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Effect of *Habrobracon hebetor* (Hymenoptera: Braconidae) Release on Moth Infestation in Stored Tobacco

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HIGHLIGHTS

- The *H. hebetor* parasitoid is effective to control the population of *Ephestia* spp.
- The potential of *H. hebetor* in stored tobacco is recorded for the first time in Brazil.
- *H. hebetor* can be part of an integrated pest management program in stored tobacco.

Abstract: During the storage period of dry tobacco and its derivatives, insect pests such as species of *Ephestia* Guenée (Lepidoptera: Pyralidae), popularly known as moths, cause damage to the product, being controlled mainly with physical practices and synthetic chemicals, although with limitations on their use and results. Some biological control agents, such as the parasitoid *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae), have the potential to control these pests. This study aimed to evaluate the effect of *H. hebetor* release in tobacco farms and industrial warehouses, on the infestation of *Ephestia* spp. adults. Parasitoids were released in tobacco farmers and tobacco industry warehouses between 2016-2018, comprising two years/crop seasons. Each release consisted of 1,000 parasitoids (four times) in tobacco farmers warehouses (70 m^2) and 30,000 (five times) in tobacco industry warehouses (8,000 m^2). Pheromone-baited sticky traps were used to weekly monitor the average number of adults of *Ephestia* spp. in warehouses with (WP) and without parasitoid release (NP). The average number of adults of *Ephestia* spp. captured in the traps in WP environments at farmers and industry level was significantly lower than the average captured in NP from the third and fifth weeks. In the following weeks, the averages of *Ephestia* spp. were always significantly lower in the WP environments until the end of monitoring. Therefore, the use of *H. hebetor* for moth control should be considered as part of a biological control program in stored tobacco environments.

Keywords: integrated pest management; post-harvest; parasitoid; *Ephestia* spp.; biological control.

INTRODUCTION

Brazil is currently the largest exporter country of leaf tobacco (*Nicotiana tabacum* L.) and the second largest producing, only behind China [1]. The crop has great economic importance by its high commercial value and capacity to employ a large number of people, both in cultivation and in industrialization [2]. It is mainly planted in South Brazil region and Rio Grande do Sul state has the largest cultivation area, with 127,000 hectares [1].

From seedling production to the post-harvest of tobacco, a set of pathogens and insects can attack the crop, causing losses in production, yield, and quality [3]. During the tobacco storage period, two groups of insects cause significant damage: the cigarette beetle *Lasioderma serricorne* Fabricius (Coleoptera: Ptinidae); and the moths *Ephestia elutella* (Hübner) (tobacco moth), *Ephestia kuehniella* (Zeller) (flour moth), and *Ephestia cautella* (Walker) (cocoa moth) (Lepidoptera: Pyralidae) [4]. Moth species occur in set, however, in different proportions depending on the region, being difficult for identification, since they are cryptic species [5].

In tobacco crop, it is estimated that approximately 3% of production is lost to the presence of insects in storage [6]. The methods currently used to control these pests are generally based on the use of chemical insecticides that are highly toxic to humans and the environment [7]. In Brazil, only phosphine (aluminum or magnesium phosphide) is authorized to be used as a phytosanitary product in the control of insects in warehouses with stored tobacco [8]. As a result, the need to develop new technologies to be adopted as management strategies increases.

Augmentative biological control is a tool, in which natural enemies, such as parasitoids, are used to decrease populations of organisms that are considered pests in agricultural systems [9]. Natural enemies of stored product pests can offer advantages over traditional chemical treatments. Parasitoids, for example, continue to reproduce as long as their hosts are available and can be released at specific points and actively spread to find the target in hidden locations [10]. This can be especially important for eliminating focal points of moth infestation that often thrive in dust accumulated in nooks and crannies of food storage environments [11].

The larval ectoparasitoid *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) stands out as a potential biocontrol agent for stored pests [12]. This species has been promising in laboratory experiments to control various pest moths present in different stored product environments [13, 14].

