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Orijinal araştırma (Original article)

Effect of host density on some biological characteristics and glycogen, total sugar and lipid levels of parasitoid *Bracon hebetor* Say, 1836 (Hymenoptera: Braconidae)[1]

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Summary

Laboratory studies were conducted to determine the effects of host density on development time, egg dispersion, fecundity, sex ratio, longevity, and glycogen, total sugar and lipid levels of *Bracon hebetor* Say, 1836 (Hymenoptera: Braconidae), parasitizing *Ephestia kuehniella* Zeller 1879 (Lepidoptera: Pyralidae) at 26 ± 2°C, 60 ± 5 % RH and continuously illuminated conditions. One pair of the parasitoids (age<24 h) were exposed to one, two, four, and eight hosts over a 24-h period during ten days. Host density had no significant effect on development time of *B. hebetor*. For both sexes, it lasted almost 14-15 days at all host densities. Longevity of females did not vary significantly with host density, whereas males had some variations. Females appeared to allocate eggs in relation to host density. However, total number of eggs laid per female was similar at all host densities. When female had only one host larva, progeny emergence was lowest compared with other host densities but percentage of male progeny did not change with host density. We also compared the glycogen, sugar and lipid reserves of recently emerged unfed female and male *B. hebetor* maintained different host densities. Host density had no significant effect on glycogen levels of female and male wasp, whereas sugar and lipid levels showed some variations in both sexes.

Key words: *Bracon hebetor*, host density, biological characteristics, glycogen, sugar, lipid

Anahtar sözcükler: *Bracon hebetor*, konukçu yoğunluğu, biyolojik özellikler, glikojen, şeker, lipit

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Introduction

Bracon hebetor Say 1836 (Hymenoptera: Braconidae) is an idiobiont, gregarious ectoparasitoid that attacks a variety of important Lepidopterous pests of stored product. The biology of *B. hebetor* has been intensively studied because of its importance as a biological control agent of the moths and also it is easy to rear in the laboratory (Benson, 1973; Taylor, 1988; Brower & Press, 1990; Heimpel et al., 1997; Baker & Fabric, 2000; Darwish et al., 2003; Gündüz & Gülel, 2005; Milonas, 2005; Magro et al., 2006; Gündüz et al., 2008).

For insect parasitoids an individual host is the entire larval food source. So, both the number of other larvae with which a host shared, and host size, stage or species, can effect the development time, size, survival and fecundity of parasitoids (Corrigan et al., 1995; Gül & Gülel, 1995; Ueno,1999a,b; Harbison et al., 2001; Röhne, 2002; Gündüz & Gülel, 2004, 2005; Milonas, 2005).

Many adult parasitoid need considerable amounts of protein, lipid and carbohydrate for survival and reproduction (Morales-Ramos et al., 1996; England & Evans, 1997; Heimpel et al., 1997; Olson & Andow, 1998; Rivero & Casas, 1999; Olson et al., 2000; Fadamiro & Heimpel, 2001; Lee et al., 2004). Such resources may be acquired by feeding upon hosts or non-host foods (e.g., sugar sources).

B. hebetor is a synovigenic parasitoid producing yolk rich (anhydropic) eggs. Female *B. hebetor* can produce eggs without host-feeding, suggesting that it stores nutrients during larval feeding and when food is not available, energy resources bound up in eggs, fat body or muscle tissue can be converted into usable energy.

In recent years many researches have studied the effects of host quality and/or quantity on biology and ecology of *B. hebetor* (Ullyett, 1945; Doutt, 1959; Taylor, 1988; Yu et al., 2003). Eliopoulos & Stathas (2008), for instance, investigated the life table parameters of *B. hebetor* parasitising *Anagasta kuehniella* Zeller 1879 (Lepidoptera: Pyralidae) and *Plodia interpunctella* (Hubner 1813) (Lepidoptera: Pyralidae). Their work was based on the comparision of parasitoid's life table parameters related to those of its hosts at various conditions of host density. Yu et al. (2003) reported the effect of host density on egg dispersion and the sex ratio of *B. hebetor*. In a previous study we reported the effect of two host species on protein, lipid and glycogen levels of female and male *B. hebetor* (Gündüz et al., 2008). However, all the forementioned studies have not deal with the relationship between the host density, life history parameters and the biochemical composition of adult parasitoids.

