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Species-Specific Effect of Parasitization of Ectoparasitoid, *Bracon hebetor* (Hymenoptera: Braconidae) on the Larval Haemocyte Counts of *Corcyra cephalonica* and *Ephestia kuehniella* (Lepidoptera: Pyralidae)

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ABSTRACT

Many studies have described post-parasitization physiological changes in insect hosts for koinobiont endoparasitoids. However, not much is known regarding idiobiont ectoparasitoids that how these parasitoids affect their target hosts' physiology. The present study aimed to determine the effect of parasitization by *Bracon hebetor* (Hymenoptera: Braconidae), an efficient idiobiont ectoparasitoid of many lepidopterous larvae, on the total haemocyte counts (THCs) of 5th instar larvae of *Corcyra cephalonica* and *Ephestia kuehniella*, two important stored product pyralid pests. Results showed that *B. hebetor* parasitization differentially affected THCs in parasitized *E. kuehniella* and *C. cephalonica* larvae. In *E. kuehniella* larvae, THCs steadily increased till 12th hour observation after a sharp decline in the very first hour of parasitization and then started decreasing from 24th hour post-parasitization. Highest THCs value (2800 ± 300 cells mL^{-1}) was observed at 6th day of parasitization. While on the contrary, *C. cephalonica* THCs exhibited a boost in first 6 hours of post-parasitization with maximum THCs value (3413 ± 139 cells mL^{-1}) at 6th hour, but then gradually decreased till the end of experiment on 9th day. Average THCs in fresh unparasitized larvae of *E. kuehniella* and *C. cephalonica* were 2078 ± 137 and 1474 ± 47 cells mL^{-1}, respectively. These findings indicate that parasitization by idiobiont parasitoids may alter the haemocyte profiles of their hosts and these responses are species-specific. However, future studies on the interaction of post-parasitization changes in haemocyte profiles and immunity mechanisms of host larvae would help in better comprehension of the effects of ectoparasitoids parasitization on their host physiology.

Key words: Bracon hebetor, *Corcyra cephalonica*, ectoparasitoids, *Ephestia kuehniella*, parasitization, total haemocyte counts.

INTRODUCTION

Pyralid moths (Lepidoptera; Pyralidae) are destructive insect pests of stored food products worldwide (Madrid and Sinha, 1982; Cox and Bell, 1991; Ghimire and Phillips, 2014). Their larvae cause substantial losses through feeding and by favoring

mold and other pathogenic development on grains. Among these moths, *Ephestia kuehniella* (Mediterranean flour moth) and *Corcyra cephalonica* (rice moth) are the most damaging pests of stored products especially in the milled products (Ghimire and Phillips, 2014). Nevertheless, these pyralid moths are being used as well in different biological control and research programs focusing on various insect predator and parasitoid studies (Vieira *et al.*, 1992; Rahman *et al.*, 2004).

Bracon hebetor (Hymenoptera; Braconidae) is an important idiobiont ectoparasitoid that has the ability to parasitize many lepidopterous larvae particularly of above mention pyralid moths (Brower *et al.*, 1996; Yu *et al.*, 2003). This species has been widely used in various host-parasitoid interaction studies because of wide range of host species they attack, their short generation time and high reproductive potential (Gündüz and Gulel, 2005). Female *B. hebetor* firstly stings and paralyzes its host larvae, usually within 15 minutes of venom injection and then lays eggs singly or in clusters on or near the body surface of paralyzed host. The paralysis is eventually lethal, however, paralyzed larvae without parasitization may live up to a month and consumed by wasp larvae (Altuntaş *et al.*, 2010). The venom blocks neuromuscular transmission at a presynaptic site and apparently has no effect on haemolymph circulation or midgut functioning (Eliopoulos and Stathas, 2008; Altuntaş *et al.*, 2010).