Its control ability has already been evaluated on many moth species like *E. elutella*, *E. kuehniella*, *E. cautella, Plodia interpunctella* (Hübner), *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) and *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) in different products and storage environments, either alone or in combination with other parasitoids [11, 15]. Studies were conducted in the storage of bulk wheat [16, 17], bulk peanuts [18], packaged cornmeal and rice [19, 20], bakeries and mills [21], and chocolate factories [22]. The authors considered aspects such as parasitism rate, number of pest adults captured in the traps, number and frequency of releases and search capacity to determine the efficiency of the parasitoid.

In stored tobacco, *H. hebetor* was already recorded parasitizing larvae of *Ephestia* spp. Guenée (Lepidoptera: Pyralidae) [23]. In the laboratory, it is known that *H. hebetor* parasites fifth instar larvae of *E. kuehniella* fed on a tobacco diet [24], being attracted by the odors of different types of tobacco [25]. The attractiveness of tobacco volatiles to *H. hebetor* females may be due to the fact that the parasitoid recognizes the odor as the substrate where it is most likely to find its host [25]. However, there is lack of information on its ability to control target pests in biggest stored tobacco environments. Thus, this study aimed to evaluate the effect of *H. hebetor* release in tobacco farms and industrial warehouses, on the infestation of *Ephestia* spp. adults.

MATERIAL AND METHODS

Parasitoid and host rearing

Habrobracon hebetor and *E. kuehniella* were reared in the Entomology Laboratory at the Universidade de Santa Cruz do Sul (UNISC), in acclimatized rooms at a temperature of 28 ± 2°C, relative humidity (RH) of 50 ± 20% and 14-h photoperiod. *Ephestia kuehniella* was reared on an artificial diet consisting of wheat flour (97%) and yeast (3%). The parasitoid was supplied with *E. kuehniella* larvae of the last instar to parasitize.

Containers of different sizes were used for parasitoid releases: 10 cm high x 10 cm diameter, for releases in tobacco farmers (with 500 parasitoids); and, 12 cm high x 16 cm wide and long (with 2,000 parasitoids) for releases in the tobacco industry warehouses. Different container sizes were used thus, the number of parasitoids released in the environments was different depending on the volume of tobacco stored and the size of the building. The sex ratio was around 0.7 females. The parasitoids were released when they began to emerge in the containers, which occurred approximately 14 days after the larvae were exposed to the parasitism. The number and frequency of parasitoids released in each of the systems were defined after a pilot experiment in the previous crop season (2015/2016).

Release in the warehouses of tobacco farmers

Twenty-two Virginia tobacco farmers associated with a tobacco company and with a history of *Ephestia* spp. infestations, were selected in locations of large-scale tobacco production in the municipalities of Arroio do Tigre (29° 19′ 22″ S, 53° 4′ 35″ W), Novo Cabrais (29° 44′ 24″ S, 52° 57′ 42″ W), Paraiso do Sul (29° 39′ 50″ S, 53° 8′ 54″ W) and Sobradinho (29° 25′ 22″ S, 53° 1′ 57″ W), within a radius of 25 km in central region of Rio Grande do Sul State, Brazil. Sixteen warehouses were designated for the release of *H. hebetor* and six are used as controls, without parasitoid release. The average size of the warehouses was 70 m^2 with an average capacity of 5.3 tons of tobacco stored at the end of the crop season. Two crop seasons were evaluated (2016/2017 and 2017/2018). At each season, different warehouses were selected, without repetition.

In each warehouse, four occasions of *H. hebetor* releases were conducted during the crop season. The first release was managed when the first bales (approximately 700 kg of dry tobacco) were stored on the site and the others at two-to-three-week intervals. Each release consisted of two containers (1,000 parasitoids).

Inside the warehouse, the containers with parasitoids were opened and placed on tobacco stacks with (an average height of 1.8 meters) at two different points. The containers remained on the site until the next release when they were collected.

Ephestia spp. was monitored inside the warehouses using pheromone-baited sticky traps by Gachon® [(Z, E)-9,12-tetradecadienyl acetate]. Monitoring started one week before the first release of *H. hebetor* and continued until the farmers started selling the tobacco to companies.