In the study reported herein, the effect of host densities on some biological characteristics of parasitoid *B. hebetor* and the interaction between host density and glycogen, total sugar and lipid levels of adult parasitoids were investigated.

Materials and Methods

Insect cultures. We used *Bracon hebetor* Say 1836 (Hymenoptera: Braconidae) and late instar larvae of Mediterranean flour moth, *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae), as parasitoid and host species, respectively, in the experiments. The methods to establish and maintain stock cultures of both host and parasitoid species are described by Gündüz & Gülel (2004). All culturing and experimental procedures were conducted at 26 ± 2^{o}C, 60 ± 5 % RH and continuously illuminated laboratory conditions.

Experimental procedure. To determine the effect of host density on development time, fecundity and sex ratio of parasitoid 1, 2, 4 or 8 late instar *E. kuehniella* were introduced into a Petri dish (diameter 9 cm) containing a piece of cotton ball soaked with 50 % honey solution. One pair of the parasitoids (age<24 h) was introduced and allowed to attack the hosts for 24 h. At 24- h intervals over the following 10- day period, the wasps were transferred to a new petri dish prepared with the same treatment. The dishes were then checked every day, the number of parasitoids emerging and the progeny sex ratio per petri dish were recorded. Each experiment was replicated ten times.

To avoid death of eggs due to the disturbing, we prepared another group of petri dishes as described previously to determine the number of eggs laid by female in each host densities. One pair of newly emerged parasitoids (age<24 h) was placed in each petri dish. They were allowed 24 h for parasitization, after which they were transferred to a new petri dish. This procedure was carried on ten days. Dishes were checked daily and all newly laid parasitoid eggs were counted and recorded.

For the longevity experiments, newly emerged female and male parasitoids were paired and placed in glass vials (10×100 mm) without diet. Parasitoids were observed daily, and longevity of each individual was recorded.

We used the common biochemical analyses protocols (Olson et al., 2000; Fadamiro & Heimpel, 2001; Lee et al., 2004) that allowed direct comparisons of glycogen, total sugar and lipid levels of newly emerged parasitoids maintained on different host densities. Adults were sexed at emergence and then they were placed singly into 1.5 ml microcentrifuge tubes and deep-frozen at – 20 for the biochemical analysis.

Wasps were removed from the freezer and then crushed individually in a mixture of 50 µl of 2 % sodium sulfate and 450 µl chloroform-methanol (1:2) in

1.5 ml microcentrifuge tubes. The mixture was centrifuged at 14 000 g for 2 min and 200 µl of the resulting supernatant was transferred to a glass test tube (10×50 mm) for the sugar assay and 200 µl was transferred to a similar glass tube for the lipid assay. The precipitate was left in the microcentrifuge tube for the glycogen assay. All tubes were heated at 90 ºC until all solution was evaporated from the lipid and glycogen tubes and approximately 50 µl of solution remained in the sugar tubes. For the lipid assay, 40 µl of sulfuric acid was added and heated at 90 ºC for 2 min. Then 960 vanillin-phosphoric acid reagent was added (van Handel, 1985), mixed and kept at room temperature for 30 min. Absorbance at 525 nm was determined with a spectrophotometer and compared with lipid standards using corn oil. For sugar assay, hot anthrone test was used. We added 950 µl anthrone reagent to the sugar tube, heated at 90 ºC for 10 min and cooled on ice. Absorbance at 625 nm was read and compared with glucose standards. With the glycogen precipitate, 1000 µl of anthrone reagent was added, mixed and heated at 90 ºC for 15 min. Absorbance at 625 nm was determined and compared with glucose standards.

Data analysis

Statistical analyses were executed using SPSS statistical software (Landau & Everitt, 2004). Differences in development time, longevity, fecundity, sex ratio, the mean number of eggs laid per female and nutrient levels of parasitoid were compared using one-way analysis of variance (ANOVA). Where the differences were significant, means were separated using Student-Newman-Keuls (SNK) multiple range test at a probability level of $P \leq 0.05$.

