

**Mots-clefs**: *E. kuehniella*, pyrales, lutte biologique, parasitoïde

## Introduction

The cosmopolitan Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), is one of the main pest of flour products around the world (Cox & Bell, 1991). Furthermore the insect can feed on a great variety of other stored products, and also on dead insects (Tarlack *et al.*, 2015). In Spain, lepidopteran species are the most abundant pests encountered in stored products facilities (Belda & Riudavets, 2013).

Three main methods may be used to control flour moths and other insect pests of stored products: chemical, physical and biological controls. Chemical control is restricted by human concerns about the presence of chemical residues in stored products, and by potential pest resistance. Physical methods involving the use of heat or cold techniques remain most often expensive. Finally, the biological method is safer but remains to be optimized in order to demonstrate its efficacy (Brower *et al.*, 1996; Schöller *et al.*, 2006).

In agricultural field and greenhouses, one biological technique is the use of banker plant systems. Banker plant systems are composed of a non-crop plant infested by an alternative pest (which do not attack the focal plant) used by the biocontrol agent in absence of the focal prey in the focal plant (to be protected) (Frank, 2010; Huang *et al.*, 2015). Banker plants allow the biocontrol auxiliary to survive within the system in absence of the focal prey and then will theoretically allow a rapid attack of the focal pest when colonization occurs. The present study is a preliminary step toward the development of a functional banker box system.

In stored products systems, a type of banker box (called the "Hohenheimer box") has been developed against the granary weevil *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) based on the rearing of the parasitoid *Lariophagus distinguendus* (Forster) (Hymenoptera: Pteromalidae) (Niedermayer & Steidle, 2013).

In this study, we presented a preliminary banker box system, which is a box containing the rearing hosts alive and the biocontrol agent (a parasitoid); from this box, hosts cannot escape from the box while the parasitoids can do it. We selected the ectoparasitoid *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) a generalist gregarious natural enemy of lepidopteran pests in stored products systems. It is one of the main biological tool in biocontrol programs (Brower *et al.*, 1996; Ghimire & Phillips, 2010), and one of the more common species of parasitoids of stored product Lepidoptera in Spain (Belda & Riudavets, 2013).

This banker box system will allow the parasitoid to go out from the box at its own pace for searching and attacking pests in the stored product facilities. This system allows releasing adult parasitoids in the best physiological conditions since they are kept with their rearing host during transportation and they can abandon the rearing cage when they are ready to do so. In the present study, we evaluated 1) the impact of the banker box on pyralid populations, 2) the optimal number of natural enemies to be introduced in the banker box, 3) the optimal aperture type of the banker box.

## Material and methods

### *Insects*

All insects, the pyralid moth *Ephestia kuehniella* and the parasitoid *H. hebetor*, came from permanent colonies held in the laboratory for plant protection of IRTA, Center of Cabrils (Barcelona, Spain). Colonies were kept in growth chambers at 16 D/8N, 28 ± 2 °C.

### *Banker box*

The set-up was composed of 3 elements (Figure 1): 1- The global cage was 15 cm high x 15,5 cm wide x 22 cm long., in plexiglas with aeration at the top to prevent excess of humidity; 2- The banker box (11 cm high x 11 cm diameter) inside the plexiglas cage. It is the device for rearing and releasing the biocontrol agents. The lid of the banker box had small holes to allow parasitoids to leave and to prevent the escaping of the pyralid larvae. The type and number of holes changed among the tests; 3- The pestbox (11 cm high x 11 cm diameter) also inside the plexiglas cage, 1.5 cm apart from the banker box. It contained the larval population of *E. kuehniella* to be controlled. The pestbox was open to allow parasitoids to enter inside, but tanglefoot was added on the top of the walls to prevent *E. kuehniella* larvae to escape.

***Bioassays***

All bioassays were carried out in the same environmental conditions as those of the rearing units. Two experiments were conducted in parallel according to logistic limitations (material and space).

At high-density, at day 1, 20 larvae of *E. kuehniella* aged of 17 days were introduced in the banker box with 5 g of wheat flour and 5 g of wheat bran. Four treatments were done: 1) Hh-3 where 3 females and 2 males *H. hebetor* were introduced, 2) Hh-6 where 6 females and 4 males were introduced, 3) Hh-9 where 9 females and 6 males were introduced and 4) a control without parasitoids. At the day 10, 20 larvae of *E. kuehniella* aged of 17 days were introduced in the pestbox with 5 g of wheat flour and 5 g of bran, a line of tanglefoot was spread at the top of the pestbox to prevent escape of the *E. kuehniella* larvae. A paper strip dripped in a solution of water + honey was added in the global cage. The banker box and the pestbox were put in the global cage at a distance of about 1.5 cm. The day 13, the parasitoids previously introduced were withdrawn from the banker box. 80 apertures of 0.97 mm diameter were pierced in the top of the banker box. The day 17, parasitoids were counted in all boxes and sexed. Each pestbox was replaced by a new one (same as the previous one). A second counting was done similarly at day 20, and a 3$^{rd}$ one at day 27. The final counting was done at day 34: all pyralids and parasitoids in the banker box, the pestbox and out of both boxes (in the global cage) were counted and sexed.

At low-density, the experiment was similar except for two points: First, the treatments were,1) Hh-1 where 1 female and 1 male *H. hebetor* were introduced, 2) Hh-3 where 3 females and 2 males were introduced and 3) a control without parasitoids. Second, 50 apertures of 1.60 mm diameter were pierced in the top of the banker box.

The number of *E. kuehniella* and of *H. hebetor* individuals were compared with Analysis of variance according to the treatments. Mean separation were carried out with the Tukey test ($\alpha = 0.05$), using the JMP 10 software. Non parametrical Wilcoxon/Kruskal-Wallis tests were done when required. The global proportion of *H. hebetor* and the proportion of females out of the banker box were arcsine transformed before the tests (Sokal & Ralf, 1981).