According to the manufacturer's recommendations, one Gachon® trap per every 300 m^2 should be used for monitoring the population [8]. Therefore, one trap was used per week in each warehouse, which was always installed on Monday and remained exposed for 48 hours, being removed on Wednesday of that same week. The trap was installed on a wall at a height of two meters and the farmers were instructed to conduct the weekly change, with the trap being preserved in a closed package. In the experiment, only the population of *Ephestia* spp. was monitored. During this period, no insecticide treatment was carried out in any warehouse. In the first (2016/2017) and second crop season (2017/2018), the monitoring took place from 12/12/2016 to 03/22/2017 and from 12/11/2017 to 03/21/2018, respectively, totalizing 15 weeks per crop season.

The experimental design consisted of 16 warehouses (farmers), randomly selected for the release of *H. hebetor* (WP) and six warehouses in which parasitoids were not released, constituting the control (NP). Each warehouse was considered a weekly replica.

Release in warehouses in tobacco industry

The experiment was conducted in two Virginia tobacco warehouses (unprocessed product) located in the municipality of Santa Cruz do Sul (29º43'59" S, 52º24'52" W), Rio Grande do Sul State, Brazil. Each environment had an area of approximately 8,000 m^2 and the capacity to store 5.5 thousand tons of tobacco. One warehouse was used for the parasitoid releases and the other as control, without releases. The volume of tobacco that had been stored inside the warehouses during the crop seasons increased as tobacco was purchased (Figure 1).

The release and monitoring methodology applied was similar to that used for tobacco farmers. Five releases were conducted during each crop season; the first one occurred when the company started buying tobacco from farmers and the others at two-to-three-week intervals. Each release consisted of 15 containers (30,000 parasitoids).

Due to the circulation of forklifts inside the warehouse, the containers with adult parasitoids were opened and placed on the floor in a row close to a side wall, with a distance of eight meters between each container, remaining in place until the occasion of the next release, when they were collected.

Ephestia spp. adults were monitored weekly using 10 traps described in the previous topic in each warehouse at a height of two meters. In the warehouse where releases occurred, the traps were placed on the same wall where the release was conducted, with a distance of ten meters between each trap. The traps were exposed weekly for 48 hours. The monitoring of moths started a week before the first release of *H.*

hebetor to verify initial infestation and continued until the moment that tobacco processing started. During the experiment no insecticide treatments were carried out in any warehouse. In the 2016/2017 and 2017/2018 crop seasons, monitoring occurred from 03/13/2017 to 07/05/2017 and from 03/19/2018 to 07/11/2018 respectively, totalizing17 weeks per crop season.

The experimental design had a warehouse in which parasitoid releases were made (WP) and another, as a control, without releases (NP). Each trap was considered a pseudo-replica with 10 weekly repetitions.