Results and Discussion

The effect of host density on mean development time (egg-to-adult) and longevity of *Bracon hebetor* Say 1836 (Hymenoptera: Braconidae) is illustrated in Table 1. Our results showed that development time of female and male parasitoids did not vary significantly with host densities (Table 1) ($F_{female}=0.362$ df=3,289 p= 0.781, $F_{male}=0.370$ df=3,341 p= 0.775) and wasp development lasted almost 14-15 days at all host densities. This is in agreement with the results of Taylor (1988) who found that host species had a small but significant effect on mean development time of *B. hebetor* but host density had no significant effect on it.

The results in Table 1 also indicate that the longevity of females did not show any noteworthy change with host density ($F_{female}=1.875$ df=3,172 p= 0.136), whereas it showed some variations in males ($F_{male}=3.738$ df=3,236 p= 0.012).

Table 1 Effect of host density on mean development time and longevity of *Bracon hebetor* Say

Host density	Development time (day) Mean±SE		Longevity (day) Mean±SE	
	Female	Male	Female	Male
1	15.06±0.19a (51)	14.40±0.16a (72)	12.95±0.53a (39)	8.33±0.27a (60)
2	14.84±0.15a (77)	14.32±0.15a (87)	12.50±0.58a (32)	7.93±0.24ab (60)
4	14.97±0.17a (87)	14.49±0.15a (92)	13.41±0.36a (61)	8.25±0.23a (60)
8	14.83±0.17a (78)	14.51±0.14a (94)	12.00±0.48a (44)	7.30±0.23b (60)

Means in the same column followed by different letters are significantly different ($P \leq 0.05$) (Student Newman Keuls Test) (numbers in parentheses are sample size).

We showed that *B. hebetor* females adjust clutch size based on host density (Fig 1). Previous studies reported that when a female *B. hebetor* wasp supplied with only one host larva every day, it never lays more than 7 or 12 eggs per host (Milonas & Savopoulou- Soultani, 1999; Yu et al., 2003). They suggested that parasitoid has a tendency to avoid laying more eggs than what the host can support. Similarly, Ulyett (1945) reported that *B. hebetor* regulates allocation of eggs among hosts by decreasing the number of eggs laid on a host as host density increases. Our results are in close agreement with these findings.

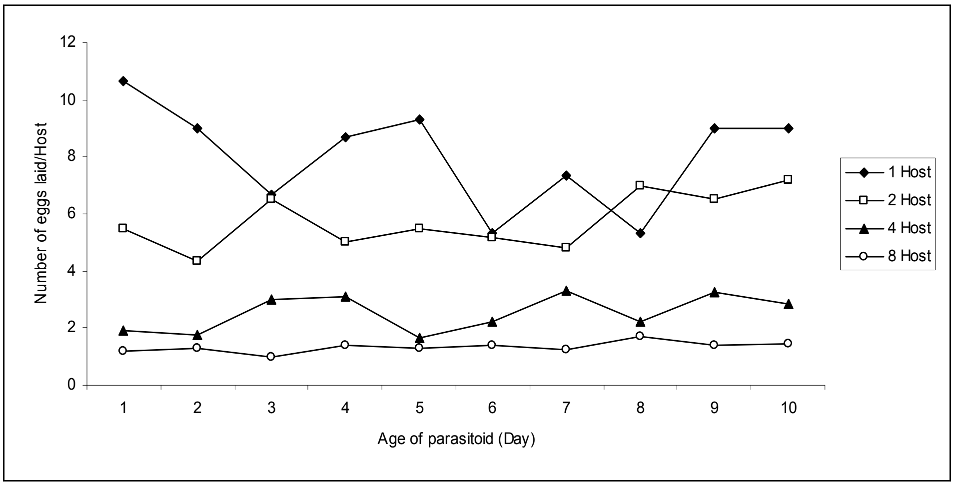


Figure 1. Relationship between the host density and the mean number of *Bracon hebetor* Say eggs laid on each host.

When we compared to total number of eggs laid by female, it can be seen that female supplied with only one host per day laid low number of eggs

(Table 2). However, when female had access to more larvae of *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae), the total number of eggs laid by female increased, but not significantly (F=1.931, df=3,8 p=0.203). Similarly, Taylor (1988) found that the total number of eggs laid by *B. hebetor* was independent of the host density. However, our findings are not in aggrement with the results of Yu et al. (2003). This was probably because of differences in strains, host species or experimental procedure between the two studies. We found that female parasitoids paralyse most available hosts, but they leave some hosts without eggs. A similar situation has also been reported by Yu et al. (2003). Thereby, this may explain why we could not determine any significant difference between different host densities.

