

REPRODUCTIVE PERFORMANCE OF THE
PARASITOID *BRACON HEBETOR* SAY
(HYMENOPTERA: BRACONIDAE) ON VARIOUS
HOST SPECIES OF LEPIDOPTERA

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PREFACE

Chapter I of this thesis is an introduction and literature review that describes the importance of stored product insect pests and biological control in storage. This initial chapter briefly describes the biology of a few stored product lepidopteran species and the larval ectoparasitoid, *Bracon hebetor* Say, selected for research purposes. The following three chapters, Chapters II, III, and IV, are formal manuscripts of the research that I conducted during my Ph.D. program and are written in compliance with the publication policies and guidelines of the Entomological Society of America (ESA). Chapter V is a general summary and concluding remarks to the dissertation.

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**Dedicated to my parents,
mother Nira Devi Ghimire and late father Jib Nath Ghimire**

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CHAPTER I
LITERATURE REVIEW

Introduction

Insect pests that are associated with stored products such as cereals, legumes, oilseeds, dried fruits, nuts, and many other value-added whole or processed food products cause substantial economic and quality losses to the products. Post-harvest losses due to stored product insects are estimated up to 10% world wide, 5-10% in the United States, and up to 10% or more in developing countries up to 20% (Adams 1977, Pimentel 1991, Boxall 1991). In addition to these quantitative losses, insect infestations significantly reduce seed viability, nutritional quality and market value of stored products. Insect infestation to stored products can occur just prior to harvest, during handling and transportation, and during storage.

Stored-product insects

The most economically important families of insects that infest stored products are in the order Coleoptera and Lepidoptera. About 600 species of beetles and 70 species of moths are associated with stored products in various part of the world (Arbogast 1991, Cox and Bell 1991). Of them 40 insect species, including about ten families of Coleoptera and four families of order Lepidoptera, are frequently encountered as pests of stored products.

Stored-product moths are among the most destructive insects of stored grain and processed food throughout the world. Larvae of these moth species do their damage by directly consuming various stored products and also by subsequent silken webbing of their food into contaminated masses. Larval feeding may also cause mold development due to increase in moisture that not only deteriorates food or grain quality, but also produces a favorable environment for other related pests.

Control methods

Several measures are available for managing insects that are associated with stored products. These control measures are categorized into five different groups: Hygienic measures (e.g., sanitation), physical and mechanical measures, chemical measures, biological measures and legislative measures (Munro 1966). Among them, the use of chemicals is one of the most widely used methods for controlling insect infestations, but recent legislative restrictions or regulatory changes limit the use of many compounds because of their potential harm to human health and the environment. In addition, stored-product pests have developed resistance to some of the major insecticides (Phillips et al. 2000, Subramanyam and Hagstrum 1996), thus reducing their effectiveness.

Recently, due to the negative impacts of pesticides, attention has been focused on adopting integrated pest management (IPM) strategies, which include physical and biological control methods as a viable option of managing stored product insects. The potential of alternative methods to pesticides in stored-product IPM have been described (Subramanyam and Hagstrum 2000). These methods include the use of resistance crop varieties, adequate storage structures, insect growth regulators, pheromones, behavior modifying chemicals, biological control agents, natural products, and physical control methods such as sanitation, structural modification, aeration and heating. In general my Ph. D. research project focuses on biological control of stored product pests as an alternative to chemical pesticides.

Biological control

The term “biological control”, first used by Smith (1919), refers to the use of predators, parasitoids, and pathogens for control of insect pests. The use of biological control agents to control insect pests in storage situations is not a new concept, but it has long been neglected, probably because of the contamination issue in food products by introducing natural enemies and the tolerance limit for insect damage (Arbogast 1983). Recently, attention has been focused on this strategy due to increased consumer concern with pesticide residues in food products and a wide-ranging negative impact of chemical insecticides to the environment. For example, the fumigant insecticide methyl bromide, once commonly used in stored product systems, is being banned due to its ability to deplete the stratospheric ozone layer (United Nations Environment Program 1992). This has led to intensified research into alternative control methods or IPM practices.

The use of beneficial insects in stored grains, raw commodities and processed food in warehouses is now acceptable after legislation passed to exempt the use of natural enemies from tolerance standards (Environmental Protection Agency 1992). All genera of parasitoids and predators that are known to attack stored product insects and are regulated by the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) are exempted for their use and occurrence as biological control agents in stored raw commodities and processed food. These include genera of parasitic Hymenoptera such as *Trichogramma*, *Bracon* (*Habrobracon*), *Venturia*, *Mesostenus*, *Anisopteromalus*, *Choetospila*, *Lariophagus*, *Dibrachys*, *Habrocytus*, *Pteromalus*, *Cephalonomia*, *Holepyris*, and *Laelius*, and predatory Hemiptera, *Xylocoris*, *Lyctocoris*, and *Dufouriellus*

(Brower et al. 1996). Thus, biological control can be a safe and viable method of stored-product protection.

***Bracon hebetor*: A potential bio-control agent**

Bracon hebetor Say (Hymenoptera: Braconidae) is a gregarious, idiobiont ectoparasitoid that attacks larvae of several species of Lepidoptera, mainly pyralid moths infesting stored products. It is an important potential biological control agent of stored product moths (Brower et al. 1996). *B. hebetor* females first paralyze their host larva by stinging and then laying variable numbers of eggs singly on or near the surface of paralyzed hosts (Antolin et al. 1995). The paralyzed host larvae are then used as food sources for developing wasps and also for the adult females. Normally the female *B. hebetor* paralyzes a number of larvae and returns afterwards to oviposit on some of them. So, they paralyze many more hosts than may be needed for oviposition at one time. Under natural conditions only a small proportion of the parasitoid larvae actually have eggs laid on them. The paralysis is ultimately fatal, though paralyzed larvae may continue to live for nearly a month if not parasitized and consumed by wasp larvae (Doten 1911, Richards and Thomsen 1932).

Host location and oviposition

The *B. hebetor* females prefer to attack and oviposit on last instar (fifth) larvae, although younger instars will also be stung and used (Benson 1973b). Ovipositing females locate their hosts via trails containing semiochemicals produced in the mandibular gland of the host larvae as they feed and deposit silk while walking or when producing their pupal cocoons (Strand et al. 1989). These compounds induce a female to decrease walking speed and begin antennal movements and probing the substrate with her

ovipositor. Once a host is located, the female *B. hebetor* injects venom that induces complete paralysis of host within 15 min. (Hagstrum and Smittle 1978). The venom blocks neuromuscular transmission at a presynaptic site and apparently has no effect on heartbeat or midgut function. The venom of *B. hebetor* may also give offspring comparative advantage over the larvae of other species. For example, larvae of the endoparasitic ichneumonid, *Venturia canescens*, are developmentally arrested when the host is paralyzed with *B. hebetor* venom (Petters and Stefanelli 1983). In this case, young *V. canescens* larvae are particularly more susceptible, but older larvae are also affected.

B. hebetor females prefer to oviposit on freshly paralyzed hosts and hosts with no eggs already on it, although they will oviposit on paralyzed hosts that are older or may have eggs on it (Hagstrum and Smittle 1978). Once a female has encountered a paralyzed host she will carefully inspect it for the presence of eggs from other females. If the eggs of another female are encountered, the female will often puncture and kill the eggs with her ovipositor. So, the females will typically engage in ovicidal behavior for up to an hour. However, this behavior depends on several factors, including host encounter rate, egg load and possibly genetics of the females because some females do not engage in ovicidal behavior (Strand and Godfray 1989, Antolin et al. 1995).

B. hebetor females continually produce eggs throughout their lifetime (synovigenic) and reproductive females are engage in host-feeding which is essential for the maturation of additional eggs (Benson 1973a, Javris and Kidd 1986). Newly-emerged females contain very few eggs and need three to four days of maturation and host-feeding to attain their maximum daily egg production (Petters and Grosch 1977).

Females that do not feed on hosts for 48 h begin to reabsorb eggs, presumably redirecting resources towards other metabolic processes (Benson 1973a).

After the host is paralyzed, the female oviposits, usually placing a clutch of several eggs on the ventral surface of the host or on the side that is in contact with the substrate (Benson 1973a, Strand and Godfray 1989). Females lay a total of 8-30 eggs per host per day depending on host size, encounter rate, and the physiological state of the ovipositing females (Benson 1973a, Hagstrum and Smittle 1977, Strand and Godfray 1989). Egg production rate and daily fecundities are highest when hosts are encountered daily. An averaged-size female, with a head capsule of 0.5-0.6mm wide, that has encountered a host every day will have daily fecundities of 10-20 eggs and a lifetime fecundities of 250-350 eggs (Hagstrum and Smittle 1977).

Host encounter rate and host feeding frequency have a greater impact on daily and lifetime fecundity, and longevity. Starved females and males can live 6-10 d and 4-10 d, respectively, at 25°C (Doten 1911, Benson 1973a), whereas females encountering host daily will live an average of 25-30 d at 25°C (Clark and Smith 1967). Starved females may live longer than males because they are able to recover resources from reabsorbed eggs, are heavier than males, and have slower weight loss rates than that for males (Griggs 1959). Females can survive on a carbohydrate based diet of honey and water and exhibit reductions in the rate of egg maturation and resorption (Benson 1973a).

Life cycle of *B. hebetor*

Egg development time varies from 12 h at temperatures of 27-34°C, to eight days when at 4-14°C. There are four larval instars with total larval developmental time 36 h to five days, depending upon rearing temperatures (Benson, 1973a). The last instar larvae spin small white cocoons before pupation, either on or near the host remains. The pupal period lasts from three to four days. The overall development time from oviposition to adult emergence is 10-13 d at 27°C (Benson 1973a, Strand and Godfray 1989). *B. hebetor* is able to live and be active in all stages between the temperatures of 14.5-40°C (Payne 1933).

Benson (1973a) observed two primary sources of developmental mortality in *B. hebetor*. First, the key mortality factor was eggs and early larval instar and this is density dependent mortality, increasing with larger clutch sizes or the presence of older larvae. The secondary mortality occurs when most larvae reach third and fourth instar, after which begins the scramble competition for remaining host resources. When clutch sizes are small (less than eight eggs) such competition is negligible, but when the clutch sizes are larger the mortality levels dramatically increase (Benson 1973a). This mortality is higher for female offspring because males develop quickly and are smaller than females, and thus use less host resources. Once larvae begin to spin cocoons mortality is very low. The occurrence of density-dependent, competitive effects on individual fitness and population size suggests that females should lay larger clutches of eggs on larger hosts. Both egg to adult survivorship and body size within broods decline with decreasing host size or increasing clutch size (Benson 1973a, Taylor 1988a, Taylor 1988b, Strand and Godfray 1989).

Taxonomy of *B. hebetor*

The taxonomic nomenclature for this species has suffered perhaps more than that of most other species in the order Hymenoptera, and is arguably in a state of disarray (Gauld and Bolton 1988, Grosch 1988). *B. hebetor* Say was first described in the genus *Bracon* by Thomas Say in 1836. Since then 24 different synonyms such as *Microbracon dorsator* Johnson & Hammer (1912), *Habrobracon junglandis* Cushman (1922) have been used by several authors (Shenefelt 1975). As was common in the early 20th century, it appears that new species names were frequently created when a biologically and ecologically similar wasp was discovered in a new country or a new host for the first time. Currently, it has been returned to the genus *Bracon* (Krombein et al. 1979). Thus, based on the taxonomic authority of the Krombein et al. (1979) work, biological studies that report the species name as “*Habrobracon hebetor*” are incorrect, and refer to *Bracon hebetor* Say *sensu stricto*.

In the United States, *B. hebetor* populations associated with stored product moths, predominantly with pyralid moths in the sub-family Phycitinae, are probably represented by one biologically distinct species. However, in other countries a wasp called *Bracon hebetor* is reported as a parasitoid of non-pyralid moths in the field and also shows potential as a parasitoid of storage moths. A recent study by Heimpel et al. (1997) claimed that a species morphologically indistinguishable from *B. hebetor* may exist in Barbados and utilizes noctuid moths in field crop habitats. In order to clarify its taxonomic status in relation to the stored product *B. hebetor*, they conducted experiments that proved pre-mating and post-mating reproductive isolation between the two geographically and ecologically separate populations in laboratory studies, and they

showed genetic distinctness indicative of reproductive isolation between the two populations, one from a storage habitat associated with phycitine species and other from a field habitat associated with Heliothine species. Thus, morphologically identical allopatric or possibly sympatric sibling species may exist and could be confused with the biologically distinct *B. hebetor* that parasitizes stored product moths. Understanding the basic biology and reproductive performance of *B. hebetor* in response to various lepidopteran host species is necessary to enhance the biological control program for the management of stored-products insects.

Objectives

The broad goal of this study is to evaluate the use of *B. hebetor* as a biological control agent against stored product moth species. Basic and applied aspects of parasitoid biology will be investigated in order to optimize its efficacy. To achieve the goal, these specific research objectives will be investigated.

1. Suitability of different lepidopteran host species for development and reproduction of *B. hebetor*
2. Effects of six pyralid host species, considered more “suitable” or preferred for wasp reproduction, on oviposition and reproductive performance of *B. hebetor*
3. Effects of parasitoid and host densities, and size of the rearing containers on mass rearing of *B. hebetor*.

These three objectives are addressed in the following three chapters of the dissertation. Each chapter is written as an independent manuscript, each intended for publication as separate peer-reviewed journal articles beyond this dissertation.

References

- Adams, J. M. 1977.** A review of the literature concerning losses in stored cereals and pulses published since 1964. *Trop. Sci.* 19:1-28.
- Antolin, M. F., P. J. Ode, and M. R. Strand. 1995.** Variable sex ratio and ovicide in an outbreeding parasitic wasp. *Anim. Behav.*, 17: 1-7.
- Arbogast, T. R. 1983.** Natural enemies as control agents for stored-product insects. In *Proceedings of the third international working conference on stored product entomology*, Oct. 23-28, 1983. Kansas State University, Manhattan, Kansas, USA
- Arbogast, T. R. 1991.** Beetles: Coleoptera, pp. 131-176. In J. R. Gorham, (ed.), *Ecology and management of food-industry pests*. Assoc. Offic. Anal. Chem. Arlington.
- Benson, J. F. 1973a.** Intraspecific competition in the population dynamics of *Bracon hebetor* Say. *J. Anim. Ecol.* 42:193-197.
- Benson, J. F. 1973b.** The biology of Lepidoptera infesting stored products, with special reference to population dynamics. *Biolog. Rev.* 48: 1-26.
- Boxall, R. A. 1991.** Post-harvest losses to insects- a worldwide review, pp. 160-175. In H.W. Rossmore (ed.), *Biodeterioration and Biodegradation Symposium*, Windsor, Ontario, Canada, 26-31 August 1990. Elsevier Applied Science, New York.
- Brower, J. H, L. Smith, P. V. Vail, and P.W. Flinn. 1996.** Biological control, pp. 223-286. In B. Subramanyam and D.W. Hagstrum (eds.), *Integrated Management of Insects in Stored Products*. Marcel Dekker, New York, USA.

- Clark, A.M. and R. E. Smith. 1967.** Egg production and adult life span in two species of *Bracon* (Hymenoptera: Braconidae). *Ann. Entomol. Soc. Am.* 60: 903-905.
- Cox, P. D., and C. H. Bell 1991.** Biology and ecology of moth pests on stored food, pp. 181-193. In J. R. Gorham (ed.), *Ecology and management of food-industry pests*. Association of Official Analytical Chemists, Arlington, VA, USA.
- Cushman, R. A. 1922.** The identity of *Habrobracon brevicornis* (Wesmael) (Hym. Braconidae). *Proc. Ent. Soc. Wash.* 24: 122-124.
- Doten, S. B. 1911.** Concerning the relation of food and reproductive activity and longevity of certain hymenopterous parasites. *Tech. Bull. Nevada Agric. Expt. Sta.* 78: 30 pp.
- Environmental Protection Agency. 1992.** Parasitic and predaceous insects used to control insects pests; exemption from a tolerance. *Fed. Reg.* 57: 14645-14646.
- Gauld, I. and B. Bolton. 1988.** *The Hymenoptera*. Oxford University Press, Oxford.
- Grisggs, R. C. 1959.** A study of succinic dehydrogenase activity in the wasp *Habrobracon juglandis* (Ashmead). *Bios*: 202-207.
- Grosch, D. S. 1988.** Genetics research on braconid wasps. *Advan. Gene.* 25: 109-184.
- Hagstrum, D. W. and B. J. Smittle 1977.** Host finding ability of *Bracon hebetor* and its influence upon adult parasite survival and fecundity. *Environ. Entomol.* 6: 437-439.
- Hagstrum, D. W. and B. J. Smittle 1978.** Host utilization by *Bracon hebetor*. *Environ. Entomol.* 7: 596-600.
- Heimpel, G. E., M. F. P Antolin, R. A. Franqui and M. R. Strand. 1997.**

- Reproductive isolation and genetic variation between two “strain” of *Bracon hebetor* (Hymenoptera: Braconidae). Biol. Control. 9: 149-156.
- Jervis, M. A. and N. A. C. Kidd. 1986.** Host-feeding strategies in hymenopteran parasitoids. Biolog. Rev. 61: 395-434.
- Johnson, W. G. and Hammer 1912.** Bull. Bur. Ent. U.S.D.A. 116:48.
- Krombein, K. V., Jr. P. D. Hurd, D. R. Smith and B. D. Bruks. 1979.** Catalog of Hymenoptera in America North of Mexico. Washington D. C., Smithsonian Institution Press.
- Munro, J. W. 1966.** Pests of stored products. Hutchinson & Co. Ltd., London 234 p.
- Payne, N. M. 1933.** The differential effects of environmental factors upon *Microbracon hebetor* Say (Hymenoptera: Braconidae) and its host *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). I. Biol. Bull. 65: 187-205.
- Petters, R. M. and D. S. Grosch. 1977.** Reproductive performance of *Bracon hebetor* females with more or fewer than the normal number of ovarioles. Ann. Entomol. Soc. Am. 70: 577-582.
- Petters, R. M. and J. Stefanelli. 1983.** Developmental arrest of endoparasitic wasp larvae (*Nemeritis canescens* Grav) caused by an ectoparasitoid wasp (*Bracon hebetor*). J. Expt. Zool. 225: 459-465.
- Phillips, T. W., R. Berberet and G. W. Cuperus. 2000.** Post-harvest integrated pest management, pp. 2690-2701. In F. J. Francis (ed.), Encyclopedia of food science and technology, 2nd ed. Wiley, New York.
- Pimentel, D. 1991.** World resources and food losses to pests, pp 5-11. In J. R. Gorham

(ed.), Ecology and Management of Food Industry Pests. Assoc. Off. Anal. Chem., Arlington.

Richards, O. W. and W. S. Thomson. 1932. A contribution to the study of the genera *Epesthia*, Gn. (including *Strymax*, Dyar) and *Plodia*, Gn. (Lepidoptera: Phycitidae) with notes on parasite of the larvae. Transac. Royal Entomol. Soc. London. 80: 169-250.

Say, T. 1936. Description of the new species of North American Hymenoptera, and observation on some already described. Boston J. Nat. Hist. 1: 209-305.

Shenefelt, R. D. 1975. Braconidae 1-8. In W. Junk (ed.), Hymenopterum Catalogus. Academic Press, The Hague, pp. 1597-1607.

Smith, H. S. 1919. On some phases of insect control by biological method. J. Econ. Ento. 12: 288-292.

Strand, M. R. and H. C. J. Godfray 1989. Superparasitism and ovicide in parasitic Hymenoptera: theory and a case study of the ectoparasitoid *Bracon hebetor*. Behav. Ecol. Sociobiol. 24: 421-432.

Subramanyam, B. and D. W. Hagstrum. 1996. Resistance measurement and management, pp. 331-397. In B. Subramanyam and D.W. Hagstrum (eds.), Integrated Management of Insects in Stored Products. Marcel Dekker, New York, USA.

Subramanyam, B. and D. W. Hagstrum. 2000. Alternatives to pesticides in stored product IPM. Kluwer Academic Publisher, United States of America, 437p.

Taylor, A. D. 1988a. Host effects on larval competition in the gregarious parasitoid *Bracon hebetor*. J. Anim. Ecol. 57:163-172.

Taylor, A. D. 1988b. Host effects on functional and ovipositional response of
Bracon hebetor. *J. Anim. Ecol.* 57: 173-184.

United Nations Environment Program. 1992. The impact of ozone layer depletion.
Nairobi, Kenya.

CHAPTER II
SUITABILITY OF DIFFERENT LEPIDOPTERAN HOST SPECIES
FOR DEVELOPMENT OF *BRACON HEBETOR* SAY
(HYMENOPTERA: BRACONIDAE)

Abstract

Bracon hebetor Say (Hymenoptera: Braconidae) is a gregarious larval ectoparasitoid of several species of Lepidoptera that are associated with stored products. The suitability of twelve potential lepidopteran host species representing four families was investigated in this study for the development and reproduction of *B. hebetor*. The Lepidoptera species used were the Indianmeal moth, *Plodia interpunctella* (Hübner), Mediterranean flour moth, *Ephesia kuehniella* (Zeller), almond moth, *E. cautella* (Walker), rice moth, *Corcyra cephalonica* (Walker), navel orangeworm, *Amyelois transitella* (Stainton), greater wax moth (laboratory reared and commercial), *Galleria mellonella* (Linnaeus) (all Pyralidae); tobacco budworm, *Heliothis virescens* (Fabricus), corn earworm, *Helicoverpa zea* (Boddie), beet armyworm, *Spodoptera exigua* (Hübner) (all Noctuidae); webbing clothes moth, *Tineola bisselliella* (Hummel) (Tineidae); and Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Gelichiidae). Experiments were conducted using Petri-dishes (100 by 15 mm) as experimental arenas. *Bracon hebetor* females were introduced singly into arenas and given a full-grown host larva every day for five consecutive days. Paralysis of the host larvae and oviposition by *B. hebetor* females were significantly affected by host species. The cumulative fecundity in the five-day period was highest on *A. transitella* (106.42 ± 5.19) and lowest on *T. bisselliella* (9.64 ± 1.28). The egg-to-adult survivorship and progeny sex ratio were also significantly affected by the host species. The highest percentage of parasitoid survival the adult stage was on *A. transitella* (84.07 ± 2.26) and zero on *T. bisselliella*. Egg to adult development time was shortest on *E. cautella* (9.75 ± 0.25 days) and longest on *G. mellonella* (12.63 ± 0.28 days). Results from the current studies suggest that *B. hebetor*

females can use a wide range of lepidopteran hosts for paralysis and oviposition. However, *B. hebetor* can not necessarily develop and reproduce on all host species that it can paralyze and oviposit on, and optimum reproduction is with the stored-product pyralid hosts. The possible application of these results for biological control of stored product insects is discussed.