Species-specific interactions that occur in the host-parasitoid systems result in diverse effects of parasitization on the total haemocyte counts (THCs) of hosts. Haemocytes play an important role in insect immunity and defense mechanisms (Strand, 2008; Kacsoh and Schlenke, 2012). Parasitoid associated factors and parasitization usually induce changes in haemocytes morphology and physiology and variations in total and differential haemocyte counts (Stettler *et al.*, 1998; Er *et al.*, 2011). Many studies have described post-parasitization physiological changes in insect hosts for koinobiont endoparasitoids (Pennacchio and Strand, 2006; Yu *et al.,* 2007; Nishikawa *et al.*, 2013; Teng *et al.*, 2016). However, there is a lack of knowledge regarding the effect of idiobiont ectoparasitoids that how these parasitoids alter their target hosts' physiology, particularly haemolymph dynamics. In this study, therefore, we compared the effect of *B. hebetor* parasitization on the haemocyte profiles (total haemocyte counts) in the haemolymph of parasitized and unparasitized larvae of *C. cephalonica* and *E. kuehniella*. We hypothesized a suppression of haemocytes in both larval hosts by idiobiont ectoparasitoid *B. hebetor* parasitization as observed in case of koinobiont endoparasitoids (Yu *et al.,* 2007). The results provide better understanding of the interactions between parasitism and bio-resources potentially available for the nutrition and larval development of idiobiont parasitic wasps within their parasitized hosts.

MATERIALS AND METHODS

Insect collection and rearing

Larvae of host pyralids, *Corcyra cephalonica* and *Ephestia kuehniella* (Lepidoptera: Pyralidae) were procured from the laboratory of Earth and Life Institute, Biodiversity

Research Center, Université Catholique de Louvain, Louvain-la-Neuve, Belgium. These host larvae were reared on rice and wheat flour diets separately in plastic boxes under controlled laboratory conditions (26 ± 1ºC, 25-35% relative humidity, 16:8 hours light and dark photoperiod). Adults of idiobiont ectoparasitoid *Bracon hebetor* (Hymenoptera: Braconidae) were collected from the field and were reared under similar controlled laboratory conditions as mentioned above on the larvae of both lepidopterous hosts separately in plastic vials (7 cm H and 3.5 cm Ø) provided with 50% sugar solution diets as well. In order to get synchronized progeny of parasitoids, five full grown larvae of each lepidopterous host species were exposed to 24 hours old mated female wasps for parasitism on daily basis. After parasitization, the parasitized larvae were removed and confined in a petri dish for adult parasitoids emergence. Similarly, both pyralid moths were reared for many generations in order to obtain synchronized progeny to be used in experimentation. All the equipment, materials and solutions used during the study were sterilized.

Parasitization and total haemocyte counts

Experimental protocol regarding the parasitization of host larvae by parasitoid wasps and post-parasitization haemocyte counts of host larvae involved the exposure of a set of five 5th instar larvae of each host species (*C. cephalonica* and *E. kuehniella*) to 24 hours old mated female of *B. hebetor* confined within sterilized plastic vials, separately. After parasitization, parasitized host larvae were removed and placed in separate petri dishes (five larvae per petri dish). Three independent petri dish sets were maintained for each lepidopterous host species and for each of different post-parasitization sampling periods viz; 1 hour, 6 hours, 12 hours, 24 hours, 3 days, 6 days and 9 days. Prior to the collection of haemolymph, parasitized larva was surface sterilized with 75% ethanol (v/v) and air-dried on paraffin film. The haemolymph was squeezed out from each of the five larvae of each treatment by cutting larval proleg with sterilized fine-tipped forceps and was pooled in a 200 µL sterilized Eppendorf tube and this pooled sample was considered as one replicate for each treatment. Unparasitized larvae were inactivated by putting on ice for few seconds and were then subjected to haemolymph collection in the same way as explained above for parasitized larvae. Total haemocyte counts (THCs) were determined by transferring 5 µL of pooled haemolymph samples in 5 µL phosphate buffered saline solution (PBS) and then this 10 µL of diluted haemolymph sample was transferred to Neubauer hemocytometer (VWR Scientifics, West Chester, PA) and counting was made under the phase contrast stereomicroscope (LEICA DMC-4500; Leica Microsystems, Bannockburn, IL). Under the microscope, a total of 10 cells of Neubauer hemocytometer were observed on random basis for the counting of haemocytes. Measurements were made for three independent replicates for each sampling period.

