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Behavioral Studies of the Parasitoid *Bracon vulgaris* Ashmead (Hymenoptera: Braconidae)

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Abstract *Bracon vulgaris* is a larval ectoparasitoid of the boll weevil, *Anthonomus grandis*, a key cotton pest We investigated the influence of parasitoid age, photoperiod and host availability on *B. vulgaris* parasitism. Five- and 10-day-old parasitoids were exposed to *A. grandis* densities of three, six, 12 and 24 larvae per female. Five-day-old females showed higher parasitism rates (F = 21.55, $P < 0.0001$), with a positive increase in parasitism rate up to 12 larvae, parasitizing an average (±SE) of 1.3 (±0.12), 3.4 (±0.35) and 4.4 (±0.45) larvae (at the densities of 3, 6 and 12 larvae, respectively), dropping to 1.4 (±0.17) when 24 larvae were offered. Next, the period of most parasitism activity during the photophase was determined by exposing five-day-old *B. vulgaris* females to different durations of light exposure (1, 3, 6, 8 and 10 h) after 12 h darkness. Results showed that the shortest time to host location occurred 8 h after light exposure, when parasitoids were most active searching for the host. A sequence of seven behavioral acts leading to successful parasitism was identified and the transitions between successive behavioral acts were quantified and depicted in an ethogram. The longest duration act was oviposition (or host acceptance), a complex behavior with several distinct phases.

Keywords Ectoparasitoid · Parasitism behavior · Boll weevil · Ethogram

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Introduction

Agricultural pest control is one of the factors that increase production costs, due to the development of pest insecticide resistance, and the great diversity of pest species found in agroecosystems. For instance, the cotton plant (*Gossypium hirsutum* L., Malvaceae) hosts between 300 and 600 species of herbivores, of which about 32 can cause significant reduction in cotton production in Brazil (Torres 2008). Approximately 20 % of the total cost of cotton production is directed toward agricultural pesticides (Alves and Serikawa 2006; Fonseca et al. 2011).

Among the pests that affect cotton production in Brazil, the boll weevil, *Anthonomus grandis* (Boheman) (Coleoptera: Curculionidae), stands out as the most important, and it is widely recognized to be the main constraint for cotton cultivation throughout Brazil. This weevil colonizes cotton plants, where it feeds and lay eggs inside fruiting structures (squares and young bolls), inducing their abscission (Neves et al. 2013). Even when bolls do not fall from the plant, the boll weevil larvae developing inside destroy cotton fibers and seeds (Santos et al. 2003; Busoli et al. 2004; Grigolli et al. 2013).

Natural biological control of the boll weevil on cotton in Brazil is mainly performed by larval parasitoids. Altogether, 13 species of boll weevil parasitoids have been described (Araújo et al. 2000; Ramalho and Wanderley 1996; Santos et al. 2013). Among these, *Bracon vulgaris* (Ashmead) and *Catolaccus grandis* (Burks) are the main biological agents responsible for reducing natural populations of boll weevil in the Northeast of Brazil (Ramalho et al. 2007; Silva-Torres et al. 2009b; Santos et al. 2013). Carvalho et al. (2000) found that *B. vulgaris* can achieve high rates of parasitism of *A. grandis* ($\cong$ 70 %), especially at the end of the cropping season. However, to avoid significant losses in cotton production, it is necessary to determine the effectiveness of a parasitoid throughout the season and in particular to know how they respond to changes in host density and distribution.

The physiological state of the parasitoid also has important implications relative to the success of parasitism. Parasitoid age, for example, can affect host location, parasitism rate, total offspring production, and offspring sex ratio (Honda and Kainoh 1998; Harbinson et al. 2001; Cooperband et al. 2003; Gunduz and Gulel 2005; Silva-Torres et al. 2009a; b; Pereira et al. 2009). In addition abiotic factors (e.g. photoperiod and temperature) influence parasitoid behavior (Ferreira et al. 2003; Silva-Torres et al. 2009a).