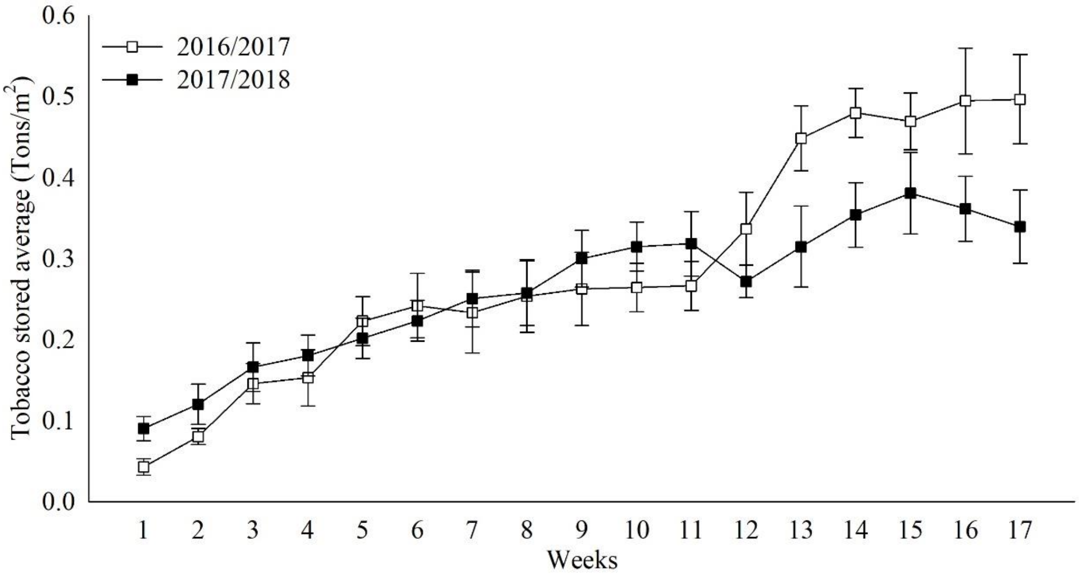

Figure 1. Average volume (± standard error) of tobacco stored/square meter/week, within the two warehouses over the weeks during the 2016-2017 and 2017-2018 crop seasons.

Data analysis

The average data were evaluated for normality by the Shapiro-Wilk test, homoscedasticity by the Hartley test, and the independence of residuals was evaluated by graphical analysis. The average number of *Ephestia* spp. adults captured by a trap in the warehouses with and without *H. hebetor* release was compared using the *t* test. In order to relate the volume of tobacco stored in the industry with the average number of insects captured over the weeks, Pearson's correlation coefficient followed by power-normal distribution was used. The analysis was conducted using the Bioestat 5.0® software at a significance level of 5% [26].

RESULTS

The average number of adults of *Ephestia* spp. captured in the traps in the warehouses with the release of *H. hebetor* at farmers was significantly lower (19.18±5.27) than that of sites without release (41.50±11.62) from the third week (t = 2.3668; df = 20; p = 0.02), in the first crop season (Figure 2 a). In the second crop season, this difference occurred from the fifth week (WP = 13.50±2.29) and (NP = 25.66±3.84) (t = 2.1602; df = 20; p = 0.04) (Figure 2 b). In the subsequent weeks, in both crop seasons, the averages of *Ephestia* spp. were always significantly higher in warehouses without parasitoid release, and the values continued to increase until the end of monitoring (Figure 2 a b).

In the industry, the average number of *Ephestia* spp. trapped was significantly lower after the third week in the environment with the release of *H. hebetor* in the 2016/2017 crop season, (WP = 22.80±8.04) and (NP = 58.30±19.18) (t = 2.0218; df = 18; p = 0.05) (Figure 3 a). In the control warehouse, without parasitoid release, the moth population continued to increase and peaked in the seventh week (Figure 3 a). In the 2017/2018 crop season, the significant difference in the average number of *Ephestia* spp. trapped occurred from the fourth week of sampling, (WP = 48.60±13.55) and (NP = 82.50±13.98) (t = 2.0409; df = 18; p = 0.05) (Figure 3 b). The number of insects captured in the control warehouse peaked in the sixth week (Figure 3 b).

In the 2016/2017 crop season, a positive correlation was observed between the volume of tobacco stored in the industry and the average number of *Ephestia* spp. trapped every week in the environment without the release of *H. hebetor* (Figure 4), and 60% of this variation can be explained by the relationship between the volume of tobacco and the number of insects trapped. On the other hand, in the environment with parasitoid release, the correlation between these parameters was not observed (p > 0.05).

In the second season, no correlation was observed between the volume of tobacco stored and the average number of captured insects, both in warehouses with release of the parasitoid ($y = 29.064x^{-0.13}$, $R^2 = 0.0065$, $p > 0.05$) and those without ($y = 267.28x^{0.6457}$, $R^2 = 0.293$, $p > 0.05$).