Key words: Lepidoptera, host suitability, stored-product insects, parasitoid, biological control

Introduction

Bracon hebetor Say (Hymenoptera: Braconidae) is a cosmopolitan, gregarious, ecto-parasitoid that attacks larvae of several species of Lepidoptera, mainly pyralid moths infesting stored-products. *B. hebetor* is considered one of the best potential biological control agents for stored-product insects in the moth family Pyralidae (Brower et al. 1996). *B. hebetor* females first paralyze their host, which are typically last stage larvae in a “wandering” phase, by stinging them, injecting a paralytic venom and then ovipositing variable numbers of eggs on or near the surface of paralyzed host. Paralyzed host larvae are then used as food sources for developing wasp larvae and adult females.

B. hebetor is primarily known as a parasitoid of pyralid moths in the sub-family Phycitinae that are associated with durable stored food products, and include the Indianmeal moth, *Plodia interpunctella* (Hübner) , Mediterranean flour moth, *Ephesia kuehniella* (Zeller), tobacco moth *E. elutella* (Hübner), driedfruit moth, *Vitula edmansae* (Packard), *Moodna sp*, and almond moth, *E. cautella* (Walker) (Richards and Thomson 1932, Reinert and King 1971, Hagstrum and Smittle 1977, 1978, Brower et al. 1996). According to Krombein et al. (1979). *B. hebetor* also attacks several other non-phycitine pyralid moths, such as the rice moth, *Corcyra cephalonica* (Stainton) (sub-family: Galleriinae), the greater wax moth, *Galleria mellonella* (Linnaeus) (sub-family: Galleriinae), grass moth *Laetilia coccidivora* (Comstock) (sub-family: Crambidae), and some species outside Pyralidae such as potato tuberworm, *Phthorimaea operculella*(Zeller) (family: Gelechiidae), Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Gelechiidae) in the Nearctic region.

Host records from Asian countries indicate that *B. hebetor* also attacks a number of non-pyralid Lepidoptera species that occur in both grain storage and field habitats (Harakly 1968, Gerling 1971, Cock 1985, Nikam and Pauer 1993, Amir-Maafi and Chi 2006). However, according to Heimpel et al. (1997), the wasp species described in these earlier works could be a distinct biological species from *B. hebetor* that can successfully attack larvae of moths in the family Noctuidae in the field. Their laboratory studies demonstrated that two geographic separate populations of *B. hebetor*, one population from a storage habitat collected in the United States and another population from a field habitat collected in Barbados, and island in the southern Caribbean region, were reproductively isolated and genetically distinct. Despite of studies such as that by Heimpel et al. (1997), it is not known whether host utilization patterns of *B. hebetor* associated with storage habitats may varies over its reportedly wide lepidopteran host range.

A good understanding host-parasitoid association is crucial to the success of biological control programs. A host's value to the reproductive fitness of a parasitoid mainly depends on the number and quality of her progeny producing from that host. Thus, physiological suitability of the host is absolutely necessary for the successful development of parasitoid progeny (Wiedenmann and Smith 1997). Similarly, a parasitoid's fitness also depends on her ability to locate and recognize its host in a complex environment and to produce a high or optimum number of viable and high-quality progeny from that host. The objective of this study was to determine the ability of *B. hebetor* from a stored-product habitat to successfully parasitize and successfully

reproduce on a range of lepidopteran host species from several families under laboratory conditions.

Material and Methods

Parasitoid origin and rearing

B. hebetor adults were collected from grain bins at the Stored Products Research and Education Center (SPREC) at Oklahoma State University in Stillwater, Oklahoma on November 2003 that were associated with the Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). The parasitoids were then cultured and mass-reared on full-grown larvae of *P. interpunctella* in the laboratory at a temperature of $29 \pm 1^\circ\text{C}$, a relative humidity of $65 \pm 5\%$, and a photoperiod of 14:10 (L: D) h. Full-grown larvae of *P. interpunctella* were obtained from a laboratory culture that was reared on a standardized diet of corn meal, chick laying mash, chick starter mash, and glycerol (Phillips and Strand, 1994) at a volumetric ratio of 4:2:2:1, respectively, at a temperature of $28 \pm 1^\circ\text{C}$, a relative humidity of $65 \pm 5\%$, and a photoperiod of 16: 8 (L:D) h.

Host species

The host species studied in these experiments were four species of phycitine Pyralidae, three species of non-phycitine pyralids, and five species of Lepidoptera from other families (Table 1). The host larvae of pyralids from Phycitinae, Gelechiidae and Teneidae were obtained from laboratory colonies at Oklahoma State University. Larvae of Noctuid species were obtained from United States Department of Agriculture Stoneville, MS and Dow AgroScience, Indianapolis and were reared on artificial diets from those facilities. The greater wax moth larvae were obtained from a local pet store, and supplied through Timberline Live Pet Foods, Inc. Marion, IL and I also maintained a culture of greater wax moths in the laboratory that originated from the Timberline Co., a commercial supplier of greater wax moth larvae. The initial culture of *A. transitella* was

obtained from USDA-ARS Commodity Protection and Quality Laboratory at Parlier, CA. The culture of *C. cephalonica* was obtained from Insects Limited Inc, Westfield, IN.

The larvae of phycitine species were obtained from our laboratory cultures, except *A. transitella*, which were obtained from the USDA ARS laboratory in Parlier, CA and that were reared on the same diet as used for rearing of *P. interpunctella* and were maintained at the same environmental condition. The *S. cerealella* moths were reared on whole wheat kernels, whereas *T. bisselliella* moths were reared on a feather-meal and brewers yeast based diet. *G. mellonella* was reared on a mixture of wheat flour, honey, glycerol, bee wax, and brewer's yeast at a weight basis ratio of 0.44:0.23:0.18:0.04:0.11, respectively (Mohaghegh and Amir-Maafi 2001). *A. transitella* was reared on a mixture of 11.355 liter of flakey red food bran, 900 ml honey, 800 ml de-ionized water, 100 gm brewer's yeast, and 10 ml Vanderzants vitamins solution (1%). *C. cephalonica* was reared on a mixture of wheat bran, wheat germ, rolled oats, glycerin, and brewer's yeast at a ratio of 1:1:1:1:0.5, respectively. All the cultures were maintained at the similar growth chamber environment as used for rearing of *P. interpunctella*.

Host suitability experiments

Experiments were conducted in the laboratory in a no-choice design using Petri-dishes (100 by 15 mm) as experimental arenas with a single wandering stage larva of each host species. According to Hagstrum and Smittle (1977), *B. hebetor* females attack wandering larvae 10-fold more than they attack concealed young larvae indicating that they rarely preferred to attacked younger larvae that are usually concealed within the infested commodity. Before the experiment, a relative sample of full-grown larvae of each host species were randomly taken from the rearing jars and larval fresh weights

were measured (n=12) by placing the individual larvae on a Denver instruments (Denver, CO, USA) M-220 electronic balance (± 0.01 mg) (Table 1). Two-day old mated *B. hebetor* females were introduced singly into experimental arenas and allowed to sting and oviposit for next five days with a fresh host given daily. After emergence of a parasitoid's adult progeny was completed (approximately two weeks), all the experimental arenas were frozen at -15°C for three days. Observations were recorded on the numbers of hosts paralyzed and parasitized, numbers of eggs laid each day on each host, egg-to-adult development time, numbers adult progeny produced on each host, egg-to-adult survivorship, and parasitoid's sex ratio. Each experiment was replicated ten to twelve times.

Data analysis

The numbers of hosts paralyzed and parasitized each day, the cumulative number of eggs and adults count after five days of oviposition, the egg-to-adult development times, egg-to-adult survivorship, and progeny sex ratio (% female of the total emerged adult progeny) were used as response variables to assess the quality and suitability of host species on the development and reproduction of *B. hebetor*. Host species were used were considered independent variable for the analysis of response variables. Data for numbers of host paralyzed and parasitized, egg-to-adult survivorship, and progeny sex ratio were analyzed by one-way analysis of variance (ANOVA) (PROC GLM, SAS Institute 2005). The differences in age-specific daily oviposition was determined by two-way repeated measure ANOVA (Proc Mixed) assuming an autoregressive covariance structure (Littell et al 1996). Data from the egg-adult developmental period of both sexes were pooled together as no statistically significant sex difference was observed, and these data were

subjected to one-way ANOVA. Because *B. hebetor* failed to produce any adult progeny on *T. basinella*, and a very few adult progeny were produced on Heliothine species (*H. virescens* and *H. zea*), these species were excluded in the statistical analysis for calculating development times. Count data for the cumulative value of eggs and adults were $\log(X + 0.5)$ and $\log(X + 1)$ transformed, respectively, and progeny sex ratio and egg-to- adult survivorship data were arcsine transformed to meet assumptions of normality and heterogeneity of variance. Means were separated using Duncan's Multiple Range Test (DMRT) ($\alpha=0.05$) and original mean values are presented in the figures.

Results

An acceptable host was defined as one that was paralyzed and received at least one or more parasitoid eggs. *B. hebetor* females used or accepted all twelve host species for paralysis and oviposition that were offered in these experiments (Fig. 1). However, the level of paralysis and oviposition varied significantly with the host species ($F = 23.96$; $df = 11, 623$; $P < 0.0001$, and $F = 32.52$; $df = 11, 623$; $P < 0.0001$ for paralysis and oviposition, respectively). *B. hebetor* females paralyzed only 42% of *H. zea* larvae that were offered and oviposited on about 50% of those hosts, while they paralyzed almost 100% of pyralid host larvae that were offered and used 100% of these for oviposition (Fig. 1). There were no significant differences observed in proportion of larvae that were paralyzed by *B. hebetor* females among the pyralid host species. However, in contrast, there were a significant differences observed in proportion of larvae that were paralyzed by *B. hebetor* females among the non-pyralid host species (Fig. 1). A similar trend was observed in proportion of host that were parasitized or oviposited.

The daily rates of oviposition varied significantly with host species ($F = 32.32$; $df = 11, 94.1$; $P < 0.0001$), age of female wasp ($F = 8.52$; $df = 4, 315$; $P < 0.0001$), and also by the interaction between host species and age of female wasps ($F = 1.81$; $df = 42, 271$; $P = 0.0029$). Daily oviposition was higher on *A. transitella* (22.4 ± 0.96 eggs/host/day) and laboratory reared *G. mellonella* (21.9 ± 1.09 eggs/♀/host) with these hosts having the maximum range of oviposition (44 and 42 eggs/♀/host), respectively) compared to other host species tested in this study (Fig. 2 and 3). These two hosts elicited increased oviposition response to *B. hebetor* females as the female wasps aged and became more experienced with the host larvae during the five-day period (Fig. 2). Significantly lower

numbers of eggs were laid on *T. bisselliella* (3.12 ± 0.23 eggs/♀/host) and *S. cerealella* (4.93 ± 0.39 eggs/♀/host) and these hosts did not elicit increased oviposition response over time (Fig. 3). Among the noctuid species, only *S. exigua* elicited increased oviposition (12.07 ± 1.86 eggs/♀/host) response to *B. hebetor* females as the female wasps aged and became experienced with host larvae (Fig. 3).

The mean total numbers of eggs laid by *B. hebetor* females over the five-day periods on twelve different hosts varied significantly ($F = 26.67$; $df = 11, 108$; $P < 0.0001$). The greatest number of eggs, in decreasing numerical order were laid on *A. transitella* (106.42 ± 5.19 eggs/♀/5 d), *G. mellonella* from laboratory-reared larva (105.10 ± 7.2 eggs/♀/5 d) and *C. cephalonica* (93.00 ± 6.94 eggs/♀/5 d) (Fig. 4). Oviposition on this group of hosts was statistically similar to that on *E. kuehniella*, commercially reared *G. mellonella* and *P. interpunctella*. Oviposition on the three leading hosts was significantly greater than on *E. cautella*, and oviposition on all seven pyralid hosts was significantly greater than on the non-pyralid host species. For example, *B. hebetor* females laid on average <10 eggs on *T. bisselliella*, ≈ 20 eggs on *S. cerealella*, and about 16-36 eggs on noctuid species during the five days of oviposition (Fig. 4).

The numbers of *B. hebetor* adult progeny produced in response to different host species was found significant ($F = 67.50$; $df = 11, 108$; $P < 0.0001$). The greatest number of adult progeny was produced on *A. transitella* (87.17 ± 5.03 adults/♀/5 d) followed by *C. cephalonica* (70.30 ± 6.88) and *E. kuehniella* (61.51 ± 5.40 adults/♀/5 d) during the five consecutive days (Fig. 5). However, there were no significant differences in the average numbers adult progeny produced on *C. cephalonica*, *E. kuehniella* and *P. interpunctella*. The lowest numbers of adult progeny (<2 adults/♀/5 d) was produced on

heliothine species whereas *B. hebetor* failed to produce any adult progeny on *T. bisselliella*. Among the noctuid species, *S. exigua* produced greater number of parasitoid progeny (12.56 ± 4.89 adults/♀/5 d).

Egg-to-adult survivorship was significantly affected by host species ($F = 69.66$; $df = 11, 480$; $P < 0.0001$). The highest percentage of parasitoid survival was found on *A. transitella* (84.07 ± 2.26) followed by *P. interpunctella* (77.75 ± 2.75) and *C. cephalonica* (75.78 ± 3.03) (Fig. 6). Although *G. mellonella* elicited a high level of oviposition response by *B. hebetor*, parasitoid survival was significantly lower on both populations of this host compared to other pyralid species (Fig. 6). *T. bisselliella* did not support the parasitoid development as there were no adult progeny produced from this host. Although few eggs were laid on *S. cerealella* larvae, this host had a significantly higher percentage of parasitoid survival (67.10 ± 5.05) compared to other non-pyralid host species (Fig. 6). Similarly, *S. exigua* supported a significantly higher percentage of parasitoid survival (26.98 ± 5.97) compared to other noctuid species (Fig. 6).

The egg-to-adult developmental duration for *B. hebetor* varied significantly with host species that were tested ($F = 16.28$; $df = 7, 65$; $P < 0.0001$). Development times were shortest on all pyralid host species (≈ 10 d) except for *G. mellonella* and *A. transitella*, compared to other host species (Fig. 7). Parasitoids larvae developed slowest on *G. mellonella* (12.6 d) host larvae compared to all other host species. There were no significant differences observed in developmental times between *S. exigua* and *A. transitella* (Fig. 7).

The parasitoid's secondary sex ratio (% females of the total adult progeny) was significantly affected by host species ($F = 3.95$; $df = 10, 380$; $P < 0.0001$). A strongly

female biased secondary sex ratio was observed on larger hosts such as *G. mellonella*, *S. exigua*, *H. virescens*, *C. cephalonica*, and *E. kuehniella*, except for *H. zea*; whereas only slightly female biased sex ratios were found on smaller hosts such as *S. cerealella*, and *E. cautella* (Fig. 8).

Discussion

Several experimental studies have shown that host suitability for parasitoid development can be influenced by many factors including environmental conditions, the ability of parasitoid to evade the host's defense mechanisms, the presence of host toxins that are detrimental to parasitoid eggs or larvae, and the nutritional adequacy of host (Vinson and Iwanntsch 1980). This study compares the development, reproduction, and survival of *B. hebetor* in twelve different host species that vary considerably in size at the full grown larval stage (Table 1). A significant effect of host species was observed on the overall performance of parasitoid, *B. hebetor*.

This study showed a higher percentage of pyralid host larvae were paralyzed and subsequently parasitized compared to *T. bisselliella* and noctuid host species. Nevertheless, no significant differences among the pyralid host species were observed on these parameters. These results are in agreement with the earlier work by Heimpel et al. (1997), in which *B. hebetor* females performed similarly on pyralid host, *P. interpunctella* and noctuid host, *H. virescens*. There are several factors that might have influenced the low level of parasitoid performance in noctuid hosts. First, noctuid larvae moved vigorously in the Petri dish arenas in response to host-seeking actions of *B. hebetor* females compared to other host species, and the noctuids may have depleted the energy necessary for pursuit by the wasps. Second, noctuid larvae were much larger and heavier than other hosts except *G. mellonella*, and *B. hebetor* venom may have been depleted more quickly when subduing the larger prey. Third, the sensitivity to *B. hebetor* venom and mechanism of venom detoxification may vary with the host species and size. Although hosts tested here are taxonomically closely related and may possibly respond

similarly to venom, Beard (1952) showed that higher levels of venom were required to paralyze *Anagasta* (= *Ephestia*) as compared to two other moths, *Plodia* and *Galleria*, although *Galleria* was much larger than *Ephestia*.

In this study, *B. hebetor* females were capable of paralyzing and subsequently parasitizing all host species that were offered. Although there was $\approx 50\%$ of the paralyzed *T. bisselliella* and noctuid host larvae were parasitized, *B. hebetor* failed to develop any adult progeny on *T. bisselliella*, whereas very few adult progeny were produced from the Heliiothine species. However, *B. hebetor* was able to develop and produce significantly higher number of adult progeny from the *S. exigua* compared to these host species. This could be due to venom selectivity that may require higher levels of venom to paralyze the host or other physiological responses of the host in response to parasitoid's larval feeding such as development of a melanized ring at the site of feeding as reported by Backer and Fabrick (2000, 2002).

Host size can affect levels of parasitism by *B. hebetor*. A full grown larva of *S. cerealella* weighs ≈ 4 mg, by far the smallest host and a *G. mellonella* larva weighs ≈ 265 mg, by far the largest host that were used in these experiments. The large host, *G. mellonella*, elicited a significantly higher number of oviposition, at ≈ 20 eggs/host/day, compared to the smallest host, *S. cerealella*, at ≈ 5 eggs/host/day. In contrast, parasitoid survival to adulthood was significantly greater in *S. cerealella* (67 %), compared to *G. mellonella* which averaged at 30%. The result indicates that *B. hebetor* females may alter their clutch size in response to host size during oviposition to avoid laying more eggs than the host can support. These findings are in agreement with the earlier works by Yu et al. (2003), in which *B. hebetor* females never laid more than 7 or 12 eggs/day when

they encountered only one host larva of the tortricid, *Adoxophyes orana* or the pyralid, *P. interpunctella*, respectively. Despite high oviposition in response to large host larvae, I observed low parasitoid survival rates in the larger hosts like *G. mellonella* and the Noctuidae species. The results suggest that parasitoid fitness may be influenced not only by the host size at oviposition, but also by its nutritional adequacy for parasitoid growth and development after oviposition as purposed by Mackauer (1986). In this study, highest parasitoid survival with higher number of adult progeny was obtained on *A. transtitella* followed by all pyralid host species, except for *G. mellonella* (Figs. 5 and 6), which was a much larger host compared to other pyralid species (Table 1).

There was a significant effect of host species on mean development time of *B. hebetor*. The duration of egg-to-adult development of *B. hebetor* generally increased as host size increased. For example, wasps reared on *G. mellonella* and *S. exigua* emerged on an average of 12.6 and 11.2 days, respectively, compared with ≈ 10 days on other hosts (Fig. 7). These results agree with a hypothesis purposed by Godfray (1994) that parasitoid development time is a compensation response to limited host resources, both qualitatively and quantitatively, such that wasps either develop slowly and utilize host resources with maximum efficiency, or they develop quickly and utilize host resources with reduced efficiency.

Two different populations of *G. mellonella* were used in these studies and there were slight but consistent differences in suitability as a host for *B. hebetor* in some response variables. The commercial *G. mellonella* larvae were obtained directly from a pet supply store where they were sold as live fish bait, while the laboratory *G. mellonella* were derived from the commercial insects, but larvae used in experiments were from

moths that had been raised one or more complete generations in the laboratory.

Laboratory *G. mellonella* appeared to be relatively higher quality hosts for *B. hebetor* than were commercial *G. mellonella* based on responses such as total eggs laid (Fig. 4) and total adult progeny produced (Fig 5). It is possible that the increased reproduction of *B. hebetor* on laboratory *G. mellonella* compared to commercial *G. mellonella* was due to a possible nutritional improvement in the laboratory moths, due to laboratory diet and rearing conditions, compared to the nutritional value of commercial moths, for which details of diet and rearing were unknown and not able to be controlled. The slightly lower quality of the commercial *Galleria* may have been due to some special treatment given the commercial moths to prolong their larval stage. Very few commercial *Galleria* larvae could successfully develop through the pupal and adult stages when held in the laboratory, thus limiting the number of sexually mature adults that were available to start my new laboratory culture of *G. mellonella*. It is suspected that commercial moths were treated with insect growth regulators or other “juvenilizing” materials to prolong their larval stage and enhance their utility as live fishing bait. This presumed commercial treatment of the commercial *Galleria* may have lowered their quality as hosts for *B. hebetor*.

The secondary sex ratio of *B. hebetor* progeny, which is the proportion of the total emerged adult parasitoids from a given mother that are female or male, was significantly influenced by the host species in this study. A female-biased progeny was emerged from the larger host species such as *G. mellonella* and *S. exigua*, at 83% and 77% females, respectively, and a slightly lower female-biased progeny emerged from the smaller host species such as *E. cautella* and *S. cerealella* at 57% and 59% females, respectively.