Although *B. vulgaris* is recognized as an important biological control agent in cotton ecosystems in northeastern Brazil, many details, such as the influence of parasitoid age, host availability, and the sequence of behavioral events involved in the parasitoid-boll weevil interaction are poorly known. Thus, the objectives of this study were to determine the effect of parasitoid age on parasitism success, the effect of host availability on parasitism rates, and to quantify parasitoid behavior under different light regimes for *B. vulgaris*. To facilitate these investigations we also identified and quantified the sequence of behavioral acts leading to successful parasitism and assessed the degree of stereotypy in parasitism behavior.

In this context, the following hypotheses were tested: i) the rate of parasitism of *B. vulgaris* is dependent on the age of the parasitoid, ii) parasitism rate is influenced by the photophase, iii) parasitism behavior of *B. vulgaris* follows an innate sequence of

behavioral events. Thus, it is expected that *B. vulgaris* increase their rate of parasitism when subjected to a greater amount of host and, that this parasitoid showed a difference in search and host handling time along the photophase.

Materials and Methods

The experiments were conducted at the Laboratory of Insect Behavior of the Universidade Federal Rural de Pernambuco (UFRPE), Recife-PE, Brazil, and at the Biological Control Unit of Embrapa Algodão, Campina Grande-PB.

Insect Host Collection

Third-instar larvae of *A. grandis* were obtained by collecting abscised cotton squares and bolls with oviposition signs from 45-day-old cotton plants (cv. BRS 7 H) cultivated in greenhouses at the UFRPE campus. The collected cotton squares were taken to the laboratory where they were gently partly opened and examined under a stereomicroscope (OLYMPUS SZ-40) to confirm the presence of weevil larva, its stage of development, and the absence of parasitism or pathogenicity. Then, the weevil larvae were kept inside the cotton square to be exposed to the parasitoid because previous observations revealed that the female parasitoid rejected host larvae encountered separate from the cotton fruiting structure.

Collection and Maintenance of Parasitoids

Flower buds and bolls were collected in an experimental cotton field of Embrapa, located in Barbalha County, CE, Brazil. Subsequently, the material was transported to the laboratory of Insect Behavior-UFRPE, placed in plastic trays lined with filter paper, and kept in Plexiglas cages (45 cm wide × 45 cm long × 30 cm high) at 25 ± 1.5 °C and 12 h daylight until adult parasitoids emerged.

Within one day of emergence, pairs of one female and one male were formed and each pair was placed in a transparent plastic container (500 ml) (Prafesta®, Mairipora, SP); for ventilation, the container lid was replaced by a piece of voile fabric (2 mm mesh) tightly secured with a rubber band. Cotton pieces soaked in water and droplets of honey placed on the container wall were offered to the parasitoids as moisture and food, respectively. The insects were placed in a BOD chamber at 25 ± 2 °C, photoperiod of 12 L:12D and 70 ± 10 % relative humidity and remained under these conditions until their use in the experiments. Males were removed from containers 24 h after pair establishment and females were assumed to have mated with them.

Parasitism Capacity of Bracon Vulgaris According to its age and Host Availability

Boll weevil-infested cotton squares and flower buds collected from 45-day-old cotton plants (cv. BRS 7 H) were exposed to *B. vulgaris* parasitism in the laboratory. Based on the instar of the larvae, those that were in the third instar of development were offered to a single female parasitoid in order to monitor the response of parasitism of *B. vulgaris*, by increasing host density. Thus, mated inexperienced female wasps were

randomly assigned to two groups, 5 and 10-days old. Females of *B. vulgaris* from each age class were individually placed in plastic pots (250 ml) closed by a large piece of voile fabric tightly secured by a rubber band. In addition, cotton pieces soaked in water and droplets of honey on the container wall were provided to the parasitoids. Four host densities were tested for each age group of female parasitoids (5 and 10): 3, 6, 12 and 24 larvae per pot, respectively, with 15 replications for the smallest host density (3) and 10 replicates each for the higher host densities. Therefore, a total of 45 adult *B. vulgaris* females were used in this experiment, being one female per replication.