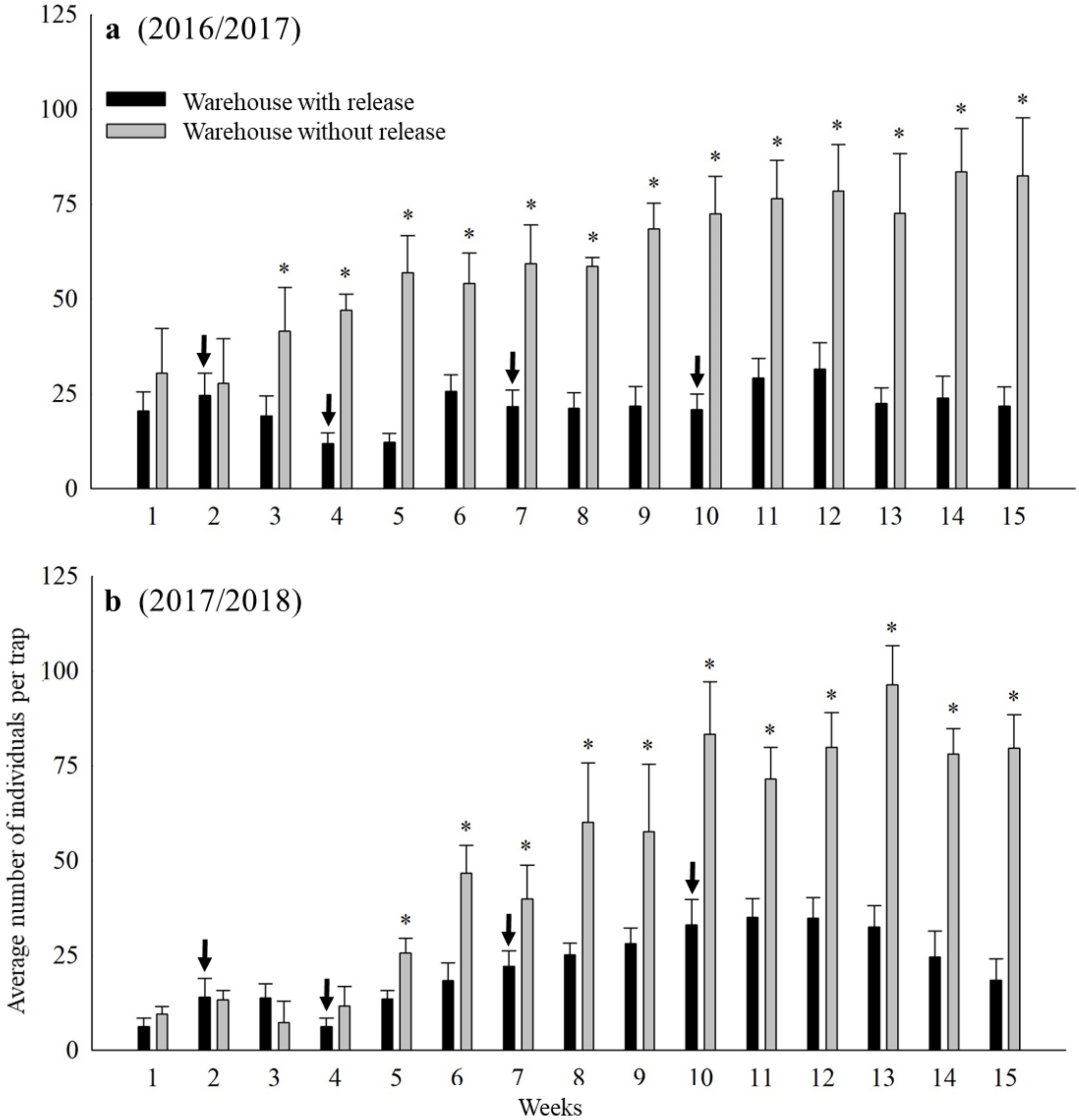

Figure 2. Average number (± standard deviation) of *Ephestia* spp. adults trapped in warehouses of tobacco farmers with (16 warehouses) and without release (6 warehouses) of *H. hebetor* weekly (arrows indicate when the releases occurred = 1,000 parasitoids) in two crop seasons (a) 2016/2017 and (b) 2017/2018. Bars with an asterisk differ significantly between treatments by the *t* test (p≤0.05).