Table 2. Effect of host density on total number of eggs laid per female, progeny production and sex ratio of *Bracon hebetor* Say

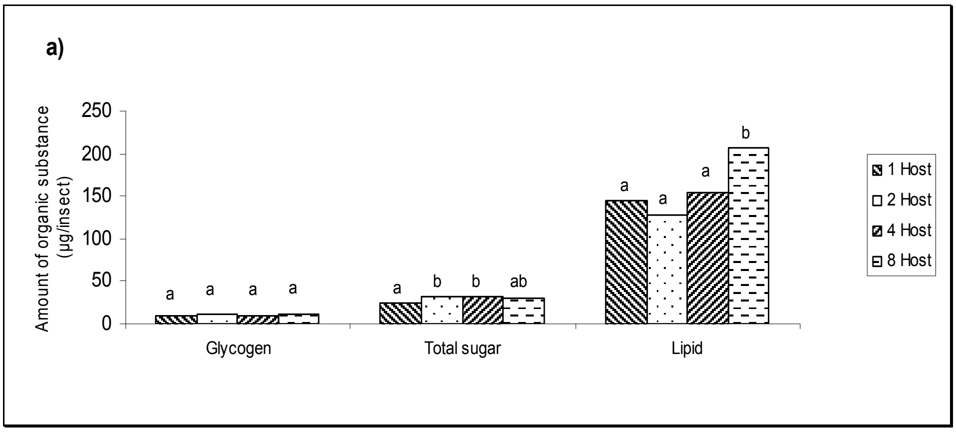
Host density	Total number of eggs laid	Progeny Production					Male sex ratio (%)
		Female		Male		Total	
		Range	Mean±SE	Range	Mean±SE	Mean±SE	
1	80.33±5.46a	3-24	13.50±2.22a	16-66	29.20±4.43a	42.70±5.75a	68.84±3.51a
2	115.00±14.29a	13-38	22.50±2.57ab	27-71	53.40±5.08b	75.90±6.64b	70.08±2.54a
4	101.33±11.44a	13-51	30.60±4.40b	22-77	47.50±5.74b	78.10±8.13b	60.80±4.34a
8	106.67±9.39a	2-50	32.10±5.27b	29-85	48.70±5.93b	80.80±3.89b	59.85±6.48a

Means in the same column followed by different letters are significantly different (P ≤ 0.05) (Student-Newman Keuls Test).

Parasitoid progeny emergence was low at the densities of one host, and plateauted at a density of two hosts (Table 2) (F=8.093, df=3,36 p=0.000). Extreme immature mortality could be possible reason for this phenomenon. Our results showed that the mean number of eggs oviposited per female with one host was more than eight. Benson (1973) stated that the larval mortality of *B. hebetor* increase when the number of eggs laid on a single host is more than eight.

Sex ratio was not significantly different among host densities (Table 2) (F=1.416, df=3,36 p=0.254). Working with *B. hebetor*, Yu et al. (2003) reported that overall progeny sex ratio (male/total) was not effected by host density. Sagara et al. (2000) obtained similar results with the Hymenopterous parasitoid, *Anagyrus kamali* Moursi (Hymenoptera: Encyrtidae) parasitizing *Maconellicoccus hirsutus* Green (Homoptera: Pseudococcidae). However, Rotary & Gerling (1973) showed that the progeny sex ratio (male/total) increased as the host/parasitoid ratio decreased in *B. hebetor.* Differences in the strains of *B. hebetor* or experimental condition could be involved in this contradictory result.

The effect of host density on glycogen, sugar and lipid levels of newly emerged female and male *B. hebetor* is shown in Figure 2a and 2b.