Pots containing host larvae and female parasitoids were maintained for 48 h in a BOD chamber at 25 ± 2 °C, photoperiod of 12 L:12D and 70 ± 10 % relative humidity. Following this exposure, weevil larvae were placed individually in Petri dishes (35 mm × 10 mm) and kept in the growth chamber until adult emergence. The number of parasitoid eggs deposited per host larva was determined by examining larvae under a stereomicroscope, and we also recorded the number of hatched larvae, pupae, and subsequent emergence of parasitoid or weevil adults.

Data were tested for normality (Kolmogorov D: normal test) and homogeneity of variance (Bartlett's test), and square root (x + 0.5) or log(x + 1) transformations were used when necessary; however, untransformed means are presented in tables and figures. The results were submitted to two-way analysis of variance (ANOVA) (wasp ages and host density) were performed to test the changes on the variables between was ages and host densities. When host density was significant by ANOVA, a Tukey studentized range test was performed to separate the means at the 5 % probability level (SAS Institute 2000).

Parasitism Behavior of Bracon Vulgaris Along the Photophase

Female *B. vulgaris* were obtained from the stock colony maintained at the Biological Control Unit of Embrapa Algodão, Campina Grande, PB. The parasitoids were reared under the conditions of temperature of 28 ± 2 °C, 12 L: 12D of photoperiod, 70 ± 10 % relative humidity. Emerging adult parasitoid wasps were placed in transparent acrylic cages measuring 44 × 35 × 45 cm (height x width x length), in which moisture was provided in water soaked cotton balls in a 10 mL plastic container. In addition, honey was provided as food in droplets on the inner wall of cages.

To determine the age and time of day of higher search activity of the parasitoid along the photophase 5-day-old females of *B. vulgaris*, previously fed, mated, and without experience of parasitism were subjected to one, three, six, eight or 10 h light intervals following the scotophase in BOD, at 25 ± 2 °C and 70 ± 10 % relative moisture. We chose a temperature of 25 °C to conduct our observations, since at this temperature the parasitoid has a better development (duration from egg to adult) and a larger number of offspring produced (Ramalho et al. 2009). Each light exposure regime had 30 replications (individual females),. Next, each female parasitoid was individually placed in a glass vial (10 cm × 2 cm) containing one gently partly opened cotton square infested with a third instar boll weevil larva and closed with cotton wool. The females of the different groups (light regimes) were observed individually under a stereomicroscope recording the sequence of behaviors until oviposition, recognized by the appearance of eggs on the host surface. Data were submitted to the non-parametric PROC FREQ of

SAS (SAS Institute 2001) followed by χ^2 tests for comparison between groups of females subjected to different light exposure regimes.

Ethogram of Host Parasitism by Bracon Vulgaris

To observe and record the steps involved in host parasitism by *B. vulgaris* we used the method of all occurrences, "ad libitum", (Yamamoto and Volpato 2007). Thus, 5-day-old female parasitoids, fed, mated, and without parasitism experience were obtained from the stock colony of Embrapa Algodão, as mentioned for the previous experiment. Based on the preliminary results, and determination of the time of the day with higher parasitism activity, 30 female parasitoids were placed individually in glass vials (10 cm × 2 cm in diameter) containing a cotton square infested with a third instar boll weevil larva, a cotton wad soaked in water, and a tiny drop of honey on the inner wall of the glass tube. The tubes were closed with cotton wool to allow venting inside the vial. Each one of the 30 female parasitoids was observed continuously from the time of its release inside the vial until location, parasitism, and departure from the host. From these observations the behavioral acts of *B. vulgaris* to locate and parasitize the host were defined. This experiment was also conducted in all other light intervals previously assessed, besides the one in which there was higher parasitism activity, to determine whether there was any significant difference in the sequence and or number of events involved in the parasitism behavior of *B.vulgaris* along the photophase.

