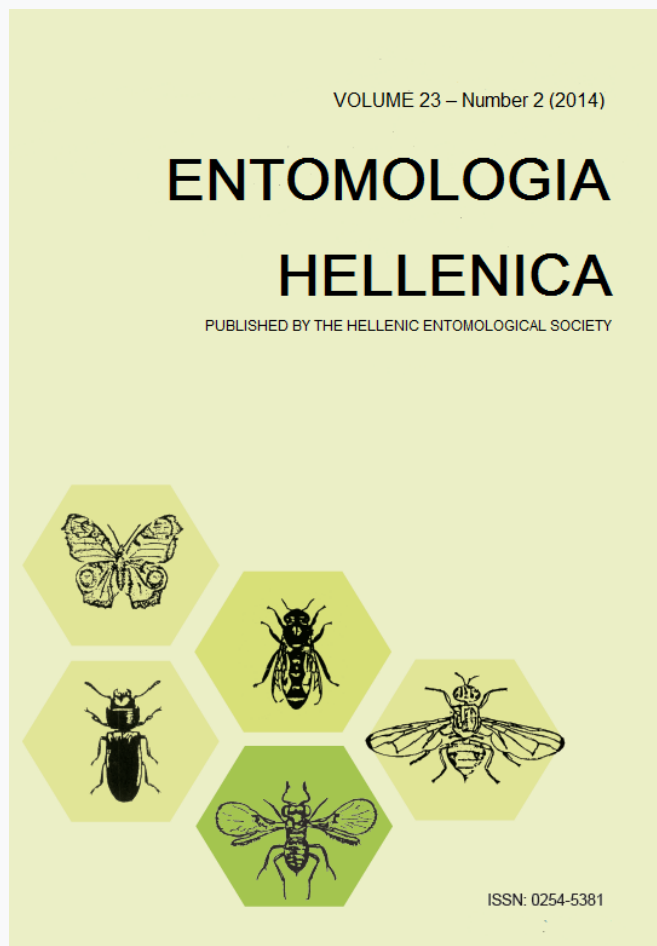


ENTOMOLOGIA HELLENICA

Vol 23, No 2 (2014)



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doi: [10.12681/eh.11537](https://doi.org/10.12681/eh.11537)

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To cite this article:

Malesios, G. A., & Prophetou-Athanasiadou, D. A. (2014). Development of the parasitoid *Bracon brevicornis* on different larval instars (L2-L5) of the Indian meal moth *Plodia interpunctella*. *ENTOMOLOGIA HELLENICA*, 23(2), 59–64.
<https://doi.org/10.12681/eh.11537>

Development of the parasitoid *Bracon brevicornis* on different larval instars (L₂-L₅) of the Indian meal moth *Plodia interpunctella*

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ABSTRACT

Bracon brevicornis (Wesmael) (Hymenoptera: Braconidae) is a gregarious parasitoid that attacks a variety of important lepidopterous pests of stored products. The aim of this study was to determine if different larval instars of *Plodia interpunctella* (Lepidoptera: Pyralidae) affected longevity and fecundity of parasitoid *B. brevicornis*. Percentage of parasitism, number of eggs laid on different larval instars of *P. interpunctella*, number of larvae, prepupae, pupae and the number of F₂ adults of *B. brevicornis* as well as the developmental time of parasitoid egg, larva, prepupa, pupa and the total developmental time from egg to adult on different larval instars of the host were recorded. Our results showed that statistically significant differences were observed among the larval instars of *P. interpunctella* in all studied parameters. In L₂ of *P. interpunctella* no eggs of *B. brevicornis* were observed. A few eggs laid on L₃ but they were not developed successfully. Concerning the development of *B. brevicornis* on L₄ and L₅ instars of *P. interpunctella* statistically significant differences were observed between the two instars. The mean number of eggs laid by *B. brevicornis* on L₄ of *P. interpunctella* per 10 days was 2.42 eggs per adult, and the mean number larvae, prepupa, pupae and adults was 1.42, 1.37, 1.26 and 1.24 respectively. The mean number of eggs laid on L₅ of *P. interpunctella* by *B. brevicornis* was 13.65, while the mean number of larvae, prepupae, pupae and adults was 9.73, 7.90, 7.44 and 7.16 respectively. In all cases the differences were statistically significant. The developmental time of *B. brevicornis* developed on L₄ instar of *P. interpunctella* was 1.12 days for egg, 2.03 for larva, 1.47 for prepupa, 6.82 for pupa and the total developmental time from egg to adult 11.45 days. The developmental time of *B. brevicornis* developed on L₅ instar of *P. interpunctella* was 1.18 days for egg, 1.68 for larva, 1.29 for prepupa, 7.76 for pupa and the total developmental time from egg to adult 11.92 days. The female and male *B. brevicornis* adult life span of F₁ generation was 16.70 and 11 days when developed on L₄ of *P. interpunctella* and 28.70 and 17.60 days when developed on L₅ of *P. interpunctella*.