These results with *B. hebetor* agree with the models proposed by Charnov (1982) and King (1994), in which the ovipositing female parasitoid controls the sex of the eggs she is laying depending on the host quality she has assessed, such as host species, host size and host age. Female parasitoids typically allocate more male progeny, which are unfertilized eggs, to smaller or otherwise lower quality hosts, while reserving more female offspring, from fertilized eggs, for larger or higher quality hosts in order to increase her reproductive fitness.

Results from this study showed that the growth, development and survival of a polyphagous parasitoid vary with the host species. In this study, *B. hebetor* females paralyzed and oviposited on most or all individuals of each host species that was presented and they reproduced to some degree from all hosts except for *T. bisselliella*. In general, if host suitability for *B. hebetor* is characterized by on response data such as mean daily fecundity, parasitoid survival to adulthood, development time, and parasitoid secondary sex ratio, then this study revealed that *A. transtitella* was the most suitable host followed by other pyralid species, except *G. mellonella*, which was a marginally suitable host, and *T. bisselliella*, which was the least suitable host of those tested. Of the noctuid species, *S. exigua* was a marginally suitable host and other two Heliothine species were very low suitability hosts. Although *B. hebetor* can be considered relatively polyphagous because it can parasitize and successfully develop on moth larvae from several families of Lepidoptera, this study validates previous observations that *B. hebetor* is a relative host specialist on stored-product pyralid moths in the sub-family Phycitinae. Despite the fact that host species significantly affected parasitoid oviposition rates, egg-to-adult development, survivorship, and reproductive success. Reproductive fitness of *B. hebetor*

can be maximized through the utilization of pyralid hosts such as *A. transtivella*, which allow for the highest levels of reproduction and parasitoid progeny survival.

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References

- Amir-Maafi M., and H. Chi. 2006.** Demography of *Habrobracon hebetor* (hymenoptera: Braconidae) on two pyralid hosts (Lepidoptera: Pyralidae). Ann. Entomol. Soc. Am. 99 (1): 84-90.
- Baker, J. E. and J. A. Fabrick 2000.** Host hemolymph proteins and protein digestion in larval *Habrocon hebetor* (Hymenoptera: Braconidae). Insect Biochem, Mol. Biol. 30: 937-946.
- Baker, J. E. and J. A. Fabrick 2002.** Unusual responses of Indianmeal moth larvae (Lepidoptera: Pyralidae) to envenomation and parasitization by braconid ectoparasitoid. J. Entomol. Sci. 37: 370-374.
- Beard, R. L. 1952.** The toxicology of *Habrobracon* venom: a study of a natural insecticide. Conn. Agric. Exp. Stn. Bull. 562. 27 pp
- Brower, J. H, L. Smith, P. V. Vail, and P.W. Flinn. 1996.** Biological control, pp. 223-286. In B. Subramanyam and D.W. Hagstrum (eds.), Integrated Management of Insects in Stored Products. Marcel Dekker, New York, USA.
- Charnov, E. L. 1982.** The theory of sex allocation. Princeton University Press, Princeton, New Jersey.
- Cock, M. J. W. (Ed.). 1985.** A review of biological control of pests in the commonwealth Caribbean and Bermuda up to 1982. Tech. Commun. Commonw. Inst. Biol. Contr. 9: 218.
- Gerling, D. 1971.** Occurance, abundance, and efficiency of some local parasitoids attacking *Spodoptera littoralis* (Lepidoptera: Noctuidae) in selected cotton fields in Israel. Ann. Entomol. Soc. Am. 64: 492-499.

- Godfray, H. C. J. 1994.** Parasitoids. Behavioral and evolutionary ecology.
Princeton University Press, NJ, USA
- Hagstrum, D. W. and B. J. Smittle 1977.** Host finding ability of *Bracon hebetor* and its influence upon adult parasite survival and fecundity. Environ. Entomol. 6: 437-439.
- Hagstrum, D. W. and B. J. Smittle 1978.** Host utilization by *Bracon hebetor*. Environ. Entomol. 7: 596-600.
- Harakly, F.A. 1968.** Biological studies on the cabbageweb-worm, *Hellila undalis* Fabr. (Lepidoptera: Crambidae-Pyraustinae). Bull. Soc. Entomol. Egypte 52: 191-211.
- Heimpel, G. E., M. F. P Antolin, R. A. Franqui and M. R. Strand. 1997.**
Reproductive isolation and genetic variation between two “strain” of *Bracon hebetor* (Hymenoptera: Braconidae). Biol. Control. 9: 149-156.
- King, B. H. 1994.** How do female parasitoid wasps assess host size during sex ratio manipulation? Anim.Behav. 48: 511-518
- Krombein, K. V., Jr. P. D. Hurd, D. R. Smith and B. D. Bruks. 1979.** Catalog of Hymenoptera in America North of Mexico. Washington D. C., Smithsonian Institution Press.
- Littell, R.C., Milliken, G.A., Stroup, W.W., and Wolfinger, R.D. 1996.** SAS system for mixed models. SAS Institute Inc., Cary, NC, USA
- Mackauer, M. 1986.** Growth and developmental interactions in some aphids and their hymenopterous parasites. J. Insect Physiol. 32: 275-280.
- Mohaghegh, J., and M. Amir-Maafi. 2001.** Laboratory rearing of the predatory bug

Andrallus spinidens (F.) (Het.:Pentatomidae) on different prey. Proceeding of second national conference on optimal utilization of chemical fertilizers and pesticides in agriculture, 2001, Karaj, Iran.

Nikam, P. K., and C. V. Pawar. 1993. Life tables and intrinsic rate of increase of *Bracon hebetor* Say (Hym., Braconidae) population on *Corcyra cephalonica* Staint. (Lep., Pyralidae), a key parasitoid of *Helicoverpa armigera* Hbn. (Lep., Noctuidae). J. Appl. Ent. 115: 210-213.

Ode, P. J., M. F. Antolin, and M. R. Strand. 1995. Brood-mate avoidance in the parasitic wasp *Bracon hebetor* Say. Anim. Behav. 49: 1239-1248.

Reinet, J. A. and E. W. King. 1971. Action of *Bracon hebetor* Say as a parasite of *Plodia interpunctella* at control densities. Ann. Entomol. Soc. Am. 64 (6): 1335-1340.

Richards, O. W. and W. S. Thomson. 1932. A contribution to the study of the genera *Epesthia*, Gn. (including *Strymax*, Dyar) and *Plodia*, Gn. (Lepidoptera: Phycitidae) with notes on parasite of the larvae. Transac. Royal Entomol. Soc. London. 80: 169-250.

SAS Institute. 2005. SAS/STAT user's guide for windows, version 9.1. SAS Institute, Cary, NC.

Vinson, S. B. and G. F. Iwantsch 1980. Host suitability for insect parasitoids. Ann. Rev. Entomol. 25: 397-419.

Wiedenmann, R. N., and J. W., Jr., Smith. 1997. Novel association and importation for biological control: The need for ecological and physiological equivalencies. Insect Sci. Appl. 17: 51-60.

Yu, S. H., M. I. Ryoo, J. H. Na, and W. I. Choi. 2003. Effect of host density on egg dispersion and sex ratio of progeny of *Bracon hebetor* (Hymenoptera: Braconidae). J. Stor. Prod. Res. 39: 385-393.

Table 1. List of lepidopteran host species and the average larval body weights (mg \pm SE) of 12 representative individuals used in this study

Family	Common Name	Scientific Name	Larval Weight
Pyralidae	Indianmeal moth	<i>Plodia interpunctella</i> (Hübner)	20.15 \pm 0.92
Sub-family	Mediterranean flour moth	<i>Ephestia kuehniella</i> (Zeller)	24.56 \pm 0.96
Phycitinae	Almond moth	<i>Ephestia cautella</i> (Walker)	18.66 \pm 1.31
	Navel orangeworm	<i>Amyelois transtitella</i> (Walker)	55.00 \pm 1.90
Pyralidae	Rice moth	<i>Corcyra cephalonica</i> (Stainton)	48.89 \pm 1.66
Sub-family	Greater wax moth	<i>Galleria mellonella</i> (Linnaeus)	
Galleriinae	i. Laboratory reared		262.78 \pm 15.17
	ii. Commercial store		264.90 \pm 12.85
Noctuidae	Tobacco budworm	<i>Heliothis virescens</i> (Fabricius)	120.70 \pm 8.79
	Corn earworm	<i>Helicoverpa zea</i> (Boddie)	172.68 \pm 75.42
	Beet armyworm	<i>Spodoptera exigua</i> (Hübner)	67.43 \pm 3.43
Gelechiidae	Angoumois grain moth	<i>Sitotroga cerealella</i> (Olivier)	4.05 \pm 0.28
Tineidae	Webbing clothes moth	<i>Tineola bisselliella</i> (Hummel)	6.55 \pm 0.69

Figure legends

Fig. 1. Proportion of hosts paralyzed and oviposited on *B. hebetor* females on twelve different lepidopteran host species. AM = almond moth, *Ephestia cautella*, IMM = Indianmeal moth, *Plodia interpunctella*, MFM = Mediterranean flour moth, *E. kuehniella*, GWM-C = Greater wax moth-commercial source, *Galleria mellonella*, GWM-L = Greater wax moth-laboratory reared, *G. mellonella*, NOW = navel orangeworm, *Amyelois transitella*, RM = rice moth, *Corcyra cephalonica*, BAW = beet armyworm, *Spodoptera exigua*, CEW = corn earworm, *Helocoverpa zea*, TBW = tobacco budworm, *Heliothis virescens*, AGM = Angoumois grain moth, *Sitotroga cerealella*, and WCM = webbing clothes moth, *Tineola bisselliella*. Bars of the same type followed by same lowercase (oviposition) or uppercase (paralysis) letters are not significantly different at $\alpha \geq 0.05$ using Duncan's Multiple Range Test (DMRT).

Fig. 2. Daily mean number of eggs laid by *B. hebetor* females during five-days oviposition periods with seven different pyralid host species. AM = almond moth, *Ephestia cautella*, IMM = Indianmeal moth, *Plodia interpunctella*, MFM = Mediterranean flour moth, *E. kuehniella*, GWM-C = Greater wax moth-commercial source, *Galleria mellonella*, GWM-L = Greater wax moth-laboratory reared, *G. mellonella*, NOW = navel orangeworm, *Amyelois transitella*, RM = rice moth, *Corcyra cephalonica*.

Fig. 3. Daily mean number of eggs laid by *B. hebetor* females during five-days oviposition periods with seven different non-pyralid host species. BAW = beet armyworm, *Spodoptera exigua*, CEW = corn earworm, *Helocoverpa zea*, TBW = tobacco budworm, *Heliothis virescens*, AGM = Angoumois grain moth, *Sitotroga cerealella*, and WCM = webbing clothes moth, *Tineola bisselliella*.

Fig. 4. Mean total oviposition of *B. hebetor* females during five-days oviposition periods with twelve different lepidopteran host species. AM = almond moth, *Ephestia cautella*, IMM = Indianmeal moth, *Plodia interpunctella*, MFM = Mediterranean flour moth, *E. kuehniella*, GWM-C = Greater wax moth-commercial source, *Galleria mellonella*, GWM-L = Greater wax moth-laboratory reared, *G. mellonella*, NOW = navel orangeworm, *Amyelois transitella*, RM = rice moth, *Corcyra cephalonica*, BAW = beet armyworm, *Spodoptera exigua*, CEW = corn earworm, *Helocoverpa zea*, TBW = tobacco budworm, *Heliothis virescens*, AGM = Angoumois grain moth, *Sitotroga cerealella*, and WCM = webbing clothes moth, *Tineola bisselliella*. Bars followed by same letters are not significantly different at $\alpha \geq 0.05$ using Duncan's Multiple Range Test (DMRT).

Fig. 5. Mean total adult *B. hebetor* produced per female resulting from eggs laid during five-days oviposition periods with twelve different lepidopteran host species. AM = almond moth, *Ephestia cautella*, IMM = Indianmeal moth, *Plodia interpunctella*, MFM = Mediterranean flour moth, *E. kuehniella*, GWM-C = Greater wax moth-commercial source, *Galleria mellonella*, GWM-L = Greater

wax moth-laboratory reared, *G. mellonella*, NOW = navel orangeworm, *Amyelois transitella*, RM = rice moth, *Corcyra cephalonica*, BAW = beet armyworm, *Spodoptera exigua*, CEW = corn earworm, *Helocoverpa zea*, TBW = tobacco budworm, *Heliothis virescens*, AGM = Angoumois grain moth, *Sitotroga cerealella*, and WCM = webbing clothes moth, *Tineola bisselliella*.

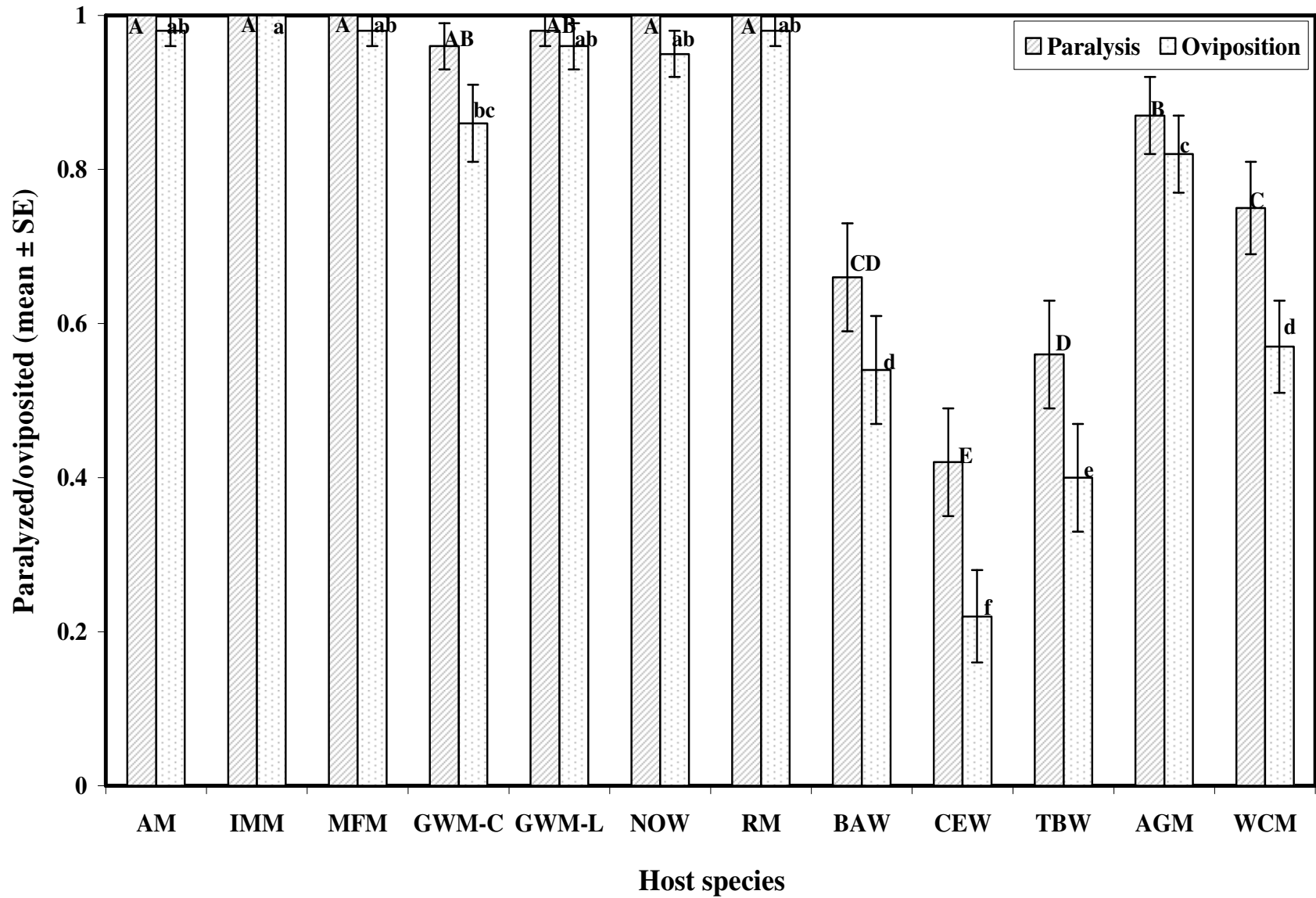
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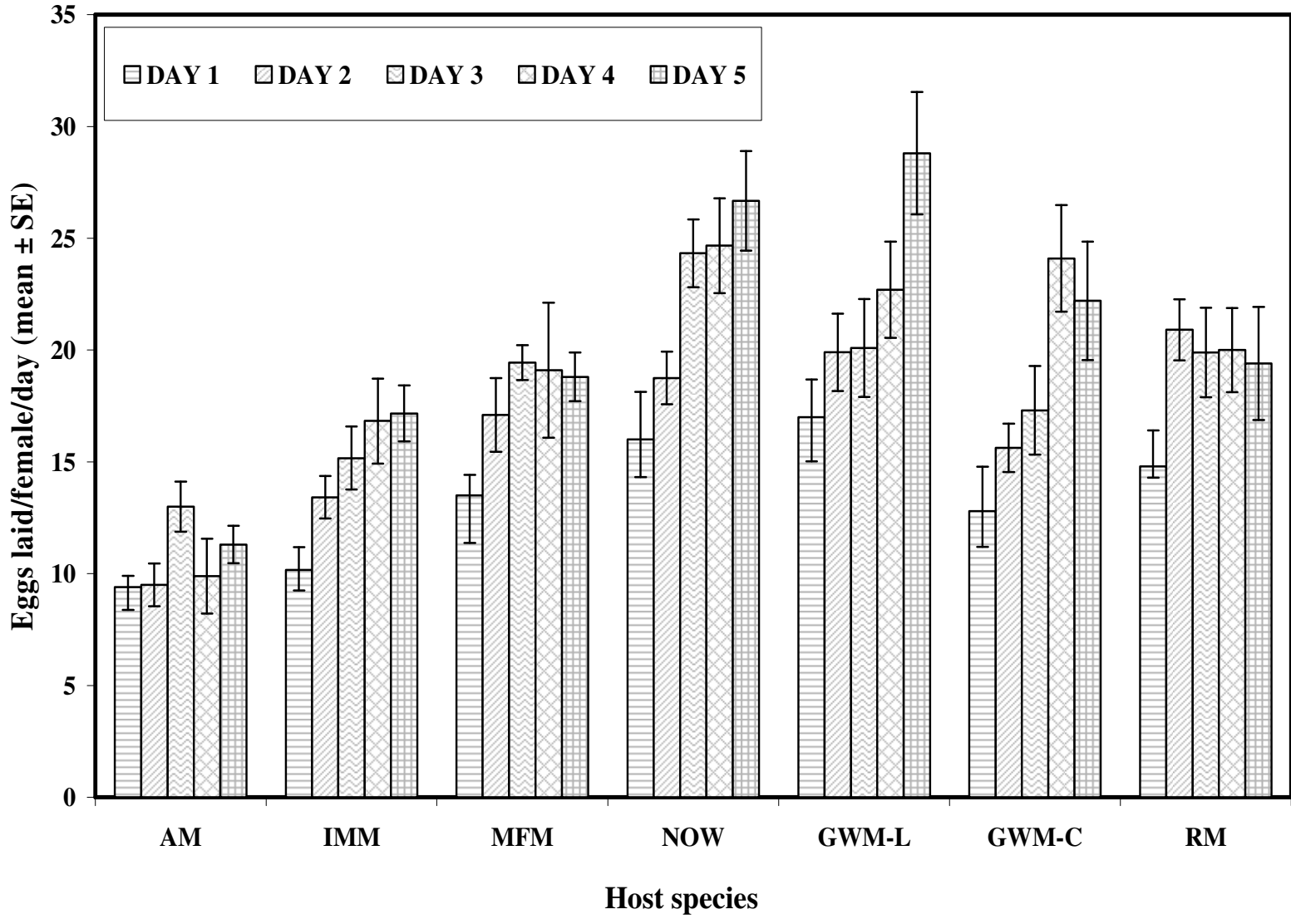
Fig. 6. Egg-to-adult survivorship of *B. hebetor* that developed on twelve different lepidopteran host species. AM = almond moth, *Ephestia cautella*, IMM = Indianmeal moth, *Plodia interpunctella*, MFM = Mediterranean flour moth, *E. kuehniella*, GWM-C = Greater wax moth-commercial store, *Galleria mellonella*, GWM-L = Greater wax moth-laboratory reared, *G. mellonella*, NOW = navel orangeworm, *Amyelois transitella*, RM = rice moth, *Corcyra cephalonica*, BAW = beet armyworm, *Spodoptera exigua*, CEW = corn earworm, *Helocoverpa zea*, TBW = tobacco budworm, *Heliothis virescens*, AGM = Angoumois grain moth, *Sitotroga cerealella*, and WCM = webbing clothes moth, *Tineola bisselliella*. Bars followed by same letters are not significantly different at $\alpha \geq 0.05$ using Duncan's Multiple Range Test (DMRT).