Data for all observed parasitism sequences were used to create a first-order Markovian behavioral transition matrix. The repetition of a single behavior (self-transition) was not included in the matrix to avoid any possible influence on the relative weight of transitions between behaviors. Following Haccou and Meelis (1992), transition probabilities were calculated from the observed frequency of a transition between two events divided by the total number of occurrence of the first event. We used the iterative proportional fitting method (Goodman 1968) to calculate the expected values of the matrix cells and the statistical significance of the individual transitions was evaluated by a log-likelihood ratio test (*G*-test) using Excel (Microsoft Office, Windows 7). The results are displayed graphically in an ethogram.

Results

Parasitism Capacity of Bracon Vulgaris According to its age and Host Availability

Parasitoid age was an important factor affecting parasitism of boll weevil larvae. There was a significant difference in the number of parasitized larvae according to the age of the parasitoid ($F_{1, 82} = 29.12$; $P < 0.0001$). Five-day-old females had a higher parasitism rate than 10-day-old females (average ± SE; 5d = 2.53 ± 0.23 larvae; 10d = 1.44 ± 0.09 larvae). In addition, there was a statistically significant difference in parasitism depending on host density ($F_{3, 82} = 19.01$, $P < 0.0001$), and the interaction of these factors ($F_{3, 82} = 10.03$, $P < 0.0001$).

Five-day-old females parasitized significantly more larvae when offered the densities of 6 and 12 larvae ($F_{3, 41} = 21.55$, $P < 0.0001$) compared to the densities of 3 and

24 larvae (Table 1). However, there was no effect of host density for 10-day-old females ($F_{3,\,41}$ = 2.23 P = 0.0995 (Table 1).

Within each host density, comparing the number of parasitized larvae depending on the age of the parasitoid, there was no age effect on parasitism of 3 and 24 larvae (P > 0.05). However, there was an age effect on parasitism rate for six larvae ($F_{1,\,18}$ = 16.44, P = 0.0007) and 12 larvae ($F_{1,\,18}$ = 42.39, P < 0.0001), when five-day-old females parasitized significantly more larvae compared to 10-day-old females (Table 1).

The number of eggs deposited at each host density level followed the same trend as the number of parasitized larvae. The number of eggs laid per host density was significantly different depending on the age of the parasitoid ($F_{1,\,82}$ = 9.53, P = 0.0028). Five-day-old females laid more eggs in comparison to 10-day-old females (average ± SE; 5d = 4.8 ± 0.54 eggs; 10d = 3.02 ± 0.25 eggs). Next, there was a significant difference in the number of laid eggs depending on host availability ($F_{3,\,82}$ = 13.30; P < 0.0001), and the interaction of these factors ($F_{3,\,82}$ = 4.04, P < 0.0098). Additionally, 5-day-old females laid significantly more eggs when offered 6 and 12 larvae ($F_{df\,=\,3,\,65}$ = 3.69, P = 0.0162) compared to 3 and 24 larvae (Fig. 1). However, there was no effect of host density on the number of eggs laid by 10-day-old females ($F_{df\,=\,3,\,65}$ = 1.97, P = 0.1220).

Within each host density, there was no effect of parasitoid age on the number of eggs deposited per larvae for 5-days-old females ($F_{df\,=\,3,\,112}$ = 1.97, P = 0.1220), while for 10-days old females there was a larger number of eggs laid when 12 larvae were offered simultaneously to the parasitoid ($F_{df\,=\,3,\,65}$ = 3.69, P = 0.0162) (Table 1). In general, 5-day-old females laid more eggs than 10-day-old females (Table 1).