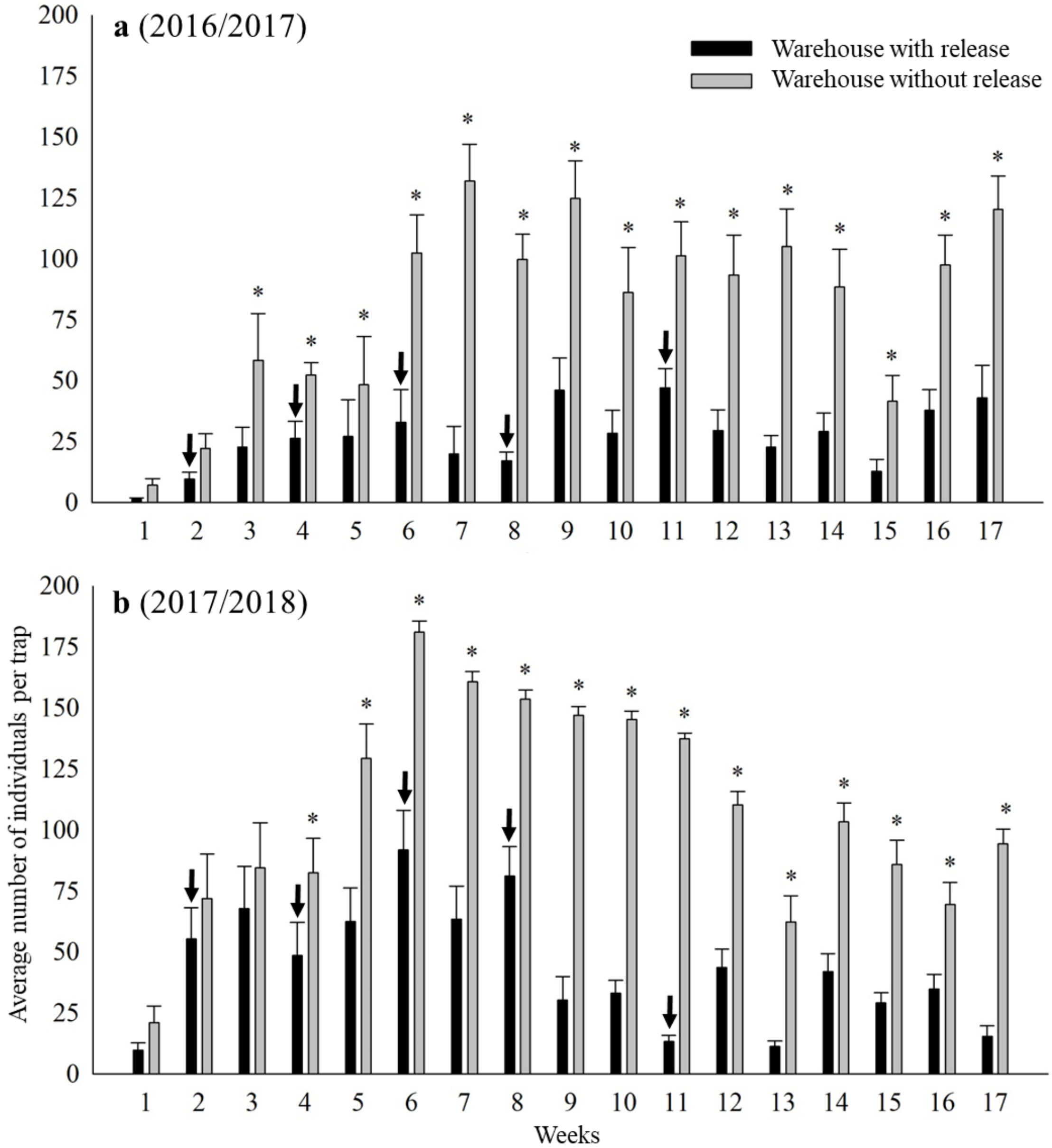

Figure 3. Average number (± standard deviation) of *Ephestia* spp. adults trapped in two warehouses in the industry with and without release of *H. hebetor* each week (arrows indicate when the releases occurred = 30,000 parasitoids) in the two crop seasons (a) 2016/2017 and (b) 2017/2018. Bars with an asterisk differ significantly between treatments by the *t* test (p≤0.05).