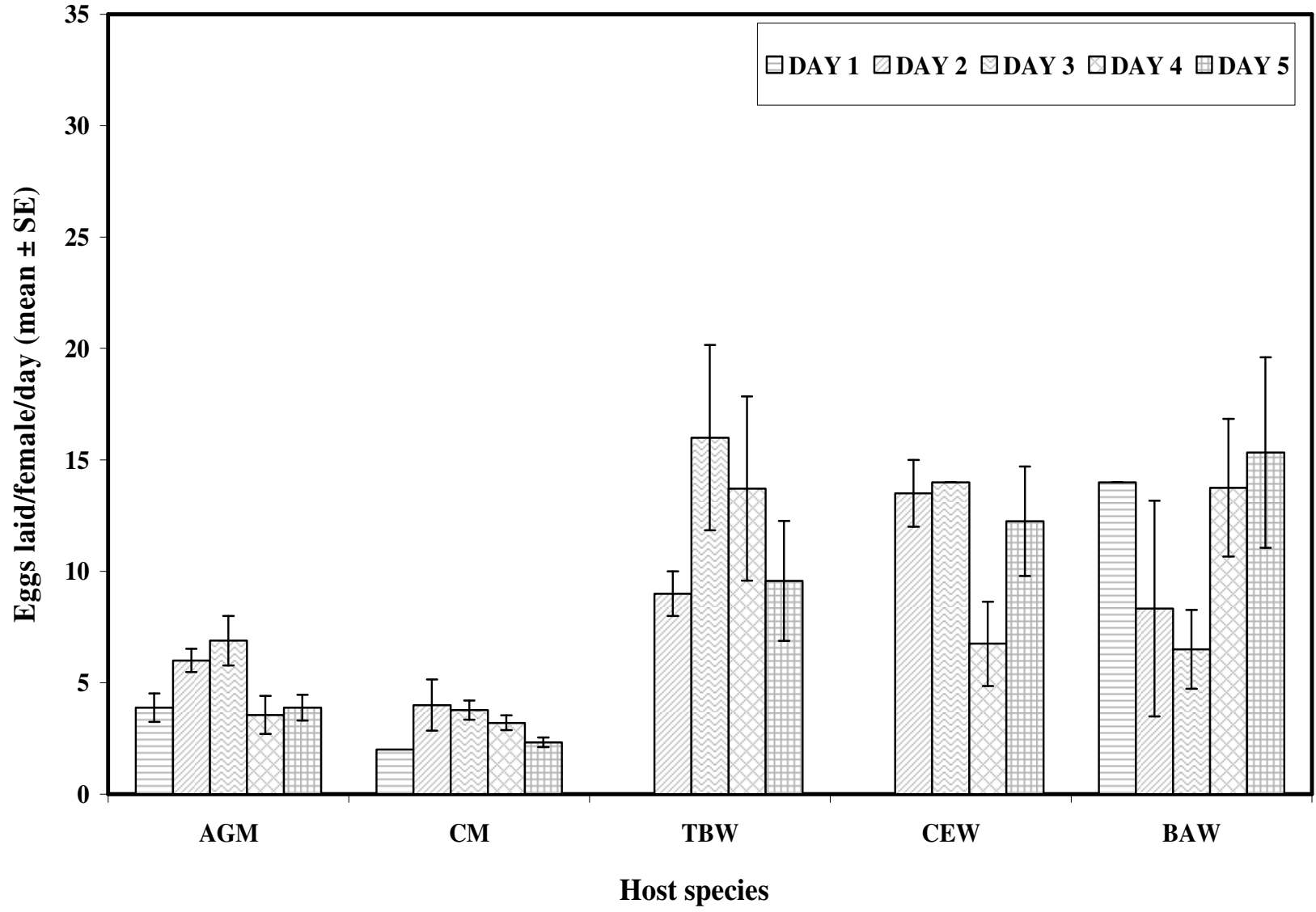
Fig. 7. Egg-to-adult developmental time, in days, of *B. hebetor* on eight different lepidopteran host species. AM = almond moth, *Ephestia cautella*, IMM = Indianmeal moth, *Plodia interpunctella*, MFM = Mediterranean flour moth, *E.*

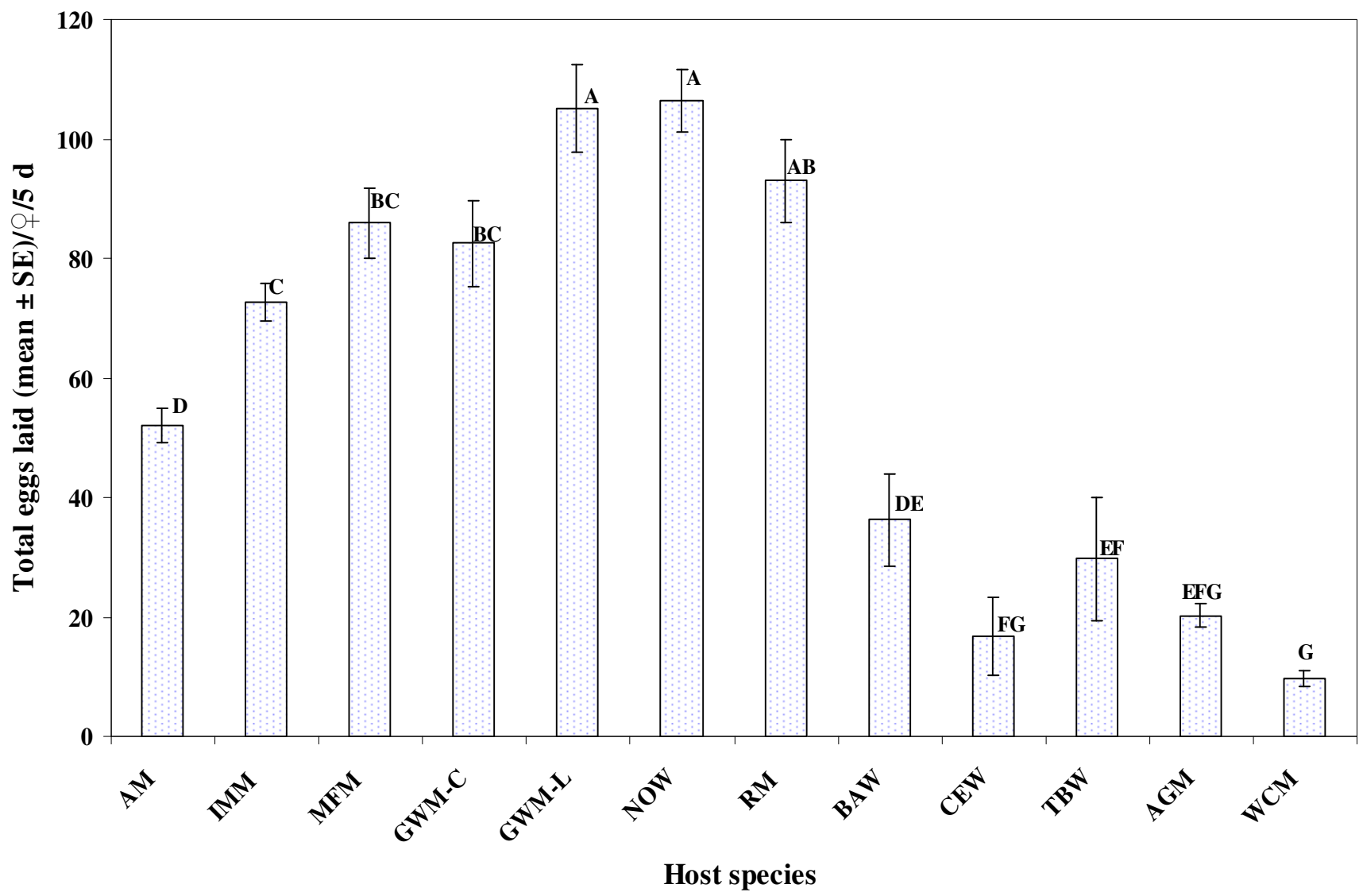
kuehniella, *Galleria mellonella*, GWM = Greater wax moth *Galleria mellonella*, NOW = navel orangeworm, *Amyelois transitella*, RM = rice moth, *Corcyra cephalonica*, BAW = beet armyworm, *Spodoptera exigua*, and AGM = Angoumois grain moth, *Sitotroga cerealella*. Bars followed by same letters are not significantly different at $\alpha \geq 0.05$ using Duncan's Multiple Range Test (DMRT).

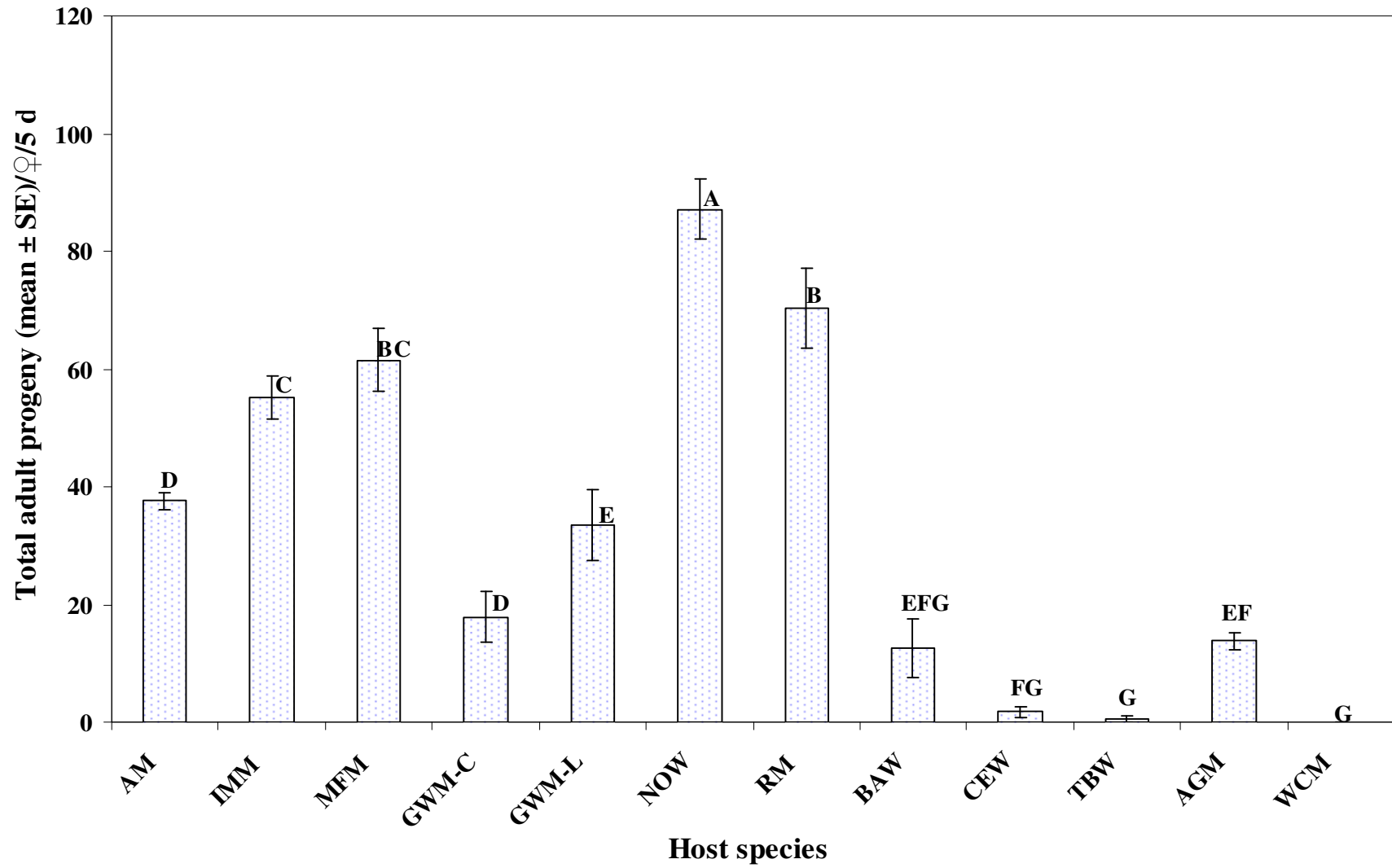
Fig. 8. Parasitoid secondary sex ratio (% females) of *B. hebetor* adult progeny produced on eleven different lepidopteran host species. AM = almond moth, *Ephestia cautella*, IMM = Indianmeal moth, *Plodia interpunctella*, MFM = Mediterranean flour moth, *E. kuehniella*, GWM-C = Greater wax moth-commercial source, *Galleria mellonella*, GWM-L = Greater wax moth-laboratory reared, *G. mellonella*, NOW = navel orangeworm, *Amyelois transitella*, RM = rice moth, *Corcyra cephalonica*, BAW = beet armyworm, *Spodoptera exigua*, CEW = corn earworm, *Helocoverpa zea*, TBW = tobacco budworm, *Heliothis virescens*, and AGM = Angoumois grain moth, *Sitotroga cerealella*. Bars followed by same letters are no significantly different at $\alpha \geq 0.05$ using Duncan's Multiple Range Test (DMRT).

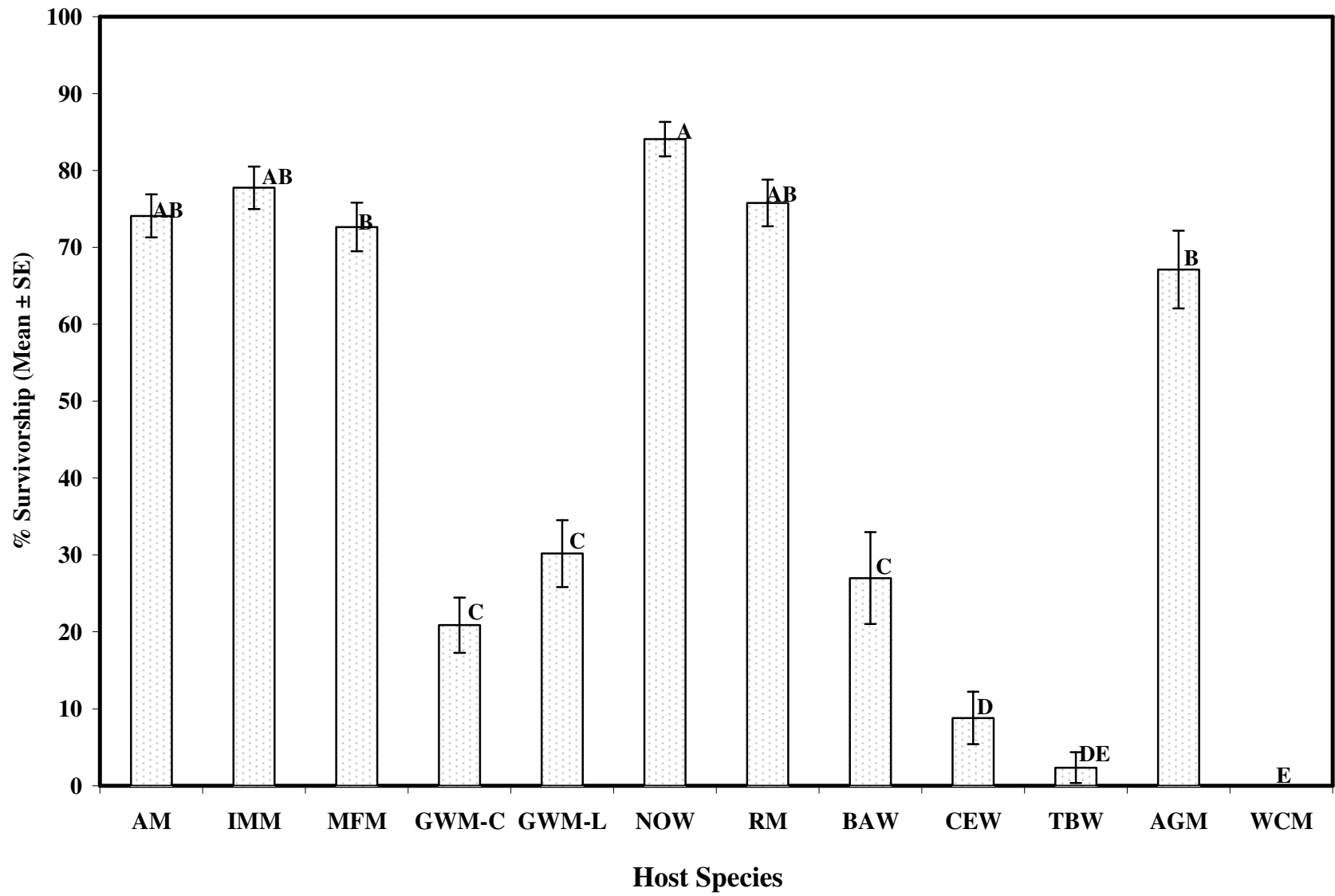


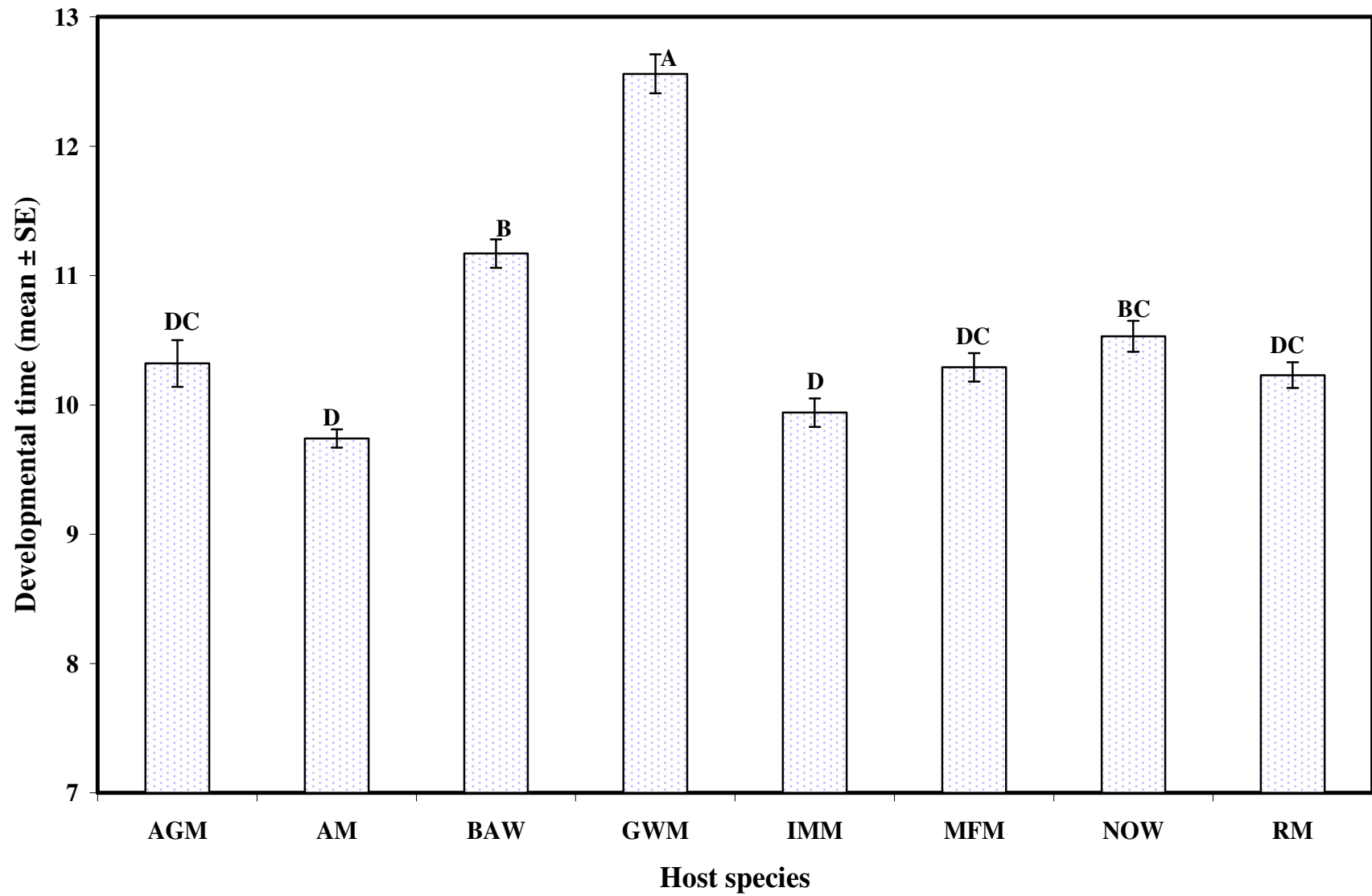


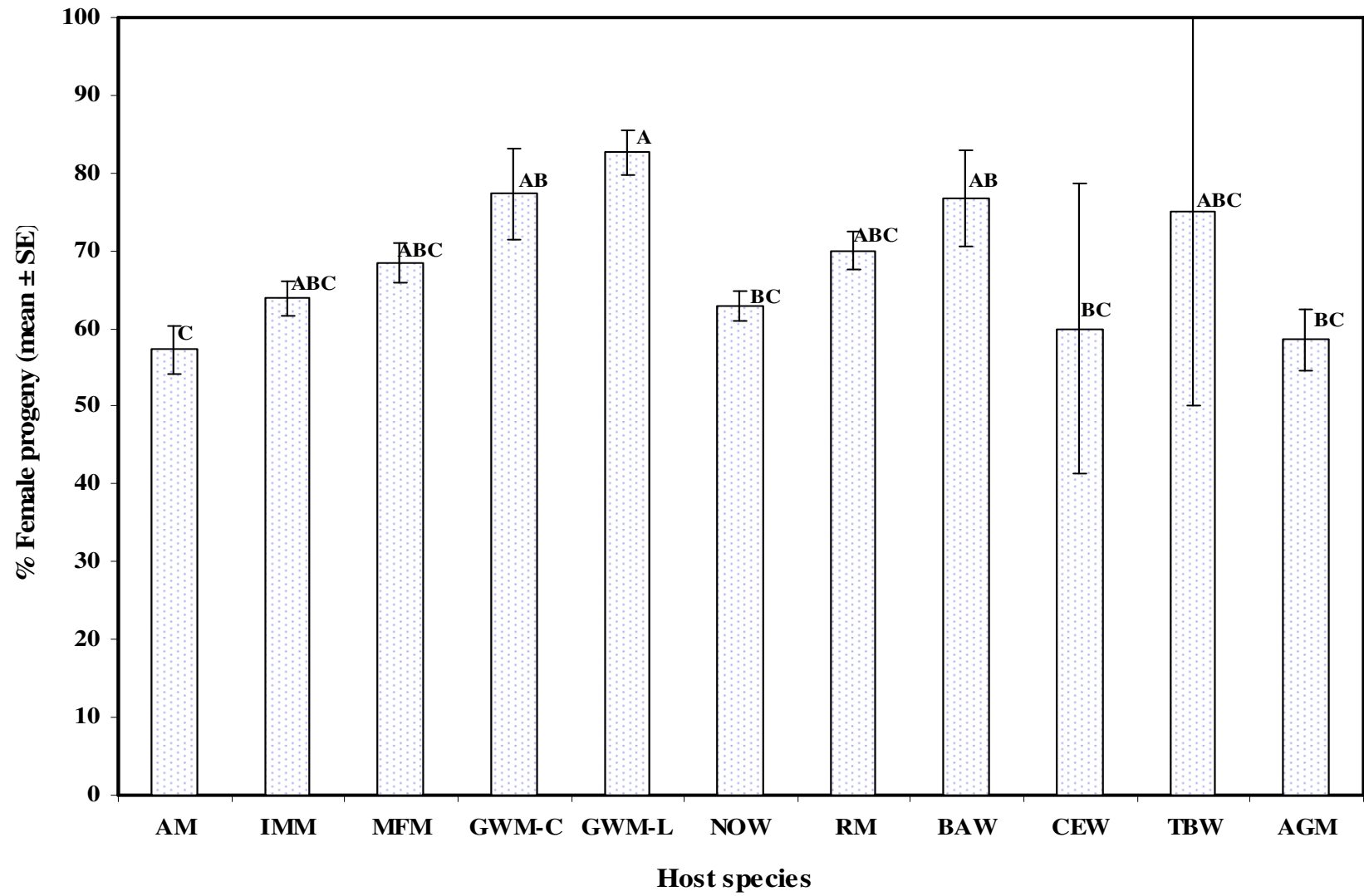












CHAPTER III

OVIPOSITION AND REPRODUCTIVE PERFORMANCE OF *BRACON* *HEBETOR* SAY (HYMENOPTERA: BRACONIDAE) ON SIX DIFFERENT PYRALID HOST SPECIES.