For pupal viability, there was a significant effect of host density for 5-day-old ($F_{3,\,41}$ = 14.08, P < 0.0001) and 10-day-old females ($F_{3,\,41}$ = 4.78, P = 0.006) (Table 1). Furthermore, comparing the ages of five and 10 days within each host density offered, there was no difference in the number of viable pupae formed in the densities of three and 24 larvae (3 L: $F_{1,\,28}$ = 0.08, P = 0.782; 24 L: $F_{1,\,18}$ = 0.91, P = 0.35). In contrast, there was a significant difference in the number of viable pupae in the densities of six and 12 larvae (6 L: $F_{1,\,18}$ = 6.83, P = 0.0176; 12 L: $F_{1,\,18}$ = 15.65; P = 0.0009), with 5-

Table 1 Characteristics of parasitized *Anthonomus grandis* exposed for 48 h to *Bracon vulgaris*

Age of parasitoid	Host density	Parasitized larvae	Number of laid eggs/larva	Number of viable pupae	Emerged adults
5 days	3	1.3 ± 0.16 b[1]	2.0 ± 0.29 a	2.0 ± 0.47 b	1.5 ± 0.47 b
	6	3.4 ± 0.20 a	2.0 ± 0.22 a	4.9 ± 0.86 a	3.6 ± 0.93 ab
	12	4.4 ± 0.16 a	1.9 ± 0.14 a	7.2 ± 0.90 a	5.2 ± 0.99 a
	24	1.6 ± 0.17 b	1.4 ± 0.27 a	1.9 ± 0.43 b	1.5 ± 0.30 b
10 days	3	1.4 ± 0.12 a	1.8 ± 0.20 b	2.1 ± 0.38 ab	0.9 ± 0.38 b
	6	1.8 ± 0.30 a	1.8 ± 0.23 ab	2.7 ± 0.45 a	1.9 ± 0.56 ab
	12	1.5 ± 0.45 a	2.7 ± 0.21 a	3.3 ± 0.37 a	2.8 ± 0.35 a
	24	1.1 ± 0.33 a	1.6 ± 0.22 b	1.3 ± 0.34 b	0.6 ± 0.26 b

[1] Values followed by the same letter in the column within the specific age of the parasitoid did not differ statistically by the Tukey test at 5 % probability
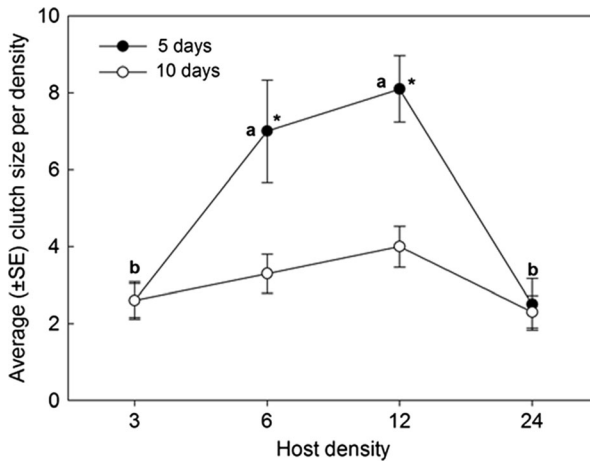
Fig. 1 Average number (±SE) of eggs laid by *Bracon vulgaris* of different ages (5 and 10 days old) as function of the host availability. Note: Means followed by different letters differ statistically as function of host density for 5 days old female ($F_{df = 3, 65} = 3.69$, $P = 0.0162$); while, 10 days old female do not differ ($F_{df = 3, 65} = 1.97$, $P = 0.1220$). *Stands for mean difference between female age within host density

day-old females having double the number of viable pupae in comparison to 10-day-old females (Table 1).

Adult emergence was at a higher rate when six ($F_{1, 18} = 2.72$, $P < 0.1167$) and 12 ($F_{1, 18} = 4.37$, $P < 0.0510$) larvae where offered, both for five-day-old ($F_{3, 41} = 6.74$, $P < 0.0008$) and ten-day-old females ($F_{3, 41} = 6.57$, $P < 0.0010$).