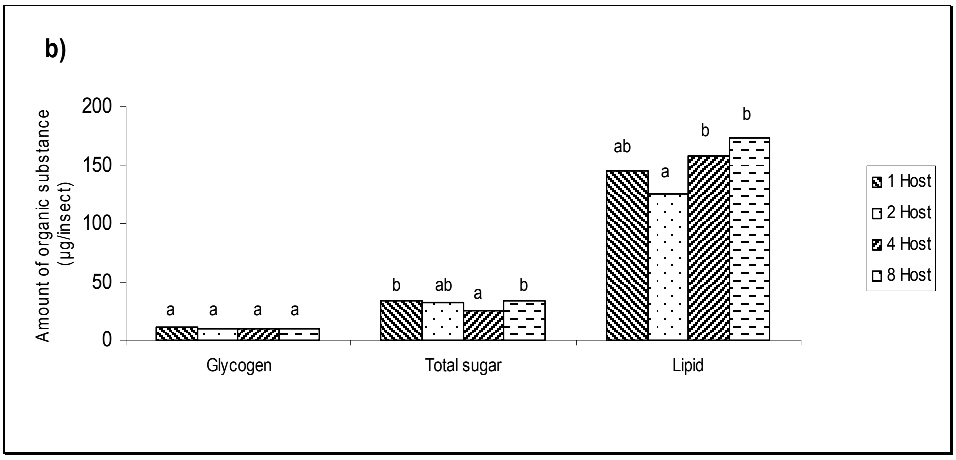


Figure 2. Glycogen, total sugar and lipid composition of female (a) and male (b) *B. hebetor* Say at various host densities.

Both female and male *B. hebetor* emerged with very small amount of glycogen reserves (Fig 2a and 2b) and host density had no significant effect on it (F_{female}=1.958, df=3,56 p=0.131, F_{male}=2.886 df=3,56 p=0.044). Working with *B. hebetor*, Gündüz et al. (2008) reported that host species had a significant effect on glycogen level of the females, but not in males.

It has been shown that glycogen can be stored only when sugar concentration in hemolymph reaches a certain threshold, whereas when sugar concentration of hemolymph has declined below a certain value, glycogen reserves are mobilized (Ziegler & Schultz, 1986; Rivero & Casas, 1999). Thus, this may explain why low levels of glycogen is detected in newly emerged female and male *B. hebetor.*

Numerous studies show that, in the field, synovigenic species can use both host hemolymph and nonhost foods such as nectar, pollen or honeydew to supply their maintenance and reproductive needs (Rivero & Casas, 1999; Rivero & West, 2002; Lee et al., 2004).

Sugar is the main energy source for adult parasitoids (Jervis & Kidd, 1986; Heimpel et al., 1997; Lee et al., 2006; Wäckers et al., 2006). The results obtained in the present work showed that total sugar level of female and male parasitoids shows some variations with host density, but no consistent trend was found resulting in either increasing or decreasing between these values (Fig 2a and 2b) (F_{female}=3.368, df=3,56 p=0.025, F_{male}=2.923 df=3,56 p=0.042).

Jervis & Kidd (1986) reported that sugar-rich foods appear to be especially important in maintaining females during the early phase of adult life and some species forage actively for sugar sources in the field. Newly emerged unfed parasitoids were used in this study. Thereby, this statement may explain why we did determine low level of total sugar in adults. Steppuhn & Wäckers (2004) obtained similar results with two braconoid parasitoids *Cotesia glomerata* (Linnaeus, 1758) (Hymenoptera: Braconidae)and *Microplitis mediator* (Haliday, 1834) (Hymenoptera: Braconidae).

Females and males emerged with a high amount of lipid deposits at all host densities (Fig. 2a and 2b). Nonsignificant change of lipid level were recorded for females when host density increase from 1 to 4. However, when female had access to eight host, lipid level was highest (Fig. 2a, F=10.486, df=3,56 p=0.000). For males, lipid levels showed slight fluctations depending upon host density (Fig. 2b, F=5.030, df=3,56 p=0.004).

Gündüz & Gülel (2010) suggested that lipids are stored only during pre-adult stages and *B. hebetor* adults have no lipogenic capabilities. Similar results have also been reported for different parasitoid species (Jacome et al., 1995; Olson et al., 2000; Rivero & West, 2002; Lee et al., 2004). The biological meaning of this result is that newly emerged individuals have to store large amounts of lipid throughout larval development. Changes in lipid stores occur because of flight, mating activities, egg production and oviposition during adult life.

Overall, the above findings collectively suggest that all host densities, used in this study are all suitable for the development and survival of *B. hebetor*. When wasps had access to more than one host, however, produce more progeny to the next generation. This is an important consideration in the utilization of biological control agents. In general, we also did determine nonsignificant differences between the glycogen, total sugar and lipid levels of parasitoids at different host densities. This situation suggest that newly emerged *B. hebetor* adults have to loaded with a certain amounts of these nutrients to sustain egg production and metabolic needs.