Abstract

Bracon hebetor Say is a gregarious, ecto-parasitoid that attack larvae of several species of Lepidoptera, mainly pyralid moths infesting stored products. *Bracon hebetor* females first paralyze their host larvae by stinging and injecting venom and then laying eggs on or near the surface of paralyzed host larvae. The paralyzed host larvae are then used as a food source for the developing wasp and also for the adult females. In this study, the potential of this parasitoid for the management of stored product moth pests was explored in a series of laboratory experiments using six different pyralid host species: Indianmeal moth, *Plodia interpunctella* (Hübner), Mediterranean flour moth, *Ephesthia kuehniella* (Zeller), almond moth, *E. cautella* (Walker), rice moth, *Corcyra cephalonica* (Stainton), navel orangeworm, *Amyelois transitella* (Walker), and greater wax moth, *Galleria mellonella* (Linnaeus). Experiments were conducted using Petri dishes (100 × 15 mm) as experimental arenas. Two-day old *B. hebetor* females were introduced singly into experimental arenas and given a single host larva every day throughout their life time. The numbers of hosts paralyzed and parasitized, numbers of eggs laid each day on each host, egg-to-adult survivorship, and progeny sex ratio were used as parameters for assessing host suitability. Paralysis of hosts by *B. hebetor* females was significantly affected by host species. *Bracon hebetor* paralyzed more than 95% of the preferred host larvae that were offered and also used about 90% of those for oviposition. Daily fecundity was highest on *G. mellonella* (22.09 ± 0.42) and *C. cephalonica* (21.64 ± 0.35) and lowest on *E. cautella* (13.39 ± 0.24). The egg-to-adult survivorship and progeny sex ratio were also significantly affected by the host species. The highest percentage of parasitoid survival was on *A. transitella* (75.69 ± 1.99) and *C.*

cephalonica (75.42 ± 2.47) and lowest on *G. mellonella* (49.71 ± 1.74). Although, *B. hebetor* can paralyze and lay eggs on several pyralid species, it can not necessarily develop and reproduce optimally on all host species that it can paralyze and parasitize. The application of these results for biological control of stored product moth pests is discussed.

Key words: stored-product pest, biological control, parasitoid, reproduction, host quality.

Introduction

The use of biological control agents in food storage situations is not a new concept, and it has long been neglected because of the potential contamination of food products by introducing natural enemies and the tolerance limit for insect damage (Arbogast 1983). Recently attention has been focused on non-chemical methods of stored-product protection, including biological control of stored-product pests, due to negative impacts of pesticides, such as restrictions on the use of certain pesticides and the evolution of insecticide resistance in pest populations (Arbogast 1984, Hagstrum et al. 1999, Phillips et al. 2000, United Nations Environment Program 2006). The use of beneficial insects in stored-product systems received government approval as a pest mitigation practice in the United States, and is exempted from a requirement for minimum tolerance levels (EPA 1992). All genera of parasitoids and predators that are known to attack stored product insects are exempted for their use and occurrence in stored raw commodities and processed food (Brower et al. 1996). Thus, biological control can be a safe and viable method of stored-product protection.

Stored-product pyralid moths (Lepidoptera: Pyralidae; Phycitinae) are among the most destructive pests of stored-food commodities because their larvae infest the value-added, finish food products that are packaged and ready for retail use. The Indianmeal moth, *Plodia interpunctella* (Hübner), Mediterranean flour moth, *Ephestia kuehniella* (Zeller), almond moth, *E. cautella* (Walker), navel orangeworm, *Amyelois transitella* (Walker), tobacco moth, *E. elutella* (Hübner) and the raisin moth, *E. figuliella* (Gregson) are among a cosmopolitan group of stored-product pests in the sub-family Phycitinae, including the rice moth, *Corcyra cephalonica* (Stainton) and the greater wax moth,

Galleria mellonella (Linnaeus) in the sub-family Galleriinae (Lepidoptera: Pyralidae) (Simmons and Nelson 1975, Chauvin and Chauvin 1985, Vick et al. 1987, Cox and Bell 1991, Johnson et al. 2000, 2002).

Bracon hebetor Say (Hymenoptera: Braconidae) is a cosmopolitan parasitic wasp commonly found in association with several species of Lepidoptera, mainly, pyralid moths infesting stored products (Krommbein et al. 1979). *B. hebetor* is considered one of the potential biological control agents stored product pests because of its ability to regulate populations of stored product moths (Simmons and Nelson 1975, Hagstrum and Smittle 1977, 1978, Press and Flaherty 1981, Brower et al. 1996). *B. hebetor* females first paralyze their host larva by stinging and then laying variable numbers of eggs on or near the surface of paralyzed hosts (Antolin et al. 1995). The paralyzed host larvae are then used as food sources for both developing wasps and also adult females. Normally the female *B. hebetor* paralyzes several larvae and returns afterwards to find and oviposit on some immobile larvae (Ullyett 1945). *B. hebetor* females paralyze many more hosts than needed for oviposition, and paralysis is always fatal, though life may continue for nearly a month if not parasitized by wasp larvae. Under the natural conditions only a small proportion of the parasitized larvae actually used for oviposition (Doten 1911, Richards and Thomason 1932).

Host quality strongly influences the main components of parasitoid fitness, such as fecundity, developmental time, survivorship, secondary sex ratio, and size of the emerging adult wasps (Charnov et al. 1982, Vinson and Iwantsch 1980, Godfray 1994). Successful identification of host quality, and adjusting the clutch size accordingly, has important consequences for the fitness of a gregarious parasitoid (Godfray 1987).

Several studies have shown that the clutch sizes of gregarious parasitoids are correlated with the size of the hosts at oviposition (Hardy et al. 1992, Zaviezo and Mills 2000). Therefore, attacking large hosts and provisioning the host with optimum clutch size maximizes the larval performance and reproduction, and is considered adaptive in terms of parasitoid fitness. In contrast, recent work by Harvey (2000) and Harvey et al. (2004) has shown that host size at the time of oviposition may have little influence on the fitness functions in some of the koinobiont species. However, little information is available on whether such a situation occurs in *B. hebetor*, a gregarious idiobiont ectoparasitoid of lepidopterous moth pests of stored food products.

The experiments presented here examine the effects of six pyralid host species, with considerable variation in larval body size, on several reproductive parameters of *B. hebetor*. Basic and applied aspects of parasitoid biology are discussed relative to optimization of efficacy for the biological control and management of stored-product moths.

Materials and Methods

Parasitoid origin and rearing

The *B. hebetor* used in this study originated from feral adults collected from grain bins at the Stored Products Research and Education Center (SPREC) at Oklahoma State University in Stillwater, Oklahoma on November 2003 that were associated with larvae of *P. interpunctella* infesting wheat grains. A laboratory culture derived from these *B. hebetor* was maintained on late-instar larvae of *P. interpunctella* in the laboratory at 29 °C, 60-70% RH, and a photoperiod of 14:10 h (L: D). Late-instar, wandering stage, *P. interpunctella* were obtained from our laboratory culture that was reared on a standardized diet of yellow corn meal, egg crumbles, chick starter, and glycerol at a volumetric ratio of 4:2:2:1, respectively, at temperature of 28 °C, relative humidity of 60-70 %, and a photoperiod of 16:8 (L:D) h.

Host species

The hosts larvae used in these experiments were four species of phycitine pyralids and two species of non-phycitine pyralids (Table 1). The larvae of the four phycitine species and *A. transitella* were obtained from our long-term laboratory cultures or those of colleagues and reared on the same diet used for of *P. interpunctella* at similar environmental conditions. Larvae of *G. mellonella* were obtained from a local pet store supplied through Timberline Live Pet Foods, Inc. Marion, IL and we maintained an ongoing culture in our laboratory originating from that the same supplier. *G. mellonella* was reared on a mixture of wheat flour, honey, glycerol, bee wax, and brewer's yeast at a weight basis ratio of 0.44:0.23:0.18:0.04:0.11, respectively. Larvae of *A. transitella* were obtained from the USDA-ARS Commodity Protection and Quality Laboratory at Parlier,

CA and we maintained its culture on a mixture of 11.355 liter of flakey red food bran, 900 ml honey, 800 ml de-ionized water, 100 gm brewer's yeast, and 10 ml Vanderzants vitamins solution (1%). Larvae of *C. cephalonica* were obtained from Insects Limited Inc., Westfield, IN and we maintained its culture on a mixture of wheat bran, wheat germ, rolled oats, glycerin, and brewer's yeast at a ratio of 1:1:1:1:0.5, respectively. All the cultures were maintained under similar growth chamber conditions as used for rearing of *P. interpunctella*.

Experiments

Experiments were conducted in the laboratory in a no-choice design using disposable plastic Petri dishes (100 × 15 mm) as experimental arenas with a single full-grown wandering stage larva of each host species. According to Hagstrum and Smittle (1977), *B. hebetor* females attack wandering larvae at a rate 10-fold more than they attack young larvae. A representative sample of full-grown larvae of each host species were randomly taken from the rearing jars and larval fresh weights were measured (n=12) by placing the individual larvae on a Denver instruments (Denver, CO, USA) M-220 electronic balance (± 0.01 mg) (Table 1) before the experiment,. *B. hebetor* females within 24 hours of emergence were kept with males for another 24 h in 500 ml glass jar and were provided honey and water assuming ample opportunity for mating was provided because 80% of virgin *B. hebetor* females mate within the first 15 min of being in the presence of male as reported by Ode et al. (1995). After 24 h, *B. hebetor* females were isolated from the males and introduced individually into experimental arenas containing a single full grown host larva. After 24 h, females were carefully moved to a new experimental arenas containing a fresh larva of a given host species. This procedure was

repeated until parasitoids died. There were 12 replicates for each host species. Experiment were conducted in growth chamber at a temperature of 29 °C, relative humidity of 60-70 %, and a photoperiod of 14:10 (L: D).h. Observations were taken consistently on 24 h period for each female parasitoid until her death, and included number of hosts paralyzed, parasitized (oviposited upon), number of eggs laid on each host, development time, longevity of female parents, life time fecundity, egg-to-adult survivorship, and secondary sex ratio (proportion of females in surviving adult progeny). Development time was the duration from the egg stage within six hours of oviposition on individual host larvae by single female *B. hebetor* until emergence of adult parasitoids. Adult emergence was measured twice daily from the beginning of adult parasitoid emergence until emergence has been stopped (up to-three weeks).

Statistical analysis

The influence of host species on the paralysis and oviposition were determined by one way analysis of variance (ANOVA) (PROC MIXED procedure, SAS institute 2005). Data on the development time of both sexes were pooled together, as no statistically significant difference between male and female development time was found, and subjected to one-way ANOVA procedures. Oviposition period, post-oviposition period, longevity of females, life time fecundity, total adult progeny, and egg-to-adult survivorship were determined by one-way ANOVA (PROC MIXED procedure, SAS Institute 2004). The differences in age-specific daily oviposition, adult progeny, and secondary sex ratio (proportion of females) were determined by two-way repeated measure ANOVA (PROC MIXED) assuming an autoregressive covariance structure (Littell et al. 1996). The age of *B. hebetor* females by host species interaction was

analyzed within LSMEANS statement and a SLICE option was used to test the overall simple effects of the factor in question.

Results

All six species of pyralid hosts exposed to *B. hebetor* females were paralyzed and used for oviposition (parasitization) (Fig. 1). However, proportions of *C. cephalonica* and *G. mellonella* larvae (0.94 and 0.96, respectively) paralyzed by *B. hebetor* females, though relatively high, were significantly lower ($F = 6.94$; $df = 5, 3324$; $P < 0.0001$) than those for *A. transitella*, *E. kuehniella*, *E. cautella* or *P. interpunctella* (Fig. 1). In contrast, proportions of parasitism were significantly higher ($F = 6.94$; $df = 5, 3323$; $P < 0.0001$) on *G. mellonella* and *P. interpunctella* (0.93 ± 0.01 and 0.91 ± 0.01 from the total paralyzed larva 473 and 456, respectively) than that of *E. kuehniella*, *A. transitella*, or *E. cautella* (Fig. 1).

The egg-to-adult developmental duration for *B. hebetor* progeny varied significantly with host species (Table 2). The shortest total egg-to-adult developmental times were observed on *E. cautella* and *P. interpunctella* (9.75 ± 0.25 and 9.95 ± 0.21 d, respectively) and longest on *G. mellonella* (12.63 ± 0.28 d) (Table 2). The total oviposition period for *B. hebetor* females also varied significantly with host species (Table 2). The longest oviposition period was observed on *E. cautella* and *E. kuehniella*, at 49.25 ± 3.07 and 48.75 ± 3.19 d, respectively, and the shortest was on *C. cephalonica*, at 33.67 ± 2.84 d (Table 2). Similarly, post-oviposition period for *B. hebetor* females was observed significantly longer on *E. kuehniella* (11.42 ± 3.07 d) than that of all other host species (2.50 ± 0.40 to 6.08 ± 1.35 d) (Table 2). Longevity of *B. hebetor* females was significantly higher on *E. kuehniella* and *E. cautella* larvae (60.17 ± 4.22 and 55.33 ± 3.54 d, respectively) than compared to that on *C. cephalonica*, *P. interpunctella* and *G. mellonella* (37.92 ± 3.53 , 38.00 ± 2.77 , and 39.42 ± 4.81 d, respectively) (Table 2).

Mean lifetime fecundities of *B. hebetor* females were significantly higher on *A. transitella*, *G. mellonella*, and *E. kuehniella* larvae (810.08 ± 46.03 , 808.00 ± 96.46 , and 800.00 ± 65.79 eggs/female, respectively) than when parasitizing *P. interpunctella* larvae (538.3 eggs/female) (Table 2). A similar trend was observed in terms of the mean number of adult progeny produced from larvae of each hosts species, except for the *G. mellonella* (Table 2). The mean number of adult progeny produced by *B. hebetor* females in their lifetimes on *A. transiella*, *E. kuehniella* and *C. cephalonica* larvae (616.92 ± 42.56 , 568.17 ± 43.21 and 551.83 ± 60.58 adults/female, respectively) were significantly higher than when utilizing *G. mellonella*, *P. interpunctella* and *E. cautella* larvae (369.25 ± 39.15 , 372.58 ± 35.56 , and 426.50 ± 31.47 adults/female, respectively) (Table 2).

Egg-to-adult survivorship of *B. hebetor* progeny was significantly influenced by the host species. The egg-to-adult survivorship of *B. hebetor* progeny was highest on *A. transitella* (75.69 ± 1.99 %) followed by *C. cephalonica* (75.42 ± 2.47 %) and *E. kuehniella* (71.69 ± 1.80 %) and lowest on *G. mellonella* larvae (49.71 ± 4.84 %) (Table 2).

Age-specific daily fecundity was significantly affected by the host species ($F = 13.33$; $df = 5, 55$; $P < 0.0001$), age of female wasp ($F = 47.02$; $df = 8, 2805$; $P < 0.0001$) and also by the interaction between host species and age of the female wasps ($F = 9.27$; $df = 35, 2805$; $P < 0.0001$). Overall, age-specific daily fecundity was higher for the first five weeks of oviposition and gradually declined until reproduction ceased (Fig. 2). The daily fecundity was highest in *G. mellonella* in week two (27.34 ± 0.71 eggs) followed by

C. cephalonica in week five (24.71 ± 0.85 eggs) and *A. transitella* in week one (22.90 ± 0.76 eggs) (Fig. 2).

The mean number of adult progeny produced per day from eggs laid in a given week on a given host was significantly affected by the host species ($F = 14.29$; $df = 5, 55$; $P < 0.0001$), age of female wasp ($F = 23.31$; $df = 8, 2805$; $P < 0.0001$) and also by the interaction between host species and age of the female wasps ($F = 9.97$; $df = 35, 2805$; $P < 0.0001$). The highest number of *B. hebetor* adults was produced from *C. cephalonica* (19.49 ± 0.91 adults) in week four followed by *A. transiella* (18.28 ± 0.61 adults) in week one and *G. mellonella* (27.34 ± 0.98 eggs) in week two (Fig. 3).

The sex ratio (proportion of the female progeny) of emerging adults was not significantly affected by the host species ($F = 1.61$; $df = 5, 55$; $P = 0.1725$). However, it was significantly affected by age of the female wasps ($F = 145.01$; $df = 9, 2632$; $P < 0.0001$) and interaction between host species and age of female wasps ($F = 4.81$; $df = 34, 2632$; $P < 0.0001$). The sex ratio of emerging adults was significantly female-biased during the first three weeks of oviposition then remained approximately 0.5 during the week four, and the switched to male-biased progeny from the oviposition resulting from >4- week- old females (Fig. 4). However, in the case of *G. mellonella*, a female bias progenies were observed only during the first two weeks (0.73 ± 0.03 and 0.71 ± 0.03 for week one and two, respectively) then it decline sharply to male bias progeny (Fig. 4).

Discussion

Bracon hebetor females first paralyze their hosts by injecting venom through the host cuticle with the ovipositor and then laying a variable number of eggs on or near the surface of paralyzed host larvae (Hagstrom and Smittle 1978). In the current study, *B. hebetor* females were able to paralyze and subsequently, oviposit on or parasitize all the host species that were offered to them. Although *B. hebetor* females paralyzed > 90% of all host species, their reproductive performance was significantly higher with phycitine species, which were *P. interpunctella*, *E. kuehniella*, *E. cautella*, and *A. transtitella*, as compared to non-phycitine species, *C. cephalonica* and *G. mellonella* (Fig. 1). In contrast to paralysis, for the case of the proportion of hosts parasitized, *B. hebetor* females performed better with non-phycitine species as compared to phycitine species, except in *P. interpunctella* (Fig. 1). The possible explanation for this could be difference in size of the host species because full-grown larvae of non-phycitine species were larger than full-grown larvae of phycitine species (Table 1) and thus may have presented a greater stimulus for oviposition. A similar explanation was given by Ghimire and Phillips (2007) for the solitary ectoparasitoid *Anisopteromalus calandrae* Howard parasitizing cowpea weevil. Whereas, better performance (more adult progeny, higher fecundity, more longevity, etc.) occurred with *P. interpunctella* because the wasps used were from a long-term colony reared on *P. interpunctella*, and presumably adapted to *P. interpunctella*, but other hosts were actually “better”.

The findings of the current study demonstrated that host species can have a significant effect on several aspects of a parasitoid’s reproductive parameters, such as

developmental time, oviposition period, lifetime fecundity, longevity, progeny production, and egg-to-adult survivorship (Table 1). The duration of the egg-to-adult development period was longest on *G. mellonella* (12.6 d), and shortest on *E. cautella* (9.7 d) and *P. interpunctella* (9.9 d). This indicates that *B. hebetor* immatures respond differently to different host resources, both qualitatively and quantitatively, by either developing slowly and utilizing host resources with maximum efficiency or by developing quickly and utilizing host resources with lower efficiency (Godfray, 1994). The duration of the oviposition period was longest on *E. kuehniella* (48.7 d) and *E. cautella* (49.2 d) and shortest on *C. cephalonica* (33.7 d) and *P. interpunctella* (34.7 d). A similar pattern was observed for the post oviposition period and longevity of parent females. The oviposition period found here for *B. hebetor* females reared on *P. interpunctella* is similar to that reported earlier by Ode et al. (1996).

Adult female longevity that is reported here when hosts were *E. kuehniella* (60.2 d) and *G. mellonella* (39.4 d) is > 3- and 2-fold longer, respectively, than those reported by Amir-Maafi and Chi (2006). This variation could be due to the fact that those authors used a different strain of *B. hebetor* that was associated with *Heliothis* spp. infesting tomato fruits and also there were differences in experimental procedures. Mean lifetime fecundity was higher (≥ 800 eggs) on larger host larvae (*G. mellonella* and *A. transitella*) as compared smaller host larvae (538 egg) such as *P. interpunctella* (Table 2). Furthermore, average daily fecundity was much higher on *G. mellonella* (>27 eggs) as compared to 17 eggs on *P. interpunctella* (Fig. 2). This difference may be explained by the possibility that *B. hebetor* females prefer to attack large hosts and lay more eggs on them, because large host should have more resources available to support their progeny.