Parasitism Behavior of Bracon Vulgaris Along the Photophase

The elapsed time after light exposure affected the activity of parasitism of *B. vulgaris* ($\chi^2 = 120.6$, $P < 0.0001$, D.F. = 4). The shortest time the parasitoid took for locating the host was after 8 h of light exposure wherein the parasitoids were more active searching for the host. After this time interval, parasitoid activity decreased proportionally to the increase in light hours. In contrast, there was no difference in parasitism itself ($\chi^2 = 4.77$, $P = 0.311$, D.F. = 4), in other words, after locating the host, the time spent by the parasitoid to insert the ovipositor and lay eggs remained unchanged, regardless of the previous light exposure regime. This result demonstrates that even taking more time to find the host, the parasitoid still was able to successfully oviposit.

Ethogram of Host Parasitism by Bracon Vulgaris

Overall, seven behavioral acts were involved in the parasitism behavior of *B. vulgaris* upon boll weevil larvae (Table 2). Overall, the parasitoid female first remained motionless (inactive) after release in the vial. Next, in most cases it started host searching (walking); otherwise it began grooming. While walking, it also performed short flights, explored the premises, and also moved the antennae. When it started grooming, it used the legs and mouthparts to clean the wings and antennae. The females of *B. vulgaris* groom by using the front legs to clean antennae and head followed by rubbing the third pair of legs against wings and gaster and then the ovipositor. After grooming, the

Table 2 Behavioral acts and durations observed during parasitism of *Anthonomus grandis* larvae by *Bracon vulgaris* (*n* = 30)

Behavioral act	Description	Duration (min ≅)
1. Static	Remaining motionless (inactive) after release.	2.11
2. Walking	Host searching, walking or short flights, exploring the premises.	3.02
3. Drumming	Antennating substrate rapidly while resting or slowly walking.	2.16
4. Grooming	Cleaning body parts using the legs and mouthparts.	1.22
5. Probing	Inserting and removing the ovipositor in the cotton square to locate the host larva.	3.23
6. Oviposition	Bending the abdomen forward and inserting its ovipositor into the cotton square, remaining motionless while injecting venom, ending with egg laying.	26.83
7. Resting	Remaining motionless on the cotton square	3.12
Total		41.60

parasitoid went straight to drumming the larva or probing. When probing, it inserted the ovipositor in the cotton square. Alternatively, the parasitoid would switch to host searching, walking in the vial. From walking, the parasitoid next either started probing or drumming the cotton square, and these behaviors could be alternated. If it started drumming it might stop and begin grooming again. On the other hand, if it started probing the larva, in most cases oviposition followed, with the female first remaining motionless above the cotton square while injecting venom,. Less commonly, when it did not accept the host immediately, it went back to grooming. Finally, following host acceptance and oviposition, in most cases the parasitoid started grooming again; less frequently, it went back to walking or remained resting on the cotton square. After resting, it eventually began to walk again in the vial. The various behavioral transitions relating to oviposition are depicted in Fig. 2.

The average time for successful parasitism (from the release of the female until oviposition on the host) was 41.6 min (range 38–120 min). Females spent the most time
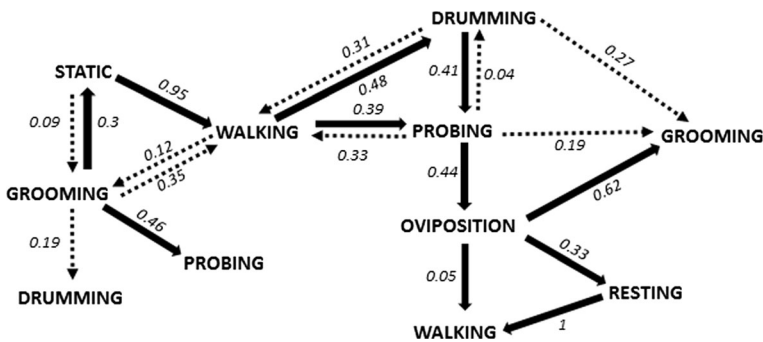