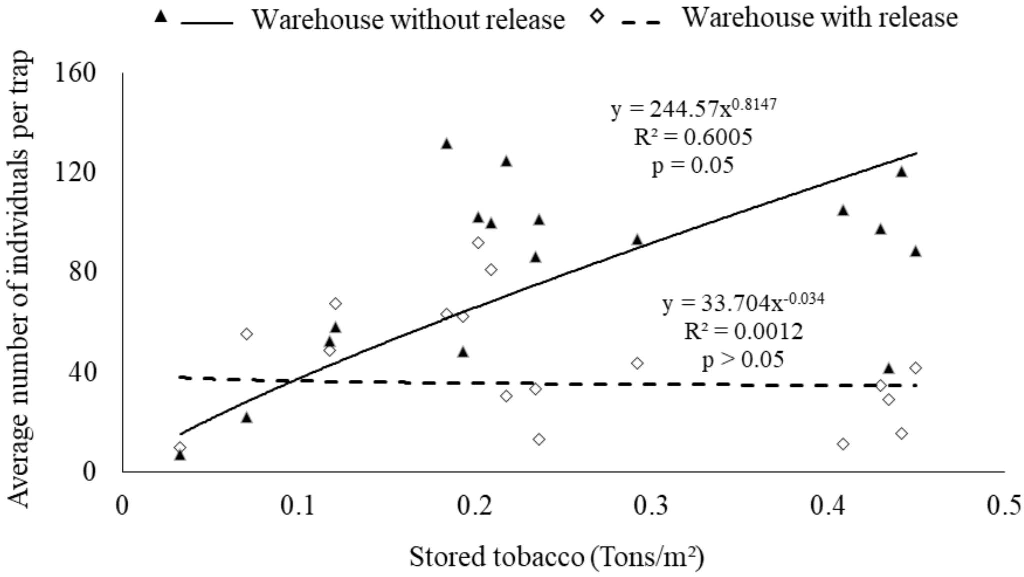

Figure 4. Correlation between the volume of tobacco stored and the average number of *Ephestia* spp. adults trapped every week in the warehouse in the industry without the release of *H. hebetor* during the 2016/2017 crop season.

DISCUSSION

The increase in the number of individuals caught in the traps of the warehouses of farmers over the weeks occurred since the volume of tobacco inside the storage environments gradually increased over the crop season, as the tobacco was harvested and cured. This increase in pest density is expected in storage environments since the volume of storage products increases, providing greater food availability for insects [27]. In addition, with the greater amount of product available, chemical clues that attract more insects increase and insect reproduction occurs at the site [28]. However, the increase in the number of *Ephestia* spp. individuals captured by pheromone traps were significantly lower in environments with the release of *H. hebetor*, showing that the parasitoid can slow the population growth of these moths even at the farmer level.

Similar to what was observed in farm warehouses, the increase in *Ephestia* spp. density associated with the increase in the volume of stored tobacco was recorded, which did not occur in warehouses with the release of parasitoids. Likewise, it happened possibly because the moth population was being controlled by the parasitoid and did not allow insect incidence to increase. In the following crop season, no correlation was observed between volume and *Ephestia* spp. trapped in the site without release, which may have occurred for the continuous flow of tobacco entering and leaving warehouses within the industry as the product was purchased and processed, without a static stock, different from the previous crop season. In addition, the volume of tobacco stored at the end of the season was lower compared to the first year.

During the storage period, there are several factors that can influence the population fluctuation of insects, such as tolerance to environmental conditions of temperature, humidity, light, quantity and quality of the food source, among others [27, 29]. However, these aspects were not evaluated in the present study. In addition, the origin of the tobacco that arrives at the industry does not have the same regularity from one crop to another and may come from different farmers or regions, with superior or inferior quality, which interferes with the infestation of insects in the warehouse.

The tobacco that entered the company was possibly already infested with *Ephestia* spp. from the farmers, since Deng and coauthors [30] described the occurrence of moths on rural properties and we also recorded their presence in the evaluated warehouses. As *H. hebetor* infests moths in the larval phase [12], moths that came from the farmers and were already in the pupal phase emerged and were trapped even after the parasitoids were released in the warehouse of industry. This explains the fact that the differences in the average density of moths per trap were only significant in the release environments from the third or fifth sampling occasion and the oscillation of individuals was registered according to the tobacco stock. Thus, we can infer that *H. hebetor* does not protect the infestation already present in the stored products, however, it will prevent their increase, reducing the next generation of moths [31]. Therefore, in order for adequate suppression of *Ephestia* spp., releases should be made as early as possible so that the parasitoids have a chance to control moth populations before they reach the action level.