Increased oviposition on larger hosts could be considered adaptive in terms of parasitoid fitness, as proposed earlier by Charnov (1982) and Godfray (1994), if the host quality is not deleteriously affected by higher parasitoid oviposition rates. However, adaptive increased oviposition on large hosts is not necessarily apparent in our study because egg-to-adult survival of *B. hebetor* progeny was lowest on *G. mellonella* (<50%), though this was the largest host (263 mg) we used in this study and females experienced the greatest lifetime fecundity with them (Table 1). On average, a higher proportion of parasitoids emerged when reared on *P. interpunctella*, *E. kuehniella*, *C. cephalonica* and *A. transitella* than when reared on *G. mellonella* (Table 2). However, highest life time fecundity and highest number of adult progeny was achieved when *B. hebetor* reared on *A. transitella*, which was the second largest host studied (55 mg). Results on parasitoid success and host size indicate that other qualitative factors of hosts are more important than size of the host. These results are similar to those of Milonas (2005), who found more parasitoid survival when *B. hebetor* reared on *P. interpunctella* compared to two other tortricid moths, *Adoxophyes orana*, and *Lobesia botrana*, which were larger.

Survival of *B. hebetor* progeny was significantly affected by the host species. Although larvae of *G. mellonella* were much larger than other hosts, parasitoid's larval mortality was much higher in this species. We observed that *G. mellonella* larvae often had a physiological response to the attack of *B. hebetor* by developing a melanized ring at the site of feeding by the *B. hebetor* larvae. Moreover, in a few cases that the body of *G. mellonella* larvae were found turned darkbrown in color and then decomposed soon after being stung by *B. hebetor* females. Parasitoid larvae could not survive on those blackened and decomposing hosts, whereas larvae of other species appeared healthy and

fresh-looking for several days after paralysis and oviposition. Similar, but more prominent observations were made by Beard (1952) with *G. melonella* larvae.

Sex ratio, the proportion of adult females produced by *B. hebetor*, was not influenced by the host species but it was clearly influenced by age of the female wasps. Wasps produced slightly female-biased progeny on all hosts resulting from oviposition by ≤ 3 -week-old females and gradually switch to male bias progeny oviposition resulted after 4-week-old females. However, in the case of *G. mellonella*, female-biased progeny were produced only by ≤ 2 -week-old females and then abruptly turned to male bias. In this case, daily fecundity was heavily peaked on week two and gradually started to decline. This shift in sex ratio could be explained by the fact that after oviposition of several clutches of eggs during the first few weeks the *B. hebetor* females probably became depleted of their sperm reserves from the initial mating, and thus could produce only males from unfertilized eggs. Ode et al. (1997 and 1998) observed a similar phenomenon in sex ratio shift with age beyond the last insemination. Furthermore, those authors demonstrated that *B. hebetor* females generally mate once in their lifetimes, and when mated females became sperm-depleted they usually were able to produce only sons and continued to lay similar numbers of eggs per day after depleting sperm reserves as before sperm was depleted. Thus, lack of provisioning females with males later in the experimental period was not the factor for producing male bias progeny by *B. hebetor* females later in their reproductive lifespan. Results from the present study revealed that *B. hebetor* females lay more eggs during the first five weeks of oviposition and produced more females during that time, and then became constrained to produce only males (Fig. 2, 3 and 4). A similar result was reported by Uçkan and Gülel (2002) for another species

of braconid wasp, *Apanteles galleriae*, a koinobiont, solitary, larval endoparasitoid reared on two lepidopteran species, *G. mellonella* and *Achoria grisellae*.

In conclusion, *G. mellonella* does not seem to be a very suitable host for *B. hebetor* because parasitoid larvae suffers from high juvenile mortality and the developmental period was relatively long on larvae of *G. mellonella*. Parasitoid survival to the adult stage on *G. mellonella* was $\approx 50\%$. This is perhaps parasitoid-induced changes in host physiology. Thus, further studies are merited particularly directed in the areas of host's endocrinology to overcome the physiological changes in response to larval feeding. Nevertheless, because *G. mellonella* is relatively easy to acquire in the private market, such as pet supply stores, this species could be considered a potential supplementary host for rearing of *B. hebetor*. However, *A. transitella* appears to be the most suitable host for the reproductive performance of *B. hebetor*. The hosts *E. kuehniella*, *C. cephalonica*, *P. interpunctella*, and *E. cautella* are also relatively optimal of *B. hebetor* based on longer reproductive lifespan of the wasps, the relatively stable daily fecundity achieved, the higher parasitoid survival rate, and the short generation time of wasps on these hosts. Reproductive fitness of *B. hebetor* can be maximized through the utilization of hosts that allow for the highest levels of parasitoid survival, which can benefit individual *B. hebetor* wasps in their natural habitat, and which can be useful for enhanced commercial mass production of wasps for purposes of biological control of stored product moth pests.

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References

- Amir-Maafi M., and H. Chi. 2006.** Demography of *Habrobracon hebetor* (hymenoptera: Braconidae) on two pyralid hosts (Lepidoptera: Pyralidae). *Ann. Entomol. Soc. Am.* 99 (1): 84-90.
- Antolin, M. F., P. J. Ode, and M. R. Strand. 1995.** Variable sex ratio and ovicide in an out breeding parasitic wasp. *Anim. Behav.*, 17: 1-7.
- Arbogast, T. R. 1983.** Natural enemies as control agents for stored-product insects. *In* Proceedings, of the third international working conference on stored product entomology, Oct. 23-28, 1983 Kansas State University, Manhattan, Kansas, USA
- Arbogast, T. R. 1984.** Biological control of stored-product insects: status and prospects, pp. 215-225. In F. J. Baur (ed.), *Insect management for food storage and processing*. American Association for Cereal Chemist, St. Paul, MN, USA.
- Beard, R. L. 1952.** The toxicology of *Habrobracon* venom: a study of a natural insecticide. *Conn. Agric. Exp. Stn. Bull.* 562. 27 pp.
- Brower, J. H, L. Smith, P. V. Vail, and P.W. Flinn. 1996.** Biological control, pp. 223-286. In B. Subramanyam and D.W. Hagstrum (eds.), *Integrated Management of Insects in Stored Products*. Marcel Dekker, New York, USA.
- Charnov, E. L. 1982.** *The theory of sex allocation*. Princeton University Press, Princeton, NJ.
- Chauvin G., and J. Chauvin 1985.** The influence of relative humidity on larval development and energy content of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). *J. Stored Prod. Res.* 21: 79-82.
- Clark, A.M. and R. E. Smith. 1967.** Egg production and adult life span in two

species of *Bracon* (Hymenoptera: Braconidae). *Ann. Entomol. Soc. Am.* 60: 903-905.

Cox, P. D., and C. H. Bell 1991. Biology and ecology of moth pests on stored food, pp. 181-193. In J. R. Gorham (ed.), *Ecology and management of food-industry pests*. Association of Official Analytical Chemists, Arlington, VA, USA.

Doten, S. B. 1911. Concerning the relation of food and reproductive activity and longevity of certain hymenopterous parasites. *Tech. Bull. Nevada Agric. Expt. Sta.* 78: 30 pp.

Environmental Protection Agency. 1992. Parasitic and predaceous insects used to control insects pests; exemption from a tolerance. *Fed. Reg.* 57: 14645-14646.

Gauld, I. and B. Bolton. 1988. *The Hymenoptera*. Oxford University Press, Oxford.

Ghimire, M. N. and T. W. Phillips. 2007. Suitability of five species of stored-product insects as host for development and reproduction of the parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae). *J. Econ. Entomol.* 100 (5): 1732-1739.

Godfray, H. C. J. 1987. The evolution of clutch size in parasitic wasps. *Am. Nat.* 129: 221-223.

Godfray, H. C. J. 1994. *Parasitoids. Behavioral and evolutionary ecology*. Princeton University Press, NJ, USA

Hagstrum, D. W. and B. J. Smittle 1977. Host finding ability of *Bracon hebetor* and its influence upon adult parasite survival and fecundity. *Environ. Entomol.* 6: 437-439.

- Hagstrum, D. W. and B. J. Smittle 1978.** Host utilization by *Bracon hebetor*.
Environ. Entomol. 7: 596-600.
- Hagstrum, D. W., C. Reed, P. Kenkel. 1999.** Management of stored wheat insect
pests in the USA. Integrated Pest Manage. Rev. 4: 127-142.
- Hardy, I. C., N. T. Griffith, and H.C. J. Godfray. 1992.** Clutch size in a parasitoid
wasp- a manipulation experiment. J. Anim. Ecol. 61: 121-129.
- Harvey, J.A., T. M. Bezmer, J. A. Elzinga, and M. R. Strand. 2004.**
Development of solitary endoparasitoid *Microplitis demolitor*: host quality does
not increase with host age and size. Ecol. Entomol. 29: 35-43.
- Harvey, J.A. 2000.** Dynamic effects of parasitism by an endoparasitoid wasp on the
development of two host species: implications for host quality and parasitoid
fitness. Ecol. Entomol. 25: 267-278.
- Johnson, J. A., K. A. Valero, M. M. Hannel, and R. F Gill. 2000.** Seasonal
occurrence of post harvest dried fruit insects and their parasitoids in a culled fig
warehouse. J. Econ. Entomol. 93: 1380-1390.
- Johnson, J. A., P. V. Vail, D. G. Brandl, J. S. Tebbets, and K. A. Valero. 2002.**
Integration of nonchemical treatments for control of postharvest pyralid moths
(Lepidoptera: Pyralidae) in almond and raisins. J. Econ. Entomol. 95: 190-199.
- Krombein, K. V., Jr. P. D. Hurd, D. R. Smith and B. D. Bruks. 1979.** Catalog of
Hymenoptera in America North of Mexico. Smithsonian Institution Press,
Washington D. C., USA.
- Littell, R.C., Milliken, G.A., Stroup, W.W., and Wolfinger, R.D. 1996.** SAS
system for mixed models. SAS Institute Inc., Cary, NC, USA

- 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. *BioControl*. 50: 415-428.
- Ode, P. J., M. F. Antolin, and M. R. Strand. 1995.** Brood-mate avoidance in the parasitic wasp *Bracon hebetor* Say. *Anim. Behav.* 49: 1239-1248.
- Ode, P. J., M. F. Antolin, and M. R. Strand. 1996.** Sex allocation and sexual asymmetries in intra-brood competition in the parasitic wasp *Bracon hebetor*. *J. Anim. Ecol.* 65: 690-700.
- Ode, P. J., M. F. Antolin, and M. R. Strand. 1997.** Constrained oviposition and female-biased sex allocation in parasitic wasp. *Oecologia*, 109: 547-555.
- Ode, P. J., M. F. Antolin, and M. R. Strand. 1998.** Differential dispersal and female biased sex allocation in a parasitic wasp. *Ecol. Entomol.* 23: 314-318.
- Phillips, T. W., R. Berberet and G. W. Cuperus. 2000.** Post-harvest integrated pest management, pp. 2690-2701. In F. J. Francis (ed.), *Encyclopedia of food science and technology*, 2nd ed. Wiley, New York.
- Press, J. W. and B. R. Flaherty. 1981.** Reproductive potential of *Bracon hebetor* Say on three moth species, *Ephestia cautella* (Walker), *Achroia grisella* (F.), and *Galleria mellonella* (L.). *J. Georgia Entomol Soc.* 16: 342-345.
- Richards, O. W. and W. S. Thomson. 1932.** A contribution to the study of the genera *Ephestia*, Gn. (including *Strymax*, Dyar) and *Plodia*, Gn. (Lepidoptera: Phycitidae) with notes on parasite of the larvae. *Transec. Royal Entomol. Soc. London.* 80: 169-250.
- SAS Institute. 2005.** SAS/STAT user's guide for windows, version 9.1. SAS

Institute, Cary, NC.

Simmons, P. and H. D. Nelson. 1975. Insect on dried fruits. U.S. Dept. Agri.

Handb. 464

Uçkan, F., and A.Gülel. 2002. Age related fecundity and sex ratio variation in *Apanteles galleriae* (Hym., Braconidae) and host effect on fecundity and sex ratio of its hyperparasitoid *Dibrachys boarmiae* (Hym., Pteromalidae). J. Appl. Ent. 126: 534-537.

Ullyett, G.C. 1945. Distribution of progeny by *Microbracon hebetor* Say. J.

Entomol. Soc. of South Africa 8:123-131.

United Nations Environment Program. 2006. Handbook for the Montreal

protocol on substances that deplete the ozone layer. UNEP Ozone Secretariat,

Nairobi, Kenya.

Vick, K. W., J. A. Coffelt, and W. A. Weaver 1987. Presence of four species of stored-product moths in storage and field situations in north-central Florida as determined with sex pheromone baited traps. Fl. Entomologist 70: 488-492.

Vinson, S. B. and G. F. Iwantsch 1980. Host suitability for insect parasitoids. Ann. Rev. Entomol. 25: 397-419.

Zaviezo, T., and N. Mills 2000. Factors influencing the evolution in clutch size in a gregarious insect parasitoid. J. Anim. Ecol. 69: 1047-1057.

Table 1. List of host species (Lepidoptera: Pyralidae), and the average larval body weight (mg \pm SE) of 12 representative individuals used in this study.

Sub-family	Common Name	Scientific Name	Larval Weight
Phycitinae	Indianmeal moth	<i>Plodia interpunctella</i> (Hubner)	20.15 \pm 0.92
	Mediterranean flour moth	<i>Ephestia kuehniella</i> (Zeller)	24.56 \pm 0.96
	Almond moth	<i>Ephestia cautella</i> (Walker)	18.66 \pm 1.31
	Navel orangeworm	<i>Amyelois transitella</i> (Walker)	55.00 \pm 1.90
Galleriinae	Rice moth	<i>Corcyra cephalonica</i> (Stainton)	48.89 \pm 1.66
	Greater wax moth	<i>Galleria mellonella</i> (Linnaeus)	262.78 \pm 15.17

Table 2. Developmental and reproductive statistics (mean \pm SE) of *B. hebetor* on six different pyralid host species.

Host species	Developmental time in days	Oviposition period in days	Postoviposition period in days	Longevity of females in days	Life time fecundity per female	Total progeny produced per female	Survival from eggs to adults in percentage
<i>P. interpunctella</i>	9.9 \pm 0.2a (9.5 - 11.5)	34.7 \pm 2.8b (14 - 44)	3.3 \pm 0.4b (1 - 6)	38.0 \pm 2.8c (18 - 46)	538.3 \pm 50.6b (216 - 754)	372.6 \pm 35.6b (137 - 554)	70.3 \pm 3.3ab (40.7 - 87.5)
<i>E. kuehniella</i>	10.3 \pm 0.2ab (9.5 - 12.5)	48.7 \pm 3.8a (21 - 64)	11.4 \pm 3.1a (1 - 31)	60.2 \pm 4.2a (22 - 82)	800.0 \pm 65.8a (328 - 1219)	568.2 \pm 43.2a (255 - 828)	71.7 \pm 1.8ab (61.9 - 83.9)
<i>E. cautella</i>	9.7 \pm 0.2a (9.5 - 10.5)	49.2 \pm 3.1a (23 - 61)	6.1 \pm 1.3b (1 - 13)	55.3 \pm 3.5ab (24 - 69)	653.9 \pm 51.6ab (249 - 896)	426.5 \pm 31.5b (201 - 545)	66.9 \pm 3.3b (37.6 - 80.7)
<i>C. cephalonica</i>	10.2 \pm 0.2ab (9.5 - 11.5)	33.7 \pm 2.8b (13 - 46)	4.2 \pm 1.1b (1 - 13)	37.9 \pm 3.5c (14 - 56)	728.4 \pm 69.6a (278 - 1081)	551.8 \pm 60.6a (245 - 836)	75.4 \pm 2.5a (60.8 - 88.1)
<i>G. mellonella</i>	12.6 \pm 0.3c (12.0 - 14.5)	36.9 \pm 5.0b (9 - 60)	2.5 \pm 0.4b (1 - 4)	39.4 \pm 4.1c (13 - 61)	808.0 \pm 96.5a (259 - 1243)	369.2 \pm 39.1b (130 - 545)	49.7 \pm 4.8c (32.1 - 82.6)
<i>A. transitella</i>	10.5 \pm 0.2b (9.5 - 11.5)	41.4 \pm 2.5ab (25 - 53)	5.5 \pm 2.3b (1 - 27)	46.9 \pm 2.8bc (26-60)	810.1 \pm 46.0a (461 - 1069)	616.9 \pm 42.6a (307 - 840)	75.7 \pm 2.0a (65.3 - 83.9)
<i>F</i>	20.65	4.28	3.36	6.76	2.77	6.28	10.42
<i>df</i>	5, 52	5, 66	5, 66	5, 66	5, 66	5, 66	5, 55
<i>P</i>	<.0001	0.0023	0.0091	<.0001	0.0247	<.0001	<.0001
<i>n</i>	66-148	12	12	12	12	12	12

Mean followed by the same letter are not significantly different by the protected LSD at $\alpha = 0.05$. Range of data (minimum to maximum) is given in the parenthesis.

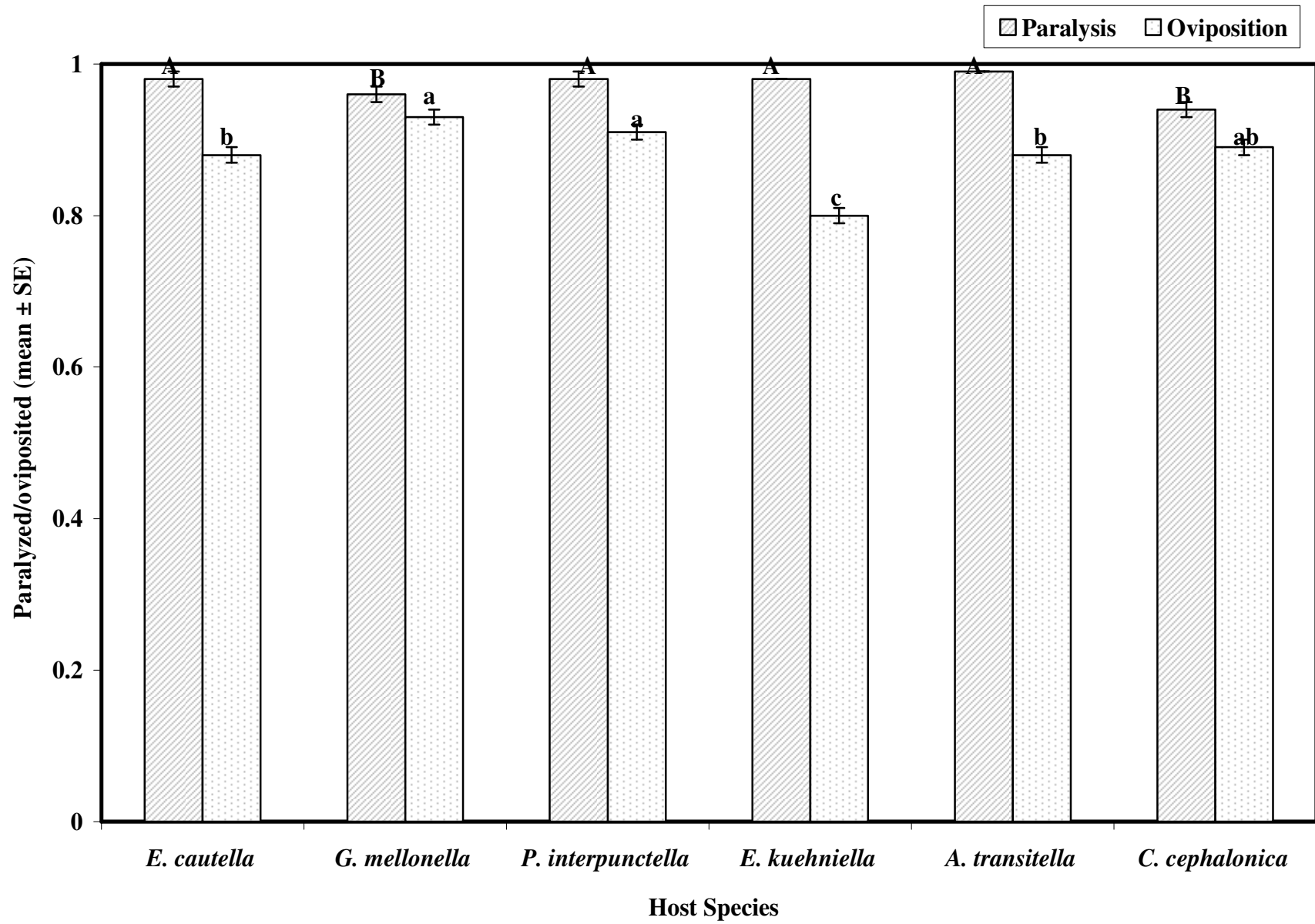
Figure legends

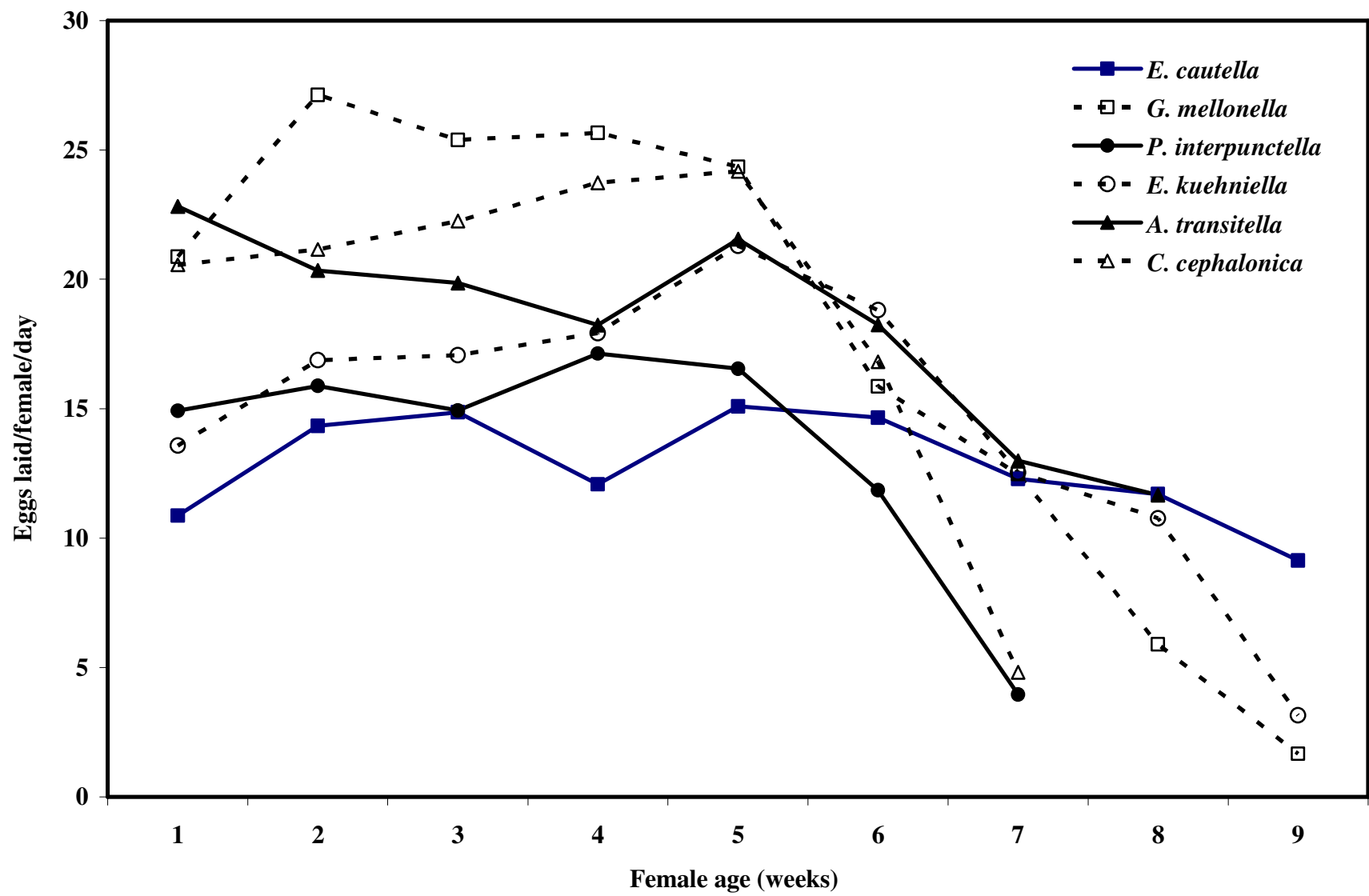
Fig. 1. Proportion of hosts that were paralyzed and parasitized (oviposited on) by *B. hebetor* females throughout their life time