Fig. 2 Sequence of parasitism behavior of *Bracon vulgaris* upon cotton squares attacked by *Anthonomus grandis*. Values represent the probability of transitions between behaviors. Solid-line arrows indicate significant transitions (*P* < 0.05) and dashed-line arrows nonsignificant transitions (*P* > 0.05). Boxes represent behavioral categories

during the host acceptance (oviposition) phase, followed by host search, probing, resting, static, and grooming, respectively (Table 2).

The behavioral repertoire as well as the sequence of events did not differ under the various light regime exposures evaluated, although, there was a significant difference in the time taken to locate the host (χ^2 = 120.6, P < 0.0001, D.F. = 4).

Discussion

Parasitoid age is a crucial factor in the success of parasitism. According Silva-Torres et al. (2009a), the potential of parasitism can be more or less favored by increasing the age of the parasitoid wasp. Thus, the higher parasitism rate obtained with 5-day-old females in this study compared to 10-day-old females indicates that the parasitism capacity of *B. vulgaris* decreases over certain age. Presumably, this results from the physiological condition of the parasitoid. According to Jervis et al. (2008), sugars, lipids, and proteins are key nutrients used in reproduction (egg production) and in the completion of the parasitoid somatic functions (maintenance and locomotion). The concentration of those compounds is directly dependent on the parasitoid's metabolism, so they tend to decline with increases in insect age. Probably, 10-day-old *B. vulgaris* females have lower amounts of such nutrients. In addition, those older females should have lower sugar content, due to aging, therefore less energy to spend on parasitism. According to Azzouz et al. (2004), there is a trade off between the action of parasitism (the host seeking behavior, venom production and maturation of eggs) and expenditures necessary for maintenance and locomotion of the parasitoid, which interferes with the success of parasitism. Also, according to Dossi and Cônsoli (2010), in some species of parasitoids the seminal fluid of males which is passed to females during copulation provides energy reserves that help support metabolic activities, vitellogenin synthesis, maturation of ovaries, and embryonic development. Further experiments are needed to investigate the effects of male contributions to the parasitism capacity and survival of *B. vulgaris* females.

Not surprisingly, host availability affected the parasitism rate (Faria et al. 2000; Makundi and Sariah 2005; Matadha et al. 2005; Faria et al. 2008). Only, 5-day-old *B.vulgaris* females responded positively to the increasing number of hosts offered up to 12 host larvae.

At higher host densities, there was a reduction in the number of larvae parasitized (Fig. 1). Sampaio et al. (2001), reported that parasitoids increase the time spent in host selection when exposed to a greater number of host options/density. Therefore, it is appears that at the higher host densities tested, *B.vulgaris* females spent more effort in host searching behavior, handling and selection; however, this effort did not always result in successful parasitism.

For 10-days-old *B.vulgaris* females, the number of parasitized larvae was lower than for younger females. In addition, the number of larvae parasitized and the number of eggs deposited was not related to host density. Differences appeared only when we considered clutch size per parasitized larva, within host density, and number of pupae formed and adults emerged; with lower means for 3 and 24 hosts offered, and highest means for intermediate values (6 and 12 hosts offered). This result suggests possible clutch size adjustment by the parasitoid females, reported for other parasitoid wasps (Bezemer and Mills 2003; Silva-Torres et al. 2010).

Interestingly, for densities of 3 and 24 host larvae, 5-day-old *B.vulgaris* females differed in the number of parasitized larvae (Fig. 1), but did not differ in the number of eggs deposited per larva (Table 1), which are correlated biological parameters. At the lowest density, possibly, this event is the result of a female strategy to avoid superparasitism, as this could affect the development of the offspring due to intraspecific competition for food resources (Hohmann and Luck 2004; Varaldi et al. 2005; Chen et al. 2006; Silva-Torres et al. 2009b). Females of *B. vulgaris*, after evaluating the physiological condition of the host larva, may reduce the number of eggs deposited per host (2.6 ± 0.49 eggs/48 h), similar to that reported by Carvalho et al. (2002) who found an average of 1.2 (± 0.21) eggs/for *B.vulgaris* parasitizing boll weevil larvae.