Statistical analysis

The effect of ectoparasitoid *B. hebetor* parasitization on the haemocyte counts of their host *C. cephalonica* and *E. kuehniella* larvae was compared using total haemocyte counts (THCs) values as dependent variable. For both host species, the difference

between THCs of unparasitized (control) and parasitized individuals at 1 hour to 9 days post-parasitization were analyzed using repeated measures general linear ANOVA models (GLM) with LSD post-hoc test at significance level of 0.05 after validation of the normal distribution. Statistical analyses were performed using software R v.3.0.2 (R development Core Team 2014).

RESULTS

B. hebetor parasitism played a significant role in increasing or decreasing total haemocyte counts (THCs) in parasitized *E. kuehniella* and *C. cephalonica* larvae throughout the time period of experiment. Initially, there was a steep decline in the THCs in *E. kuehniella* larvae observed at 1st hour post-parasitization (1113 ± 112cells/mL) as compared to the control (unparasitized) larval THCs (2100 ± 114cells/mL) (Fig. 1). Later on, THCs increased steadily up to 12 hours post-parasitization (1113 ± 112 to 2040 ± 271 cells/mL), and then started decreasing at 24th hours post-parasitization (1513 ± 138 cells/mL). Highest THCs was observed at 6th day of parasitism with 2800 ± 300 cells/mL. THCs in unparasitized control larvae ranged from 1981 to 2175 cells mL^{-1} for *E. kuehniella*.

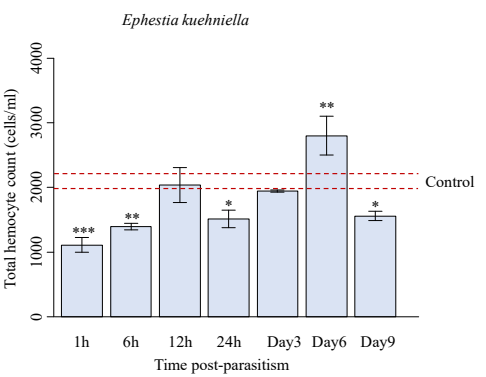

Fig. 1. Effect of parasitization by *Bracon hebetor* on total haemocyte counts (THCs) of 5th instar larvae of *Ephestia kuehniella*. Bars represent mean values of THCs ± standard errors (n = 15). Dotted red lines show THCs variation in control (unparasitized) larvae. Asterisk symbols '***', '**' and '*' show significance between parasitized and unparasitized larvae at $P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively.

Similarly, *B. hebetor* parasitization induced significant variations in the THCs of *C. cephalonica* (Fig. 2). As a result of *B. hebetor* venom injection, there was a significant increase in the THCs of parasitized larvae of *C. cephalonica* after 1st hour of larval parasitization (2220 ± 42 cells/mL) in comparison with the freshly extracted haemolymph of control larvae (1440 ± 99 cells/mL). These haemocyte numbers gradually increased up to 6th hour observation with highest and significant THCs values observed at 6th hour (3413 ± 139 cells/mL). THCs then gradually decreased along with the time reaching its significant lowest THCs values at day 9 (860± 111 cells/mL). The average THCs value in unparasitized control larvae of *C. cephalonica* was 1473 ± 47 cells mL^{-1} (Fig. 2).