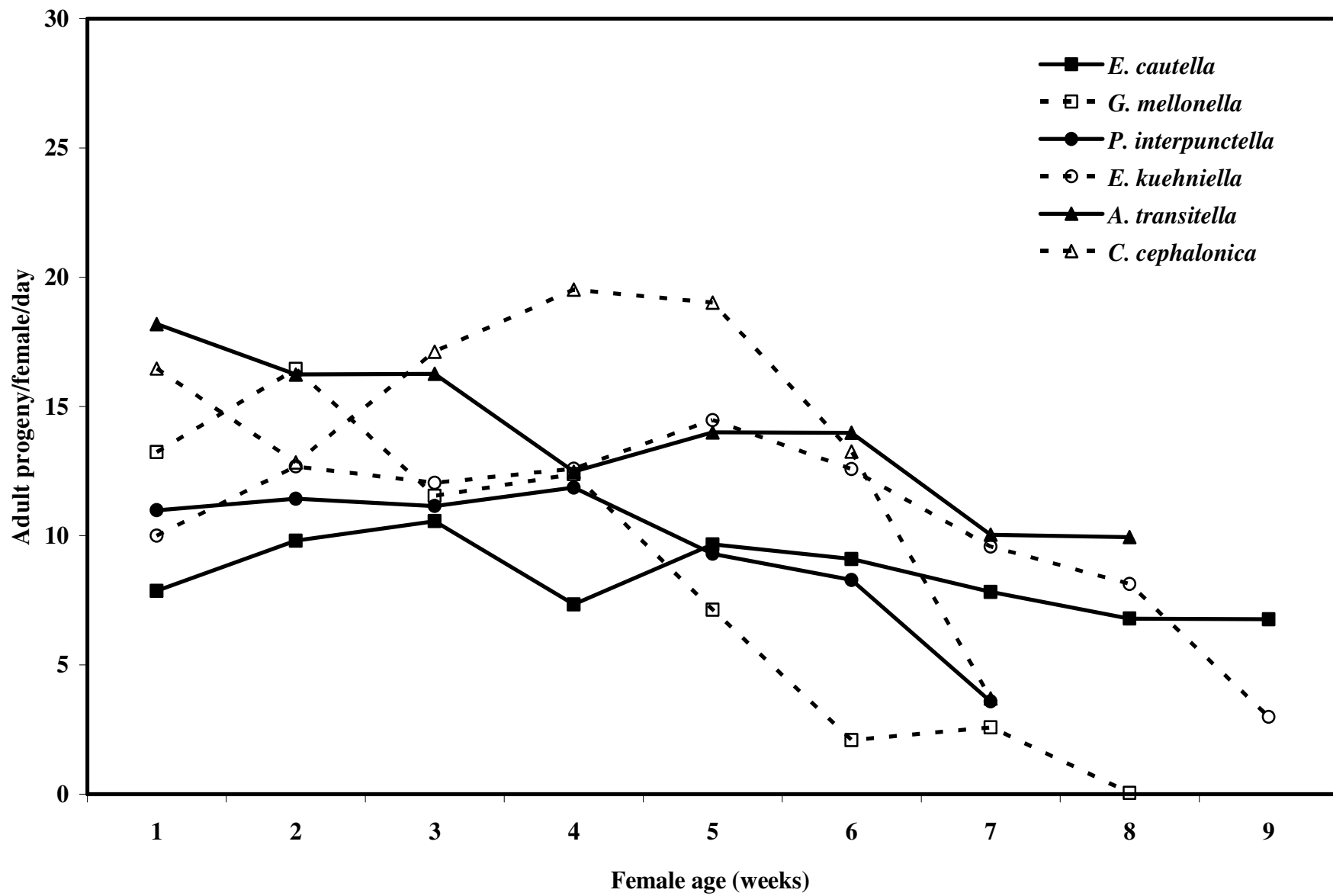
Fig. 2. Daily oviposition by female *B. hebetor* each week on six different pyralid hosts over a nine-week period

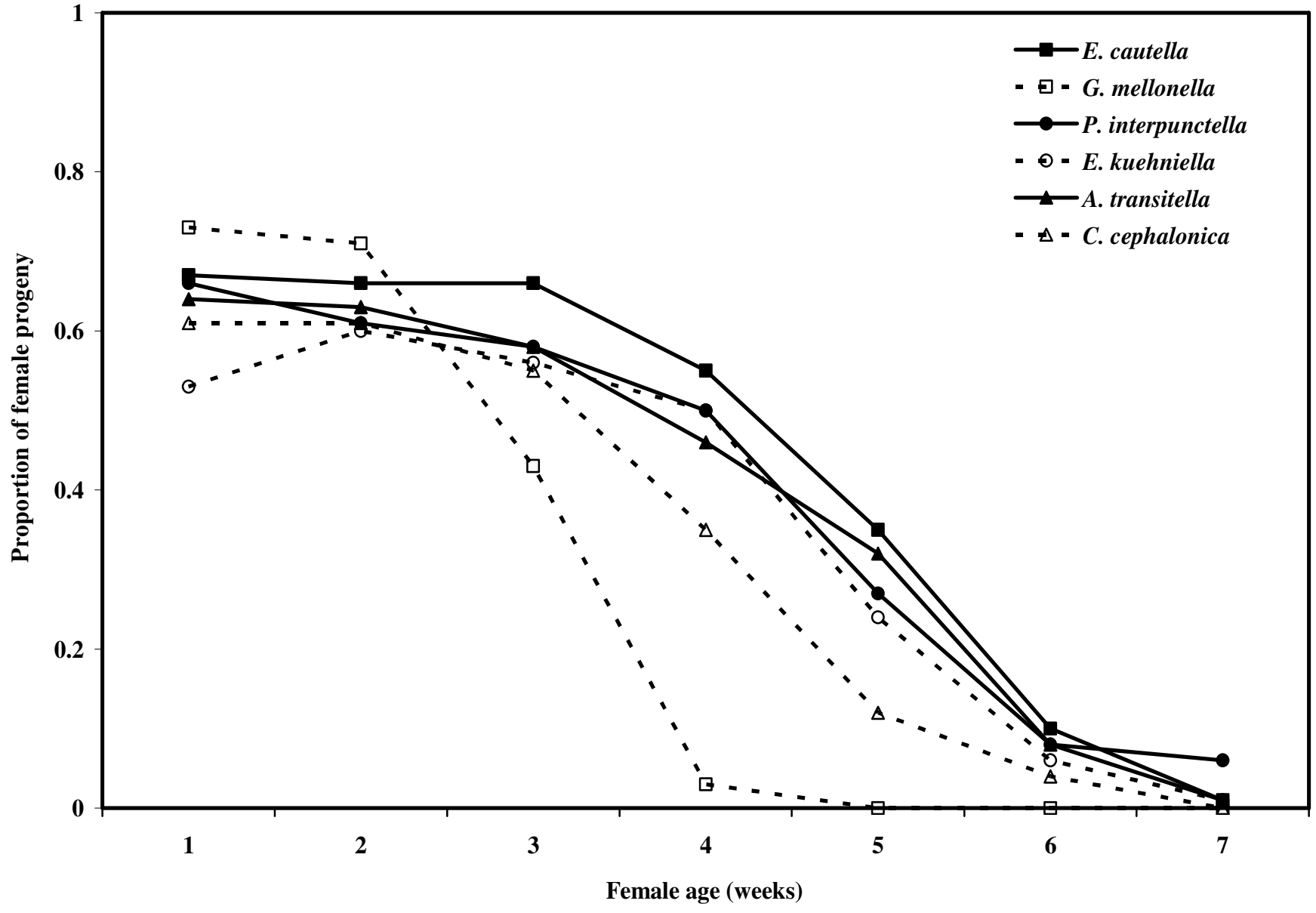
Fig. 3. Mean adult *B. hebetor* produced per day from eggs laid in a given week on six different pyralid hosts over a nine-week period

Fig. 4. Mean daily sex ratio (females/total) of *B. hebetor* progeny produced on six pyralid hosts in a given week over a seven-week period









CHAPTER IV

**MASS REARING OF *BRACON HEBETOR* SAY (HYMENOPTERA:
BRACONIDAE) ON LARVAE OF INDIANMEAL MOTH, *PLODIA
INTERPUNCTELLA* (LEPIDOPTERA: PYRALIDAE): EFFECTS OF HOST
DENSITY, PARASITOID DENSITY, AND REARING CONTAINERS.**

Abstract

Bracon hebetor Say (Hymenoptera: Braconidae) is a larval parasitoid of several species of Lepidoptera in the family Pyralidae including the Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), which is a major insect pest of post harvest commodities and finished products in the United States. Rearing methods for *B. hebetor* were investigated in the series of laboratory experiments designed to enhance the mass rearing of *B. hebetor* for biological control of *P. interpunctella* and other stored products pyralid moths. In these experiments, the effects of parasitoid density, host density, and size of the rearing containers on adult progeny production and secondary sex ratio of *B. hebetor* were tested. In parasitoid density experiments, a density of eight male-female pairs of *B. hebetor* produced a higher number of progeny (188 adults) on 50-last instar *P. interpunctella* larvae than the densities of one and two pairs of *B. hebetor*. Similarly, in a host density experiment, a density of 50-last instar *P. interpunctella* larvae produced a significantly higher number of parasitoid progeny (160 adults) among the tested host densities than when two pairs of *B. hebetor* were used. In experiments that assessed the size of the rearing containers, a glass jar with a volume of 250 ml (\approx 8 ounce “jelly jar”), produced higher number of parasitoid progeny (166 adults) than other sizes of containers when two pairs of *B. hebetor* were used. The parasitoid’s secondary sex ratio was female-biased in all experiments and there were no significant effects on sex ratio from variation in parasitoid density, host density, or size of the rearing containers.

Key words: Biological control, stored product pest, laboratory rearing, parasitoid, wasp

Introduction

The Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) is a worldwide destructive pest of stored grains, dried fruits, nuts and many other value-added food products and process foods. Infestation of *P. interpunctella* is widespread can be common and widespread in food processing facilities, flour mills, warehouses, retail stores and bulk grain bins (Doud and Phillips 2000). Damage is mainly caused by larval feeding on food products and by subsequent production of silken webs and frass left by the larvae (Brower 1988, Na and Ryoo 2000). Larval feeding may also provide a conducive environment for mold development due to increase in moisture and temperature that decrease the quality and quantity of stored products (Abdel-Rahman et al. 1969). For many years the management of *P. interpunctella* has traditionally involved the use of fumigants, aerosols and contact chemical insecticides. However, this moth species has become resistant to many commonly used insecticides (Zettler 1973). Moreover, insecticides pose a direct risk to human health and the environment due to the presence of their residue in food products and in processing facilities where workers are exposed. Also, legislative restrictions have limited the use of fumigants in the food industry, such as the banning of the use of methyl bromide due to its effects on the depletion of the atmospheric ozone layer (United Nation Environment Program 1992).

In recent years, interest has been focused in the development of non-chemical strategies such as cultural, physical, biological, varietal, bio-rational and genetic control measures in place of conventional pesticides for the management of stored product insects (Subramanyam and Hagsturm 2000, Fields and White 2002, Phillips 2006). Of these strategies, the use of natural enemies, such as parasitoids and predators, is an

important component of stored product protection and has many advantages over chemical control.

Bracon hebetor Say (Hymenoptera: Braconidae) is a cosmopolitan, gregarious, ecto-parasitoid that attacks the wandering stage pyralid moth larvae, including *P. interpunctella* infesting stored-products (Benson 1974). *Bracon hebetor* is considered as potential biological control agents of stored product moths (Brower et al. 1996) and has had some use in commercial pest control. The most extensive research with *B. hebetor* focused on the host-finding and utilization, and sex allocation (Hagstrum and Smittle 1977, 1978, Antolin et al. 1995, Ode et al. 1996, 1997, 1998). Taylor (1988a, 1988b) studied the influence of host age, host freshness, and wasp nutritional status on parasitism by *B. hebetor*. Although *B. hebetor* has been produced and sold commercially for management of stored product moths (Schoeller et al. 2006), there are apparently no published scientific studies that document efficacy of mass-production of the parasitoid, *B. hebetor*. Such information is essential to develop biological control programs for the suppression of storage moth populations through augmentative or inundative releases of parasitoids. The overall and long-term objective of this study is to develop and improve methodologies for mass-rearing *B. hebetor*. Specific goals in this paper were to determine the effect of (a) parasitoid density, (b) host density, and (c) size of the rearing containers on mass rearing and production of adults of *B. hebetor* in laboratory condition.

Materials and Methods

Parasitoid origin and rearing

B. hebetor adults were collected from grain bins at the Stored Products Research and Education Center (SPREC) at Oklahoma State University in Stillwater, Oklahoma on November 2003 that were associated with Indianmeal moth (IMM), *P. interpunctella*. The parasitoid was reared on full grown larvae of *P. interpunctella* in the laboratory at a temperature of $29 \pm 1^\circ\text{C}$, a relative humidity of $65 \pm 5\%$, and a photoperiod of 14:10 (L: D) h. The larvae *P. interpunctella* were obtained from a laboratory culture that was reared on a standardized diet of corn meal, chick laying mash, chick starter mash, and glycerol at a volumetric ratio of 4:2:2:1, respectively, at a temperature of $28 \pm 1^\circ\text{C}$, a relative humidity of $65 \pm 5\%$, and a photoperiod of 16:8 (L: D) h.

Parasitoid and host density experiments

Plastic yogurt cups, approximately 236.6 ml (8 oz), were used as experimental arenas and were fitted into glass jars for easy handling and adequate aeration through the metal screen. In both experiments, *B. hebetor* adults within 48 h of emergence were released into each experimental arena and allowed to sting and oviposit for next five consecutive days. In the parasitoid density experiment, 50 last instar of *P. interpunctella* larvae were placed in a yogurt cup and one of four different densities of parasitoids were introduced in the cup: one, two, four or eight male-female pairs of *B. hebetor*. In the host density experiment, two pairs *B. hebetor* were introduced into five different densities of hosts: 10, 20, 30, 40, or 50 last instar *P. interpunctella* larvae per yogurt cup. Experimental containers were held in a growth chamber at a temperature of $29 \pm 1^\circ\text{C}$, a relative humidity of $65 \pm 5\%$, and a photoperiod of 14:10 (L: D) h. The emergence of

parasitoids was monitored daily after one week until the emergence was ended (2-3 week). Observations were made on the number of adult parasitoid emerged and parasitoid's secondary sex ratio (proportion of females). Both experiments were conducted at the same conditions used for rearing of *B. hebetor* as mentioned above.

Size of rearing containers experiments

In this experiment five different sizes of the glass canning jars were chosen, 118.3 ml (4 oz jelly jar), 236.6 ml (8 oz jelly jar), 473.1 ml (16 oz pint jar), 946.2 ml (32 oz quart jar), and 1,892.5 ml (64 oz half gallon jar). Fifty last instar of *P. interpunctella* larvae were placed in these glass jar arenas. Two male-female pairs of *B. hebetor* that had emerged in the previous 48 h were introduced into each experimental jar and allowed to sting and oviposit for five consecutive days. All other procedures were followed as mentioned above in the parasitoid and host density experiments section.

Data analysis

The numbers of adult parasitoid progeny and the parasitoid's secondary sex ratio (%female) were used as response variables to assess the effect of parasitoid and host density, and also the effect of size of the rearing containers. Parasitoid density, host density and size of the rearing containers were used as independent variable for the analysis of response variables. Each experiment was replicated ten times except for 236.6 ml container size which had 20 replicates. Data for numbers of adult parasitoids and the parasitoid's secondary sex ratio were analyzed by one-way analysis of variance (ANOVA) (PROC MIXED, SAS Institute 2005). Relationship between container size and adult progeny production was measured with regression analysis (PROC REG, SAS Institute 2005).

Results

The total number of parasitoid progeny produced from the 50 last instar *P. interpunctella* larvae in the *B. hebetor* density experiment differed significantly in response to *B. hebetor* release density ($F = 7.83$; $df = 3, 39$; $P = 0.0003$). The highest number *B. hebetor* adults was produced from a density of eight pairs (187.89 ± 12.28) followed by four (163.83 ± 16.65), two (141.18 ± 17.29) and, one pair (90 ± 11.54) of *B. hebetor* (Fig. 1). There was no significant difference observed in parasitoid progeny resulted from between the eight and four pairs, and between four and two pairs, of *B. hebetor* density (Fig. 1). The *B. hebetor* progeny produced in these experiments were consistently female-biased (greater than 50% females) (Fig. 2), and that these proportions did not vary significantly across the treatments ($F = 2.02$; $df = 3, 39$; $P = 0.102$).

In the *P. interpunctella* host density experiment, the total number of *B. hebetor* adults produced in response to different host densities was found to be significant ($F = 28.15$; $df = 4, 42$; $P < 0.0001$). A significantly higher number of parasitoid progeny was produced from the density of 50 last instar *P. interpunctella* larvae (160.0 ± 8.61 adults) compared to the other host densities when two pairs of *B. hebetor* were used (Fig. 3). The lowest number of parasitoid progeny was produced from the density of 10 last instar *P. interpunctella* larvae (49.1 ± 3.11) (Fig. 3). The host density with 30 and 40 *P. interpunctella* larvae produced almost equal numbers of parasitoid progeny. Similarly, the number of parasitoid progeny did not differ significantly between host density of 10 and 20 larvae (Fig. 3). The parasitoid's secondary sex ratio (proportion of females) did not vary significantly with the host density ($F = 0.41$; $df = 4, 42$; $P = 0.797$), though a

consistently female-biased (greater than 50% females) progeny were produced from all the tested host density (Fig 4).

The number of parasitoid progeny produced in rearing containers of different sizes did not vary significantly ($F = 1.81$; $df = 4, 45.9$; $P = 0.1429$). The highest number of parasitoid progeny was produced from the 236.6 ml. (8 oz jelly jar) size container (165.5 ± 6.23) and the lowest number of parasitoid progeny was produced from the largest containers of 1,892.5 ml (half gallon jar) (139.2 ± 10.6), when two pairs of *B. hebetor* introduced into a density of 50-last instar *P. interpunctella* larvae (Fig. 5). As in the other experiments, the proportion of females also did not vary significantly in response to size of the rearing containers ($F = 1.68$; $df = 4, 43.5$; $P = 0.1730$), although there was a majority of female parasitoid progeny observed in all sizes of the rearing containers tested (Fig. 6). The relationship between the number of adult parasitoid produced could be described as a linear function of the size of the rearing containers as follows: $y = 165.39 - 0.0157x$ ($F = 5.82$; $df = 1, 58$; $P = 0.0190$; $r^2 = 0.0912$), where y is the number of parasitoid progeny produced and x is the size of the rearing container in liters when two pairs of *B. hebetor* were introduced introduced with 50 last instar *P. interpunctella* larvae for 5 days (Fig. 7). Although this analysis shows a significant negative relationship between progeny production and size of the rearing container, the r^2 of 0.0912 indicates a very weak relationship.