KEY WORDS: *Bracon brevicornis*, Braconidae, host size effect, *Plodia interpunctella*.

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Introduction

Bracon brevicornis (Wesmael) (Hymenoptera: Braconidae) is a gregarious, larval ectoparasitoid that attacks many Lepidoptera species including some serious stored products pests. *B. brevicornis* female first paralyzes its host by stinging and then lays a variable number of eggs on the ventral surface of the larva (Antolin et al. 1995). In Greece many Lepidoptera species, occurring in stored products (*Plodia interpunctella* (Hübner) *Ephestia kuehniella* (Zeller), *E. elutella* (Hübner)) have been reported to be parasitized (Eliopoulos et al. 2002).

Gregarious parasitoid species laid several eggs in, or on a single host. As the number of offspring per host increases, host resources per individual decline, resulting in the development of smaller adults. Therefore, fitness is affected not only by host size but by the number of parasitoids developing in the host as well (Charnov and Skinner 1984, Waage 1986, Godfray 1994).

Host size, age and species are critical biotic factors that affect offspring fitness of parasitoids. The most common component of host quality that has been studied is host size. For insect parasitoids an individual host is the entire larval food resource and has a major influence on parasitoid fitness and determines the maximum amount of available food resources (Temerak 1984). Host quality influences main components of parasitoid fitness such as survival from egg to adult, parasitoid development time, parasitoid size and fecundity of adults. In general, large hosts contain more resources and they are considered to be qualitatively superior, in terms of parasitoid fitness (Godfray 1994).

For insects like *B. brevicornis* whose female firstly paralyzes and then lays her eggs on the host, the size of the host is critical for the parasitoid's fitness, as it determines the quality and the quantity of provided food (Taylor 1988b, Godfray 1994, Milonas 2005).

In the study reported herein an aspect that influences the biology of *B. brevicornis* such as host quality was investigated. Specifically the influence of different host larval instars on survival and duration of development and adult life span of *B. brevicornis* was examined.

Materials and Methods

Insect cultures

The strain of *B. brevicornis* used in this study originated from wild adults emerged from parasitized larvae of *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) from Nea Moudania Chalkidikis in 2010.

A laboratory culture was maintained on 5th larval instar of *P. interpunctella* for about 31 generations. Adult parasitoids were maintained in pairs in 5 cm diameter Petri dishes and fed with drops of a 50 % honey solution, placed on the cap. A pair of parasitoids was inserted into a Petri dish containing a 5th instar larva of *P. interpunctella*. After 24 h adult parasitoids were returned carefully in a new Petri dish, with fresh larva and Petri dishes with the parasitized larva were isolated until adult emergence.

Plodia interpunctella adults were kept in 330 ml plastic jars with a sieve replacing the cap of the plastic jars. Within each jar, the moths were provided with a cotton wool with a 10 % sugar solution. Larvae maintained in plastic jars with cap, capacity of 100 ml, and fed with artificial diet (Damos et al. 2009) (Table 1).

Plodia interpunctella and parasitoids were maintained under the same rearing conditions at 25±1°C, 50–60% RH and 16L:8D photoperiod.

TABLE 1. Ingredients of artificial diet (by Ashby et al. 1985).