Özet

Konukçu yoğunluğunun parazitoit *Bracon hebetor* Say, 1836 (Hymenoptera: Braconidae)'un bazı biyolojik özellikleri ile glikojen, toplam şeker ve lipit miktarına etkisi

Konukçu yoğunluğunun *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae)'yı parazitleyen *Bracon hebetor* Say, 1836 (Hymenoptera: Braconidae)' un gelişim süresi, yumurta dağılımı, verim, eşey oranı, ömür uzunluğu ve glikojen, toplam şeker ve lipit seviyeleri üzerine etkisini belirlemek amacıyla, 26 ± 2^{o}C, 60 ± 5 % RH ve sürekli aydınlık koşullarda laborotavuar çalışmaları yapılmıştır. Bir çift parazitoite (Yaş<24 saat) on gün boyunca 24 saatlik peryotlarla bir, iki, dört veya sekiz konukçu verilmiştir. Konukçu yoğunluğu *B. hebetor*'un gelişim süresini önemli ölçüde etkilememiştir. Her iki eşey için, tüm konukçu yoğunluklarında gelişim süresi yaklaşık 14-15 gün sürmüştür. Dişilerin ömür uzunluğu konukçu yoğunluğuyla önemli derecede değişmezken, erkeklerde bazı varyasyonlar göstermiştir. Dişiler konukçu yoğunluğuna bağlı olarak yumurtalarını paylaştırırlar. Bununla birlikte, dişi başına bırakılan toplam yumurta sayısı tüm konukçu yoğunluklarında benzerdir. Dişiye tek larva verildiğinde, oğul döl çıkışı diğer konukçu yoğunluklarından daha düşük olmuş ancak erkek döl yüzdesi konukçu yoğunluğuyla değişmemiştir. Ayrıca, farklı konukçu yoğunluklarında yetiştirilen yeni ergin beslenmemiş dişi ve erkek *B. hebetor*'un glikojen, şeker ve lipit miktarları da karşılaştırılmıştır. Konukçu yoğunluğu dişi ve erkek parazitoitlerin glikojen seviyesinde önemli bir etkiye sahip değilken, şeker ve lipit seviyeleri her iki eşeyde bazı varyasyonlar göstermiştir.

References

Baker, J. E. & J. A. Fabrick, 2000. Host hemolymph proteins and protein digestion in larval *Habrobracon hebetor* (Hymenoptera: Braconidae). **Insect Biochemistry and Molecular Biology, 30** (10): 937-946.

Benson, J. F., 1973. Intraspecific competition in the population dynamics of *Bracon hebetor* Say (Hymenoptera:Braconidae). **The Journal of Animal Ecology, 42** (1): 105-124.

Brower, J. H. & J. W. Press, 1990. Interaction of *Bracon hebetor* (Hymenoptera: Braconidae) and *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) in suppressing stored -product moth populations in small inshell peanut storages. **Journal of Economic Entomology, 83** (3): 1096-1101.

Corrigan, J. E., J. E. Laing & J. S. Zubricky, 1995. Effects of parasitoid to host ratio and time of day of parasitism on development and emergence of *Trichogramma minitum* (Hymenoptera: Trichogrammatidae) parasitizing eggs of *Ephestia kuehniella* (Lepidoptera: Pyralidae). **Annals of Entomological Society of America, 88** (6): 773-780.

Darwish, E., M. El-Shazly & H. El- Sherif, 2003. The choice of probing sites by *Bracon hebetor* Say (Hymenoptera:Braconidae) foraging for *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). **Journal of Stored Products Research, 39** (3): 265-276.

Doutt, R. L., 1959. Distribution of eggs by Microbracon (Hymenoptera: Braconidae). **Ecology, 40** (2): 302-303.

Elipoulus, P. A. & G. J. Stathas, 2008. Life tables of *Habrobracon hebetor* (Hymenoptera: Braconidae) parasitizing *Anagasta keuhniella* and *Plodia interpunctella* (Lepidoptera: Pyralidae): Effect of host density. **Journal of Economic Entomology, 101** (3): 982-988.