## Results and discussion

***Impact of the parasitoid initial number on biocontrol of* E. kuehniella**

At high infestation rates (3 to 9 *H. hebetor*; 0.97 mm apertures), all treatments with parasitoids drastically affected *E. kuehniella* populations, with 99% of the pests destroyed (Kruskal Wallis, Khi2 = 28.70, $p < 0.001$). No difference was observed between treatments involving parasitoid ($p > 0.05$), while both were different from the control ($p < 0.001$). At low infestation rates (1 to 3 *H. hebetor*; 1.60 mm apertures), both initial densities provided an efficient control of *E. kuehniella* in the pestbox ($F = 58.57$, df = 2.20, $p < 0.001$). More than 85% of the pyralid were killed in the presence of parasitoid. No difference was detected between the 1-Hh and the 3-Hh treatments ($p = 0.472$) and both were different from the control ($p < 0.001$).

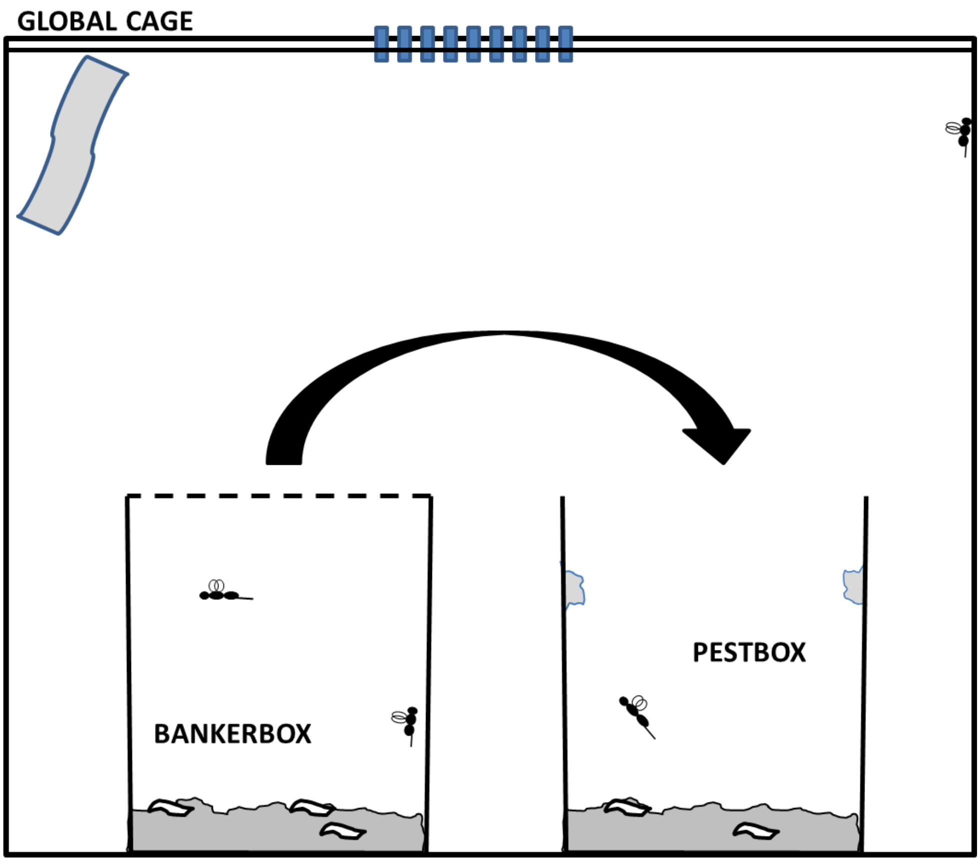


Figure 1. The bankerbox system for biocontrol of pyralid pests.

***Impact of parasitoid initial number on parasitoid production***

At high parasitoid infestation rate (3 to 9 *H. hebetor*; 0.97 mm apertures), no difference was observed between treatments in the mean number of *H. hebetor* produced. The results demonstrated that there is no decrease in the number of parasitoid produced in the banker box with the lower initial infestation level: 3 females *H. hebetor*. Female production was higher in the 3 Hh treatment than in the 9 Hh treatment. At low parasitoid infestation rate (1 to 3 *H. hebetor*; 1.60 mm apertures), the mean number of *H. hebetor* produced did not differ when the initial density was 1 or 3 female parasitoid. Female production was also similar among treatments.

The results demonstrated that the minimal number of parasitoid in the banker box could be 1 or 3 females. However, globally, according to the higher risk of mortality with only 1 initial female, the optimal initial density corresponded to 3 females and 2 males *H. hebetor*.

***Impact of the aperture type of the banker box on parasitoid immigration***

The migration rate of *H. hebetor* adults out of the banker box increased with the widening of the aperture. The immigration rate with a diameter of 1.60 mm was almost twice the rate with a diameter of 0.97 mm. The same pattern was observed with the proportion of *H. hebetor* females out of the banker box. Widening the apertures (even while decreasing also the number of apertures) of the banker box benefits greatly to the exit of the parasitoids, and more specifically to the male’s exit.

Another type of banker box, called the “Hohenheimer box” developed in Germany against the granary weevil *S. granarius* (L.) (Coleoptera: Curculionidae) allows the rearing and release of *L. distinguendus* (Forster) (Hymenoptera: Pteromalidae) over four months (Niedermayer & Steidle, 2013).

The present banker box developed against pyralid pests provided excellent results in a laboratory environment. However, these results have to be confirmed in a real commercial situation. Also, the temporal window of release of the parasitoid has to be extended.

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