Ingredients	Quantity (gr)
Dried beans (<i>Phaseolus vulgaris</i>)	450
Beer yeast	64,09
Pastry agar	31,00
Ascorbic acid	6,49
Nipagin	4,09
Sorbic acid	2,49
Formaldehyde 37%	4 ml
Distilled water	1280 ml

Bioassays

Before any experiment, one virgin *B. brevicornis* female was placed in a Petri dish, with a few drops of honey solution, a male was added after 3 hours, and the pair left for 24 hours, in order to be mated. After 24 hours in each Petri dish the suitable larval instar of *P. interpunctella* (L₂, L₃, L₄, or L₅), was inserted (Temerak 1983). Host larvae were exposed to adult parasitoids for 24 hours. The parasitoids were removed to a new Petri dish (of 5 cm diameter) with a fresh host of the studied instar every 24 h, for 10 days. Each Petri dish with parasitized *P. interpunctella* larva was isolated until adult emergence.

Percentage of parasitism, number of eggs laid on different larval instars of *P. interpunctella*, number of larvae, prepupae, pupae and the number of *B. brevicornis* adults were recorded. The developmental time of parasitoid egg, larva, prepupa, pupa and the total developmental time from egg to adult on different larval instars of the host were also recorded.

Statistical analysis

The influence of host size (different host larval stage) was determined by one-way ANOVA. Fisher's Least Significant Difference (LSD) values were estimated to

test the differences among treatments, using the IBM SPSS Statistics 21 program.

Results and Discussion

Parasitic ability. In L₂ *P. interpunctella* only 4 eggs of *B. brevicornis* were observed. In L₃ 13 eggs laid but they were not developed successfully beyond the first larval instar. Concerning the parasitic ability of *B. brevicornis* on L₄ and L₅ of its host, statistically significant differences were observed between the two host instars (Table 2).

Our results support the expectation that the larger hosts, being more suitable for larval development, would be preferred by parasitoids (Le Masurier 1987, Taylor 1988b) showed that the host's size and species affect growth and development of larvae of the gregarious parasitoid *Bracon hebetor* (Say) (Hymenoptera: Braconidae). Specifically, survival was lower and competition more intense on smaller hosts.

Many parasitoids do adjust clutch sizes in response to host size (Salt 1961, Waage 1986), and some prefer 'better' hosts (Cornell and Pimentel 1978).

Developmental time

Our results showed that larval stage of *P. interpunctella* had not any significant effect on parasitoid developmental time within each developmental stage (egg, larva, prepupa and pupa) and total development period of the parasitoid (Table 3). Similar results were reported for *B. hebetor* (Taylor 1988a, Milonas 2005).

Adult life span

The female and male *B. brevicornis* adult life span of F1 generation was 16.70 and 11 days when developed on L₄ of *P. interpunctella* respectively and 28.70 and 17.60 days when developed on L₅ of *P. interpunctella* (Bakr et al. 2014), in their work concerning the biological aspect of *B. brevicornis* when reared on different hosts

(*Ephestia kuehniella*, *Galleria mellonella*, *Corcera cephalonica*, *Sesamia cretica*, *Spodoptera littoralis* and *Pectinophora gossypiella*), found that the type of host had great impact on the durations of the immature stages and longevity of the

parasitoid. The mean duration of male longevity of parasitoids was longer when reared on *E. kuehniella* and shorter when reared on *C. cephalonica*. The mean duration of female parasitoids was highest when parasitoids reared on *S. cretica*.

TABLE 2. Mean number of progeny/female/day of *B. brevicornis* developed on L₄ and L₅ instars of *P. interpunctella*.

Stage of <i>B. brevicoryne</i>	Developed on L ₄	Developed on L ₅
Eggs	2.42±0.28a*	13.65±0.28b
Larvae	1.42±0.22a	9.73±0.20b
Prepupae	1.37±0.50a	7.90±0.20b
Pupae	1.26±0.44a	7.44±0.22b
Adults	1.24±0.30a	7.16±0.24b

*Numbers in a row followed by a different letter are significantly different.

TABLE 3. Developmental time of *B. brevicornis* stages developed from eggs laid on L₄ or L₅ larvae of *P. interpunctella*.