England, S. & E. W. Evans, 1997. Effects of pea aphid (Homoptera: Aphididae) honeydew on longevity and fecundity of the alfalfa weevil (Coleoptera: Curculionidae) parasitoid *Bathyplectes curculionis* (Hymenoptera: Ichneumonidae). **Environmental Entomology, 26** (6): 1437-1441.

Fadamiro, H. Y. & G. E. Heimpel, 2001. Effects of partial sugar deprivation on lifespan and carbohydrate mobilization in the parasitoid *Macrocentrus grandii* (Hymenoptera: Braconidae). **Annals of Entomological Society of America, 94** (6): 909-916.

Gül, M. & A. Gülel, 1995. Parasitoid *Bracon hebetor* (Say) (Hymenoptera: Braconidae)'un biyolojisi ve konak larva büyüklüğünün verim ve eşey oranı üzerine etkisi. **Turkish Journal of Zoology, 19**: 231-235.

Gündüz, N. E. A. & A. Gülel, 2004. *Bracon hebetor* (Say) (Hymenoptera: Braconidae) erginlerinde konukçu türünün ve besin tipinin ömür uzunluğuna etkisi. **Türkiye Entomoloji Dergisi, 28** (4): 275-282.

Gündüz, N. E. A. & A. Gülel, 2005. Ergin yaşı ve konukçu türünün parazitoit *Bracon hebetor* (Say) (Hymenoptera: Braconidae)'un gelişme süresine etkisi. **Ondokuz Mayıs Üniversitesi Ziraat Fakültesi Dergisi, 20** (2): 31-36.

Gündüz, E. A., A. Gülel & Ö. V. Işıtan, 2008. İki konukçu türün, larva ektoparazitoiti *Bracon hebetor* (Say, 1836) (Hymenoptera: Braconidae)'da protein, lipit ve glikojen miktarlarına etkisi. **Türkiye Entomoloji Dergisi, 32** (1):33-42.

Gündüz, E. A. & A. Gülel, 2010. Protein, lipid and glycogen levels in the parasitoid Bracon hebetor Say (Hymenoptera: Braconidae). **Turkish Journal of Zoology, 34**: 243-248.

Harbison, J. L., J. C. Legaspi, S. L. Fabritius, R. R. Saldana, B. C. Jr. Legaspi & A. Enkegaard, 2001. Effects of age and host number on reproductive biology of *Allorhogas pyralophagus* (Hymenoptera: Braconidae) attacking the Mexican rice borer (Lepidoptera: Pyralidae). **Environmental Entomology, 30** (1):129-135.

Heimpel, G. E., M. F. Antolin, R. A. Franqui & M. R. Strand, 1997. Reproductive isolation and genetic variation between two "strains" of *Bracon hebetor* (Hymenoptera: Braconidae). **Biological Control, 9**: 149-156.

Jacome, I., M. Aluja, P. Liedo & D. Nestel, 1995. The influence of adult diet and age on lipid reserves in the trotopical fruit fly *Anastrepha serpentina* (Diptera: Tephritidae). **Journal of Insect Physiology, 41** (12): 1079-1086.

Jervis, M. A. & N. A. C. Kidd, 1986. Host-feeding strategies in Hymenopteran parasitoids. **Biological Reviews, 61**: 395-434.

Landau, S. & B. S. Everitt, 2004. A Handbook of Statistical Analyses Using SPSS. Chapman & Hall/CRC Press, Boca Raton, London, New York, Washington, D.C. 354 pp.

Lee, J. C., G. E. Heimpel & G. L. Leibee, 2004. Comparing floral nectar and aphid honeydew diets on the longevity and nutrient levels of a parasitoid wasp. **Entomologia Experimentalis et Applicata, 111** (3): 189-199.

Lee, J. C., D. A. Andow & G. E. Heimpel, 2006. Influence of floral resources on sugar feeding and nutrient dynamics of a parasitoid in the field. **Ecological Entomology, 31** (5): 470-480.

Magro, S. R., A. B. Dias, W. R. Terra & J. R. P. Parra, 2006. Biological, nutritional, and histochemical basis for improving an artificial diet for *Bracon hebetor* (Say) (Hymenoptera: Braconidae). **Neotropical Entomology, 35** (2): 215-222.