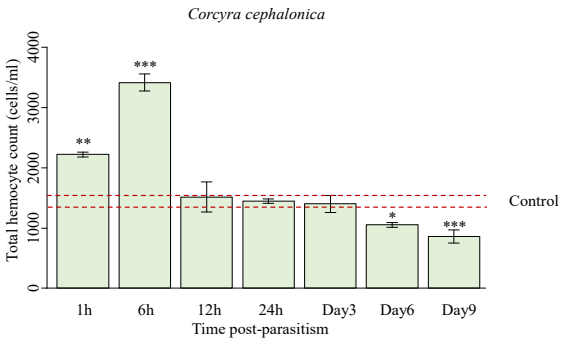

Fig. 2. Effect of parasitization by *Bracon hebetor* on total haemocyte counts (THCs) of 5th instar larvae of *Corcyra cephalonica*. Bars represent mean values of THCs ± standard errors (n = 15). Dotted red lines show THCs variation in control (unparasitized) larvae. Asterisk symbols '***', '**' and '*' show significance between parasitized and unparasitized larvae at $P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively.

DISCUSSION

Results of the study showed that *B. hebetor* parasitization had a differential effect on the larval THCs of both host species. In the first few hours post-parasitization, a sudden suppression and then gradual increase in haemocyte counts were observed in *E. kuehniella*; while on the contrary, parasitization in *C. cephalonica* larvae exhibited a stimulation in THCs in the initial hours of observations indicating a boost in the production of haemocytes within *C. cephalonica* larvae in response to parasitization by *B. hebetor*.

Parasitization may induce species-specific effects on the host physiology including haemolymph kinetics as demonstrated by Stettler *et al.* (1998). In our study, the haemocyte profiles varied significantly throughout the time period of experiment in larvae of both lepidopterous species (*C. cephalonica* and *E. kuehniella*) in response to parasitization by an ectoparasitoid *B. hebetor*. In *E. kuehniella,* higher haemocyte counts were observed in the late stages of parasitization as compared to earlier ones. Similar post-parasitization haemocyte profile patterns have been observed in case of endoparasitoid *Cotesia chilonis* by Teng *et al.* (2016) where haemocyte counts in *Chilo suppressalis* showed a boost from 72 hours post-parasitization till 9th day of experiment. Some other studies also observed a post-parasitization increase in total haemocyte counts in various insect pest species parasitized by endoparasitoid wasps such as in *Malacosoma disstria* parasitization by *Hyposoter fugitivus* (Stoltz and Guzo, 1986) and in *Pseudoplusia includens* parasitization by *Microplitis demolitor* (Strand and Noda, 1991).

Although parasitization by ectoparasitoids have been shown causing perturbations and suppression of many physiological and developmental aspects of host lepidopterous larvae including haemolymph enzymatic activities (Weaver *et al.*, 1997; Mosson *et al.*, 2000; Richards and Edwards, 2000), but there is a paucity of knowledge regarding their effect on host haemolymph profiles. Nonetheless, one study

by Richards and Edwards (2000) has demonstrated both quantitative and qualitative changes in haemolymph proteins and haemocytes by ectoparasitoid parasitization and these findings are in line with our findings.

However, sometimes host parasitization by parasitoids results in considerable THCs suppression. In our study, for instance, the parasitism by same parasitoid species resulted into a gradual decrease in THCs in parasitized *C. cephalonica* larvae. These results are in agreement with those of many previous works done on endoparasitoids (Doucet and Cusson, 1996; Yu *et al.*, 2007; Nishikawa *et al.*, 2013) and ectoparasitoids (Richards and Edwards, 2000; Yu *et al.*, 2003). This THCs reduction would most probably be due to the stimulation of immune-suppression mechanisms (Stettler *et al.*, 1998) or hematopoietic organ histolysis and circulating haemocytes cell death (Teramoto and Tanaka, 2004; Altuntaş *et al.*, 2010).

In brief, present study demonstrated a differential effect of parasitization by an idiobiont ectoparasitoid *B. hebetor* on the total haemocyte counts of 5th instar larvae of *C. cephalonica* and *E. kuehniella*. THCs in *C. cephalonica* stimulated upon parasitization and then gradually decreased till 9th day of observation, while this pattern was inverse in case of *E. kuehniella*. However, future focus on the regulation of immunity mechanisms of these lepidopterous larvae by *B. hebetor* ectoparasitoids would help in better understanding of the post-parasitization changes in haemocyte profiles.

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