Stage of <i>B. brevicoryne</i>	Developed on L ₄	Developed on L ₅
Eggs	1.12±0.03a**	1.18±0.02a
Larvae	2.03±0.06a	1.68±0.03a
Prepupae	1.47±0.08a	1.29±0.04a
Pupae	6.82±0.09a	7.76±0.04a
Developmental time from egg to adult	11.45a	11.92a

*Numbers in a row followed by a different letter are significantly different.

Our results show that the host larval instar strongly affects certain aspects of the biology of *B. brevicornis*, such as the development and survival of progeny. Specifically, survival was lower on smaller hosts (L4 larvae). Similar results of host size influence on *B. hebetor* had been referenced by Taylor (1988b) and Godfray (1994). It is likely that host size eventually affects parasitoid fitness, since adult size especially of females, influences their fecundity and longevity (Antolin et al. 1995). In conclusion, host larval instar L₅

seems to be more suitable for the parasitoid development, on a mass rearing program.

Acknowledgements

We wish to thank lecturer G. Menekses (Aristotle University of Thessaloniki) for statistical analysis of data.

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Ανάπτυξη του παρασιτοειδούς *Bracon brevicornis* σε διαφορετικές προνυμφικές ηλικίες (L₂-L₅) του εντόμου *Plodia interpunctella*

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ΠΕΡΙΛΗΨΗ

Το *Bracon brevicornis* (Wesmael) (Hymenoptera: Braconidae) είναι ένα αγελαίο παρασιτοειδές που προσβάλλει μια ποικιλία σημαντικών λεπιδόπτερων εντόμων των αποθηκευμένων προϊόντων. Ο σκοπός αυτής της μελέτης ήταν να καθοριστεί εάν τα διαφορετικά προνυμφικά στάδια του *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) επηρεάζουν τη προνυμφική ανάπτυξη, τη μακροζωία και τη γονιμότητα του παρασιτοειδούς *B. brevicornis*. Οι παράμετροι που μετρήθηκαν είναι η ικανότητα παρασιτισμού, η ωοτοκία, η προνυμφική ανάπτυξη, ο μέσος αριθμός των απογόνων καθώς και η διάρκεια ανάπτυξης του παρασιτοειδούς *B. brevicornis*, σε διαφορετικά προνυμφικά στάδια/ηλικίες του ξενιστή του. Τα αποτελέσματα μας έδειξαν ότι παρατηρήθηκαν στατιστικά σημαντικές διαφορές μεταξύ των προνυμφικών σταδίων του *P. interpunctella* σε ότι αφορά, την ικανότητα παρασιτισμού την ωοτοκία, την προνυμφική ανάπτυξη και το μέσο αριθμό των απογόνων του. Συγκεκριμένα στο στάδιο L₂ του *P. interpunctella* δεν ήταν δυνατή η ωοτοκία και η ανάπτυξη του παρασιτοειδούς. Στο στάδιο L₃, ενώ παρατηρήθηκε μια μικρή ωοτοκία δεν παρατηρήθηκε περαιτέρω ανάπτυξη του *B. brevicornis*. Σε ότι αφορά την ανάπτυξη του παρασιτοειδούς παρατηρήθηκαν στατιστικά σημαντικές διαφορές μεταξύ του L₄ και L₅ του *P. interpunctella*. Σχετικά με τη διάρκεια ανάπτυξης του παρασιτοειδούς, και στα δύο προνυμφικά στάδια δεν υπήρχαν στατιστικά σημαντικές διαφορές. Ο συνολικός χρόνος από αυγό έως ενήλικο ήταν 11,45 ημέρες, στο στάδιο L₄ και 11,92 ημέρες, στο στάδιο L₅. Όσον αφορά τη μέση διάρκεια ζωής των ενηλίκων F1 ήταν 16,70 και 28,70 για τα θηλυκά και 11,00 και 17,60 για τα αρσενικά των σταδίων L₄ και L₅ αντίστοιχα. Συμπερασματικά το στάδιο L₅, φαίνεται να είναι το πλέον κατάλληλο στάδιο για την μαζική εκτροφή του *B. brevicornis*, το οποίο μπορεί να χρησιμοποιηθεί στο πλαίσιο της βιολογικής αντιμετώπισης εχθρών αποθηκευμένων προϊόντων, όπως είναι το *P. interpunctella*.