Milonas, P. G. & M. Savopoulou-Soultani, 1999. Temperature-dependent functional response of *Bracon hebetor* parasitising larvae of *Adoxophyes orana* in the laboratory. **(IOBC/WPRS), (OILB/SROP) Bulletin, 22** (11): 93-100.

Milonas, P. G., 2005. Influence of initial egg density and host size on the development of the gregarious parasitoid *Bracon hebetor* on three different host species. **Biological Control, 50** (3): 415-428.

Morales-Ramos, J. A., M. G. Rojas & E. G. King, 1996. Significance of adult nutrition and oviposition experience on longevity and attainment of full fecundity of *Catolaccus grandis* (Hymenoptera: Pteromalidae). **Annals of Entomological Society of America, 89** (4): 555-563.

Olson, D. M. & D. A. Andow, 1998. Larval crowding and adult nutrition effects on longevity and fecundity of female *Trichogramma nubilale* Ertle and Davis (Hymenoptera: Trichogrammatidae). **Environmental Entomology, 27** (2): 508-514.

Olson, D. M., H. Fadamiro, J. G. Lundgren & G. E. Heimpel, 2000. Effects of sugar feeding on carbohydrate and lipid metabolism in a parasitoid wasp. **Physiological Entomology, 25** (1): 17-26.

Rivero, A. & J. Casas, 1999. Incorporating physiology into parasitoid behavioral ecology: the allocation of nutritional resources. **Researches on Population Ecology, 41** (1): 39-45.

Rivero, A. & S. A. West, 2002. The physiological costs of being small in a parasitic wasp. **Evolutionary Ecology Research, 4** (3): 407-420.

Rotary, N. & D. Gerling, 1973. The influence of some external factors upon the sex ratio of *Bracon hebetor* Say (Hymenoptera: Braconidae). **Environmental Entomology, 2** (1): 134-138.

Röhne, O., 2002. Effect of temperature and host stage on performance of *Aphelinus varipes* Förster (Hym., Aphelinidae) parasitizing the cotton aphid, *Aphis gossypii* Glover (Hom., Aphididae). **Journal of Applied Entomology, 126** (10): 572-576.

Sagarra, L. A., C. Vincent, N. F. Peters & R. K. Stewart, 2000. Effect of host density, temperature, and photoperiod on the fitness of *Anagyrus kamali*, a parasitoid of the hibiscus mealybug *Maconellicoccus hirsutus*. **Entomologia Experimentalis et Applicata, 96** (2): 141-147.

Steppuhn, A. & F. L. Wäckers, 2004. HPLC sugar analysis reveals the nutritional state and the feeding history of parasitoids. **Functional Ecology, 18** (6): 812-819.

Taylor, A. D., 1988. Host effects on functional and ovipositional responses of *Bracon hebetor*. **The Journal of Animal Ecology, 57** (1): 173-184.

Ueno, T., 1999a. Reproduction and host-feeding in the solitary parasitoid wasp *Pimpla nipponica* (Hymenoptera: Ichneumonidae). **Invertebrate Reproduction and Development, 35** (3): 231-237.

Ueno, T., 1999b. Host-size-dependent sex ratio in a parasitoid wasp. **Researches on Population Ecology, 41** (1): 47-57.

Ullyett, G. C., 1945. Distribution of progeny by *Macrobracon hebetor* Say. **Journal of Entomological Society of South Africa, 8**: 123-131.

Van Handel, E., 1985. Rapid determination of total lipids in mosquitoes. **Journal of the American Mosquito Control Association, 1**: 302-304.

Wäckers, F. L., J. C. Lee, G. E. Heimpel, K. Winkler & R. Wagenaar, 2006. Hymenopteran parasitoids synthesize 'honeydew-specific' oligosaccharides. **Functional Ecology, 20** (5): 790-798.

Yu, S. H., M. I. Ryoo, J. H. Na & W. I. Choi, 2003. Effect of host density on egg dispersion and the sex ratio of progeny of *Bracon hebetor* (Hymenoptera: Braconidae). **Journal of Stored Products Research, 39** (4): 385-393.

Ziegler, R. & M. Schultz, 1986. Regulation of carbohydrate metabolism during flight in *Manduca sexta*. **Journal of Insect Physiology, 32** (12): 997-1001.