When *B. vulgaris* took the least time to find the host occurred 8 h after light exposure, when the parasitoids were more active searching for the host. This is consistent with the tendency for daytime parasitism as seen in other parasitic Hymenoptera (Quicke 1997; Lewis and Whitfield 1999; Tunçbilek and Ayvaz 2003). At this time, higher luminosity and higher temperature due to solar heat favors parasitoid locomotion and stimulates flight muscle activity reducing energy costs for thermoregulation when compared with the early morning and late afternoon, as noted by Kovac et al. (2009). Moreover, it is possible that boll weevil larvae inside the fruiting structures of cotton plants are most active during the photophase, which would facilitate their discovery by the parasitoid through the perception of vibrations caused by the and feeding of the host (Dweck et al. 2008; Wang et al. 2010; Ahmed et al. 2013); however this information was not verified in this study and needs further assessment.

Besides the information about the influence of abiotic and biological factors affecting parasitism, observations of parasitoid behaviors are extremely important for Integrated Pest Management (IPM) programs. Information obtained can facilitate both mass rearing of parasitoids and maximization of natural and augmentative biological control (Mehrnejad and Copland 2006; Rukmowati-Brotodjojo and Walter 2006). Thus, ethograms or behavioral repertoires are important tools used to better understand and evaluate parasitoid behavior towards hosts (Del-Claro et al. 2002).

The relatively small number of behavioral acts characterizing *B. vulgaris* parasitism of *A. grandis* can be explained as a consequence of our experimental protocol of simply confining the parasitoid wasp with its host. Earlier phases of the host finding process thus were not observed in our study, but likely include several additional behaviors as reported for other species of parasitoids (Sinzato and Prezoto 2000; Zara and Balestieri 2000; Del-Claro et al. 2002). Similar to our results, Mills and Kuhlmann (2004) studying the parasitism behavior of *Trichogramma platneri* (Nagarkatti) and *Trichogramma pretiosum* (Riley) reported the same number of behavioral acts as we found for *B.vulgaris*.

The parasitism behavior of *B. vulgaris* can be divided into two phases. The first is locating the reproductive structure of cotton (squares and buds), in which the boll weevil larva develops. The second is finding the exact location of the host within the fruiting structure. Sensory structures (antennae and ovipositor) are essential to both phases, ensuring successful parasitism (Alves et al. 2014) either by sensing volatiles released from attacked plants and detecting host signs such as feces and other chemical clues (Xiaoyi and Zhongqi 2008; Dweck 2009; Li et al. 2011).

Among the behavioral acts observed in *B.vulgaris*, the one with the longest duration (host acceptance or oviposition) ($\cong$ 26.83 min) was expected because it is a behavior composed of several interdependent steps. As part of this behavior, the parasitoid inserts its ovipositor first through the cotton square and then the host's cuticle, then deposits injects venom to paralyze the host, then and finally lays its eggs (Brown and Anderson 1998; Ne'non et al. 1997; Moreau et al. 2009; Shah et al. 2012).

The results of this study provide valuable data that can improve the use of *B.vulgaris* in augmentative biological control programs. Knowledge of the most appropriate age and time of wasp release for maximizing parasitism rates, is especially important. Knowledge of the behavioral and biological limitations of parasitoids will enable pest management programs to better tailor strategies for parasitoid use. *Bracon vulgaris* parasitism behavior has been shown to be affected by different factors, both biotic (age and host density) and abiotic (photophase). Older females did not respond significantly to an increase in the density of hosts offered, whereas younger females (5-day-old) only responded to host availability at intermediate host densities.

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