The effectiveness of *H. hebetor* has already been recorded, in the control of *P. interpunctella* in releases inside a chocolate factory for four years [22]. The authors observed that the presence of *H. hebetor* reduced the number of *P. interpunctella* adults captured in the pheromone traps inside the factory. Adarkwah and Schöller [17] evaluated the parasitism capacity of *H. hebetor* in *P. interpunctella* larvae in wheat stored in bulk and verified parasitism between 50 and 80 % of the larvae according to the density of parasitoids released. The combination of the release of a larval parasitoid species associated with an egg parasitoid, in this case, *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae), improved the control of *E. kuehniella* and *P. interpunctella* in heated mills and bakeries [21]. Population suppression of *P. interpunctella* and *E. cautella* by *H. hebetor* was also recorded in peanut warehouses, being 60 and 90%, respectively [18].

In Brazil, the use of parasitoids in storage or processing facilities is still incipient. There is a record of the use of *H. hebetor* to control *E. elutella* in about 1,500 tobacco farmers in Brazil [23]. However, the author only reports the use, without informing data regarding the number, time, release interval, dose, or control efficiency.

In our study, we observed that *H. hebetor* is able to be established in stored tobacco environments and act in the control of associated pests despite nicotine present in tobacco which is described as an insect repellent and is frequently used as an insecticide [32, 33]. This substance, like insecticides of the neonicotinoid group, acts as competitive modulator of nicotinic acetylcholine receptors and has a rapid effect that leads to hyperexcitation of the nervous system, which can be fatal for many insects [34]. The fact that the release of *H. hebetor* reduced the population of *Ephestia* spp., shows that this parasitoid species is adapted to the taco storage environment, which is toxic to other organisms. This adaptation has already been proven in a study that showed that *H. hebetor* presented a positive chemotactic response when responding to odors from different types of tobacco, especially to that of Virginia [25]. In addition, *H. hebetor* is able to reproduce and develop in the host reared on a diet containing tobacco [24]. Thus, although the offspring from released females has not been studied in the present study, there may have been offspring emergence and the second generation could reinforce the control of the target populations in warehouses.

Although *H. hebetor* is commercialized in some countries, only a few companies sell this parasitoid species, with examples of success only on a small scale [11]. Despite the great potential of the species demonstrated in our study, one of the obstacles to the use of parasitoids in storage environments is that natural enemies can be considered as a type of contaminant present at the site [35]. However, some countries already have specific legislation on this subject, such as the United States, which allowed the increased release of beneficial insects in stored products. All genera of parasitoids and predators commonly known to control stored product pest insects were exempt by the Environmental Protection Agency (EPA) of a tolerance requirement on stored whole grains and packaged foods in warehouses, provided that insects do not become a food component [36]. In addition, according to the same authors, it was demonstrated that the fragments of insect pests were reduced in grains treated with parasitoids. During tobacco processing specifically, the product passes through several sieves and air flows that remove small foreign particles that may be present [37]. This technique can also remove dead insects or fragments from the product being processed.

While releasing parasitoids into storage environments is in itself easy and does not require skilled workers, decisions about when and where to release them, are not. As with any other control technique, the warehouse situation must be analyzed and the anticipated storage or processing steps must be taken into account. In addition, an integrated post-harvest pest management system must comprise different strategies, such as hygiene, technological and biotechnological methods, physical, chemical and biological control. These techniques must be harmonized in order to grant protection of human health and the environment together with efficient pest control.

CONCLUSION

Our study presents the first records of the practical application of the use of *H. hebetor* in mass releases in tobacco storage, demonstrating the potential of the species for moth control. The number and frequency of releases of *H. hebetor* since the beginning of tobacco storage in farmers and tobacco industry warehouses, showed that the parasitoid reduced the number of captured adults of *Ephestia* spp. under these conditions.

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Conflicts of Interest: The authors declare no conflict of interest.

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