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Discussion

The results of the *B. hebetor* density experiments show that difference in parasitoid density with a fixed number hosts significantly affected the number of parasitoids developing from these hosts. Thus, considering the strong potential for parasitoid density to affect the number of parasitoid progeny produced, it was decided to use of the lowest densities, i. e. a density of two *B. hebetor* females, for the two other subsequent experiments on host density and size of the rearing container in order to maximize the possible effects of these treatments. As parasitoid density increased from one to eight *B. hebetor* females, daily mean number of parasitoid progeny/female/day decreased from 18 to 4.7 adults. Thus, the reproductive fitness of individual female *B. hebetor* decreased with increased density of parasitoids introduced into containers. There are several factors that might have caused low numbers of parasitoid progeny in this experiment with a high density *B. hebetor*. First, at a density of eight *B. hebetor* females there was only 1.25 available hosts/female/day. It is possible that the parasitoid may have suffered with higher level of immature mortality as purposed by Benson (1973) and Yu et al. (2003). In those previous studies, they reported that larval mortality of *B. hebetor* parasitizing *Cadra* (= *Ephestia*) *cautella* (Walker), and *P. interpunctella*, increased abruptly when the number of eggs on a host exceed approximately 8 and 10, respectively, suggesting competition among the larval parasitoids. Second, *B. hebetor* females may avoid laying more eggs than could complete development on a host, as purposed by Yu et al. (2003), in which *B. hebetor* females optimized oviposition and did not lay more than 7 or 12 eggs/day when they encountered only one host larva of the tortricid,

Adoxophyes orana or the pyralid, *P. interpunctella*, respectively. Despite reduced progeny production per female observed at higher parasitoid introduction densities, the maximum number of progeny produced in this experiment came from containers with 8 male-female pairs of *B. hebetor*, which satisfies the objective of this study to develop a method to maximize production of wasp progeny in a mass rearing context. Additionally, a potential benefit of using a higher density of parental *B. hebetor* in a mass-rearing context is that the genetic variability, and that “quality” of the progeny might be improved by promoting out-breeding and avoiding deleterious effects of inbreeding (e.g., Antolin and Strand 1995; Ode et al. 1996) with a larger parental group of wasps in each containers.

In the host density experiments, a density of 50 last instar *P. interpunctella* larvae produced significantly more parasitoid progeny (160 adults) among the tested host densities. This study showed that more adult parasitoid progeny were produced as host density increased. The results from this study are not in accord with the earlier finding by Taylor (1988a, 1988b), in which he reported the total numbers of eggs laid by *B. hebetor* was independent of the host density. The difference between these data and Taylor’s results could be due to a difference in the parasitoid populations or experimental procedures between the two studies. For example, we measured the number of adult progeny ultimately produced after egg, larval and pupal development, whereas Taylor measured the total number of eggs laid by *B. hebetor* females, and did not account for mortality of life stages after that. However, the results from the current agree more recent work by Yu et al. (2003), in which *B. hebetor* females were able to allocate eggs in relation to density.

In the experiment with the size of the rearing containers, glass jars of 236.6 ml (8 oz jelly jar) produced higher numbers of parasitoid progeny (166 adults) compared to containers with larger volumes. The results suggest that the number of parasitoid adult progeny decreases with an increase of rearing container size (Fig. 7). This result could be explained by the possibility that *B. hebetor* females spent more time in host searching activities in larger containers, and less time actually parasitizing hosts, as compared to the same activities in smaller containers.

Although female *B. hebetor* are capable of regulating the progeny sex ratio on a host based on the total number of fertilized eggs laid, the overall progeny sex ratio, in all experiments reported here was not affected by parasitoid density, host density, or size of the rearing containers. Parasitoid progeny sex ratios in other studies have been variable, ranging from a male bias to strongly female bias progeny (Reinert and King 1971, Antolin and Strand 1992, Antolin et al. 1995). These variations may be due to the differences in the parasitoid strains of *B. hebetor*, the host species tested, host and parasitoid density or test arenas. The experiments reported here did not test extremes of such treatments to influence a significant change in sex ratio.

The results from this study indicate that laboratory, commercial-scale rearing of *B. hebetor* can be maximized through the utilization of a host density of 50 last instar *P. interpunctella* larvae in a relatively small container of about 250 ml. (8 oz. jelly jar) and with eight male-female pairs of *B. hebetor* to allow adequate host utilization and wasp development over a five-day period in controlled environmental conditions. Economic costs of mass rearing biological control agents should be minimized for the benefits of such activities to be profitable and effective for the pest management

activity (Schoeller et al. 2006). The small rearing container with maximum was production in a 5-day cycle reported here could facilitate more cost-effective mass-rearing of *B. hebetor* for biological control of stored product moths.

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References

- Abdel-Rehman, H. A., C. M. Christensen, and A. C. Hodson. 1969.** The relationship between *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) and stored grain fungi. *J. Stored Prod. Res.* 4: 331-337.
- Antolin, M. F., P. J. Ode, and M. R. Strand. 1995.** Mating system of *Bracon hebetor* (Hymenoptera: Braconidae). *Ecol. Entomol.* 17: 1-7.
- Antolin, M. F. and M. R. Strand. 1992.** Variable sex ratio and ovicide in an outbreeding parasitic wasp. *Anim. Behav.*, 49: 589-600.
- Benson, J. F. 1973.** Intraspecific competition in the population dynamics of *Bracon hebetor* Say. *J. Anim. Ecol.* 42:193-197.
- Benson, J. F. 1974.** Population dynamics of *Bracon hebetor* Say (Hymenoptera: Braconidae) and *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) in a laboratory ecosystem. *J. Anim. Ecol.* 43: 71-86.
- Brower, J. H. 1988.** Population suppression of the Almond moth and Indianmeal moth (Lepidoptera: Pyralidae) by release of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) into simulated peanut storages. *J. Econ. Entomol.* 81: 944-948.
- Brower, J. H, L. Smith, P. V. Vail, and P.W. Flinn. 1996.** Biological control, pp. 223-286. In B. Subramanyam and D.W. Hagstrum (eds.), *Integrated Management of Insects in Stored Products*. Dekker, New York, USA.
- Doud, C. W. and T. W. Phillips. 2000.** Activity of *Plodia interpunctella* (Lepidoptera: Pyralidae) in and around flour mills. *J. Econ. Entomol.* 93: 1842-1847.
- Fields, P. G. and N. D. G. White. 2002.** Alternatives to methyl bromide treatments for

- stored-products and quarantine insects. *Annu. Rev. Entomol.* 47: 331-359.
- Hagstrum, D. W. and B. J. Smittle 1977.** Host finding ability of *Bracon hebetor* and its influence upon adult parasite survival and fecundity. *Environ. Entomol.* 6: 437-439.
- Hagstrum, D. W. and B. J. Smittle 1978.** Host utilization by *Bracon hebetor*. *Environ. Entomol.* 7: 596-600.
- Na, H. J. and M. I. Ryoo. 2000.** The influence of temperature on development of *Plodia interpunctella* (Lepidoptera: Pyralidae) on dried vegetable commodities. *J. Stored Prod. Res.* 36: 125-129.
- Ode, P. J., M. F. Antolin, and M. R. Strand. 1996.** Sex allocation and sexual asymmetries in intra-brood competition in the parasitic wasp *Bracon hebetor*. *J. Anim. Ecol.* 65: 690-700.
- Ode, P. J., M. F. Antolin, and M. R. Strand. 1997.** Constrained oviposition and female-biased sex allocation in parasitic wasp. *Oecologia*, 109: 547-555.
- Ode, P. J., M. F. Antolin, and M. R. Strand. 1998.** Differential dispersal and female biased sex allocation in a parasitic wasp. *Ecol. Entomol.* 23: 314-318.
- Phillips, T. W. 2006.** The Science and Technology of Post harvest Insect Control: Challenges, Accomplishments and Future Directions, pp. 211-222. In: J. Heaps (ed.), *Insect Management for Food Storage and Processing*, 2nd Ed. Am. Assoc. Cereal Chem.
- Reinert, J. A. and E. W. King. 1971.** Action of *Bracon hebetor* Say as a parasite of *Plodia interpunctella* at control densities. *Ann. Entomol. Soc. Am.* 64 (6): 1335-1340.

- SAS Institute. 2005.** SAS/STAT user's guide for windows, version 9.1. SAS Institute, Cary, NC.
- Scholler, M., P.W. Flinn, M. Grieshop, and E. Zdarkova. 2006.** Biological Control of Stored Product Pests. In: J.W. Heaps, Editor, Insect Management for Food Storage and Processing. St. Paul, MN. AACC International. p. 67-87.
- Subramanyam, B., and D. W. Hagstrum. 2000.** Alternatives to pesticides in stored product IPM. Kluwer Academic Publisher, United State of America
- Taylor, A. D. 1988a.** Host effects on larval competition in the gregarious parasitoid *Bracon hebetor*. J. Anim. Ecol. 57:163-172.
- Taylor, A. D. 1988b.** Host effects on functional and ovipositional response of *Bracon hebetor*. J. Anim. Ecol. 57: 173-184.
- United Nations Environment Program. 1992.** The impact of ozone layer depletion. Nairobi, Kenya.
- Yu, S. H., M. I. Ryoo, J. H. Na, and W. I. Choi. 2003.** Effect of host density on egg dispersion and sex ratio of progeny of *Bracon hebetor* (Hymenoptera: Braconidae). J. Stor. Prod. Res. 39: 385-393.
- Zettler, J. L., L. L. McDonald, L. M. Redlinger, and R. D. Jones. 1973.** *Plodia interpunctella* and *Cadra cautella* resistance in strain to malathion and synergized pyrethrins. J. Econ. Entomol. 66: 1049-1050.

Figure legends

Fig. 1. Effect of parasitoid density on progeny production of *B. hebetor* with a density of 50- last instar of *P. interpunctella* larvae. Bars followed by the different letters are significantly different at $\alpha \leq 0.05$ using least significantly different (LSD) procedures.

Fig. 2. Effect of parasitoid density on progeny sex ratio (proportion of females) of *B. hebetor* with a density of 50- last instar of *P. interpunctella* larvae.

Fig. 3. Effect of *P. interpunctella* larval density on the parasitoid progeny production resulting from two pairs of *B. hebetor*. Bars followed by the different letters are significantly different at $\alpha \leq 0.05$ using least significantly different (LSD) procedures.

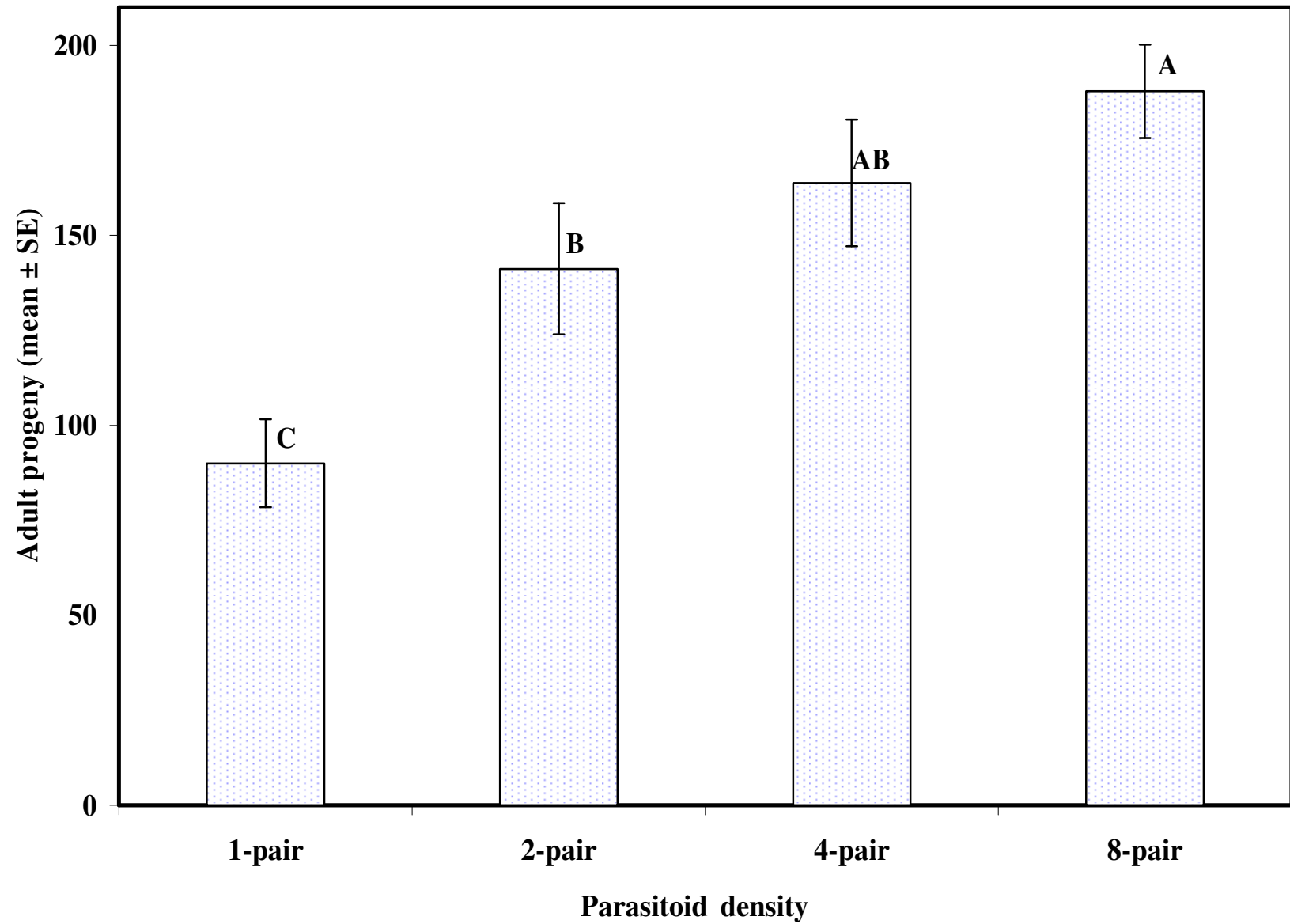
Fig. 4. Effect of *P. interpunctella* larval density on the parasitoid's secondary sex ratio (proportion of females) resulting from two pairs of *B. hebetor*

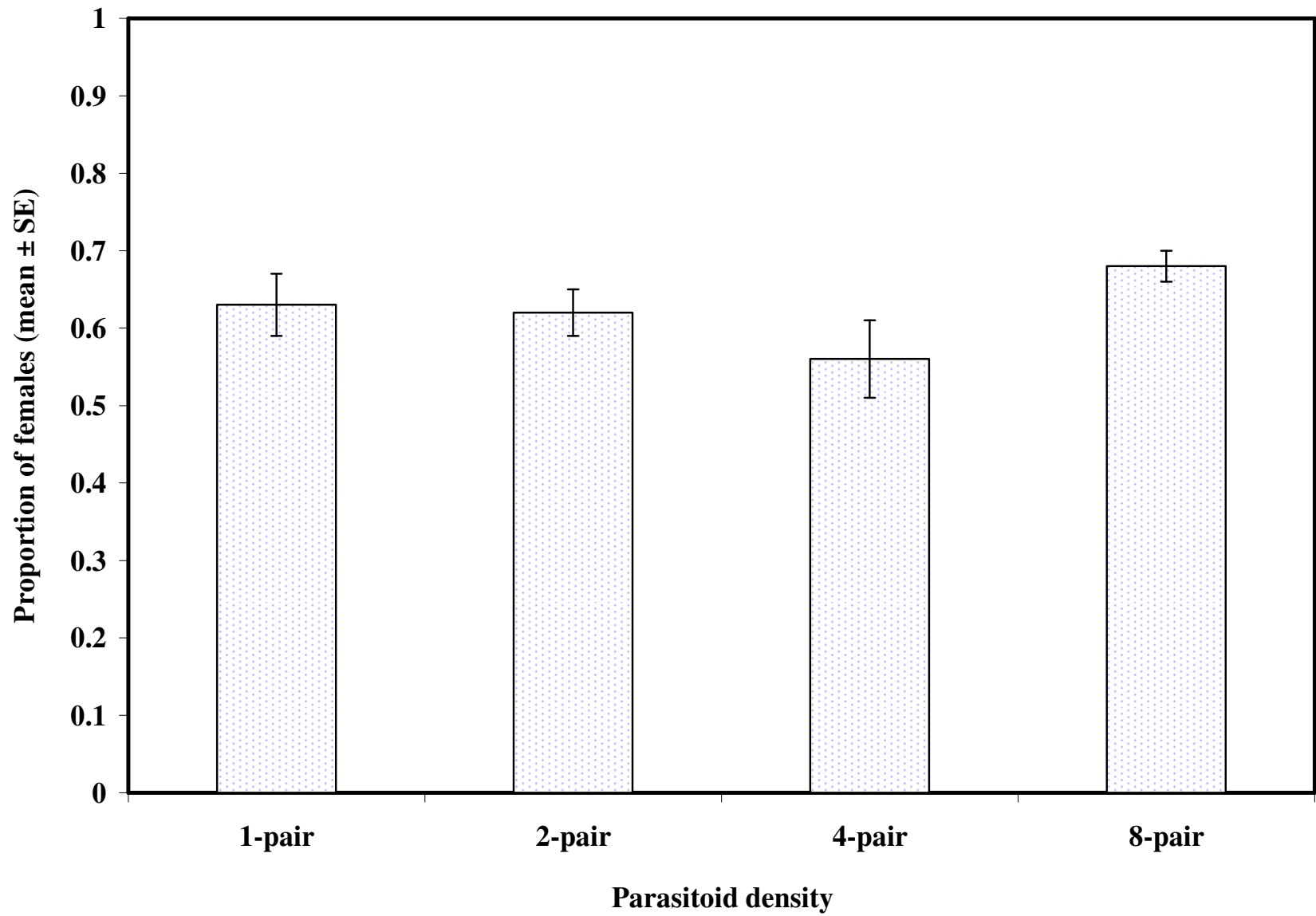
Fig. 5. Effect of size the rearing containers on parasitoid progeny production when two pairs of *B. hebetor* released with a density of 50-last insatr *P. interpunctella* larvae.

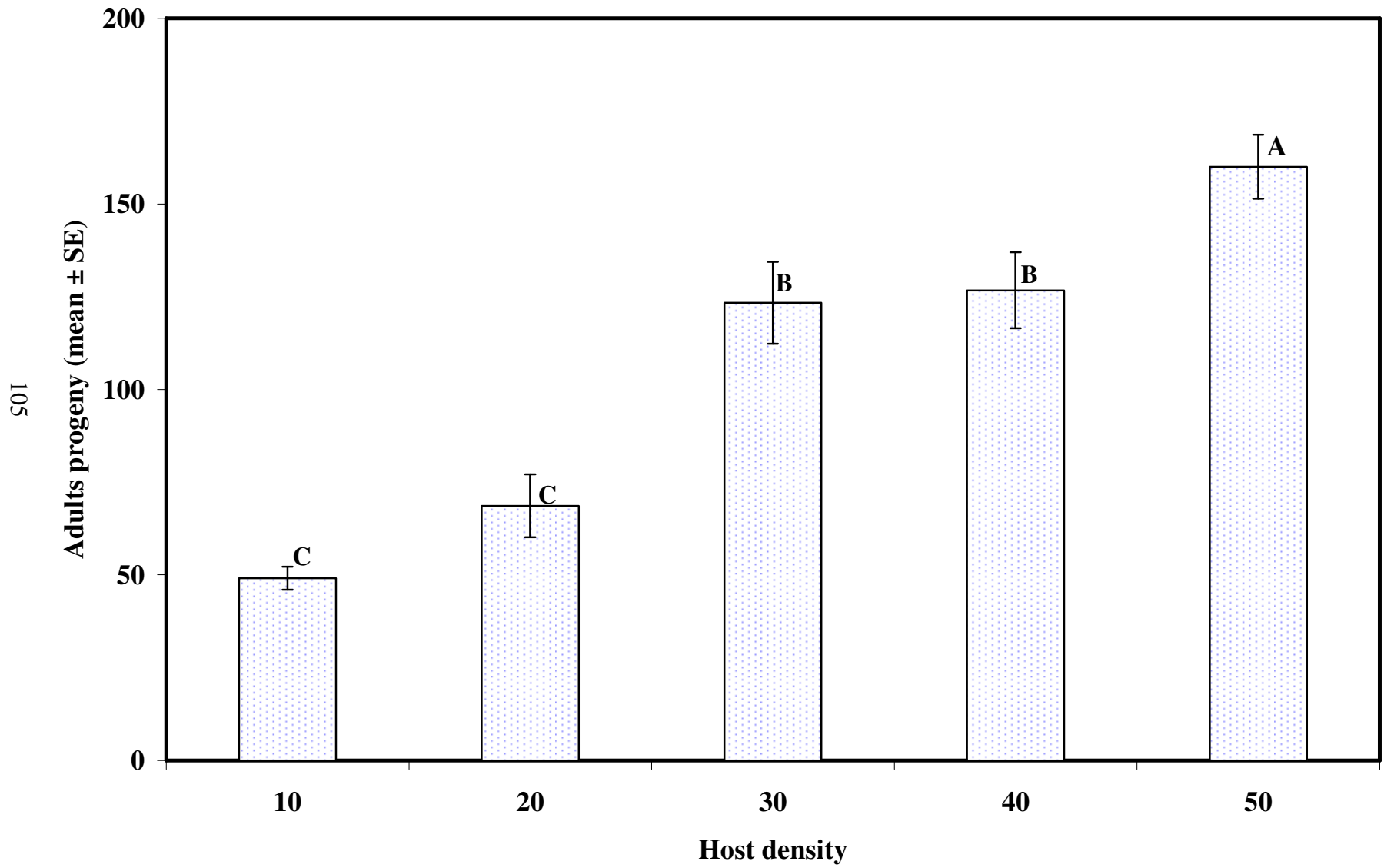
Fig. 6. Effect of size of rearing containers on parasitoid's secondary sex ratio (proportion of females) with a density of 50-last insatr *P. interpunctella* larvae.

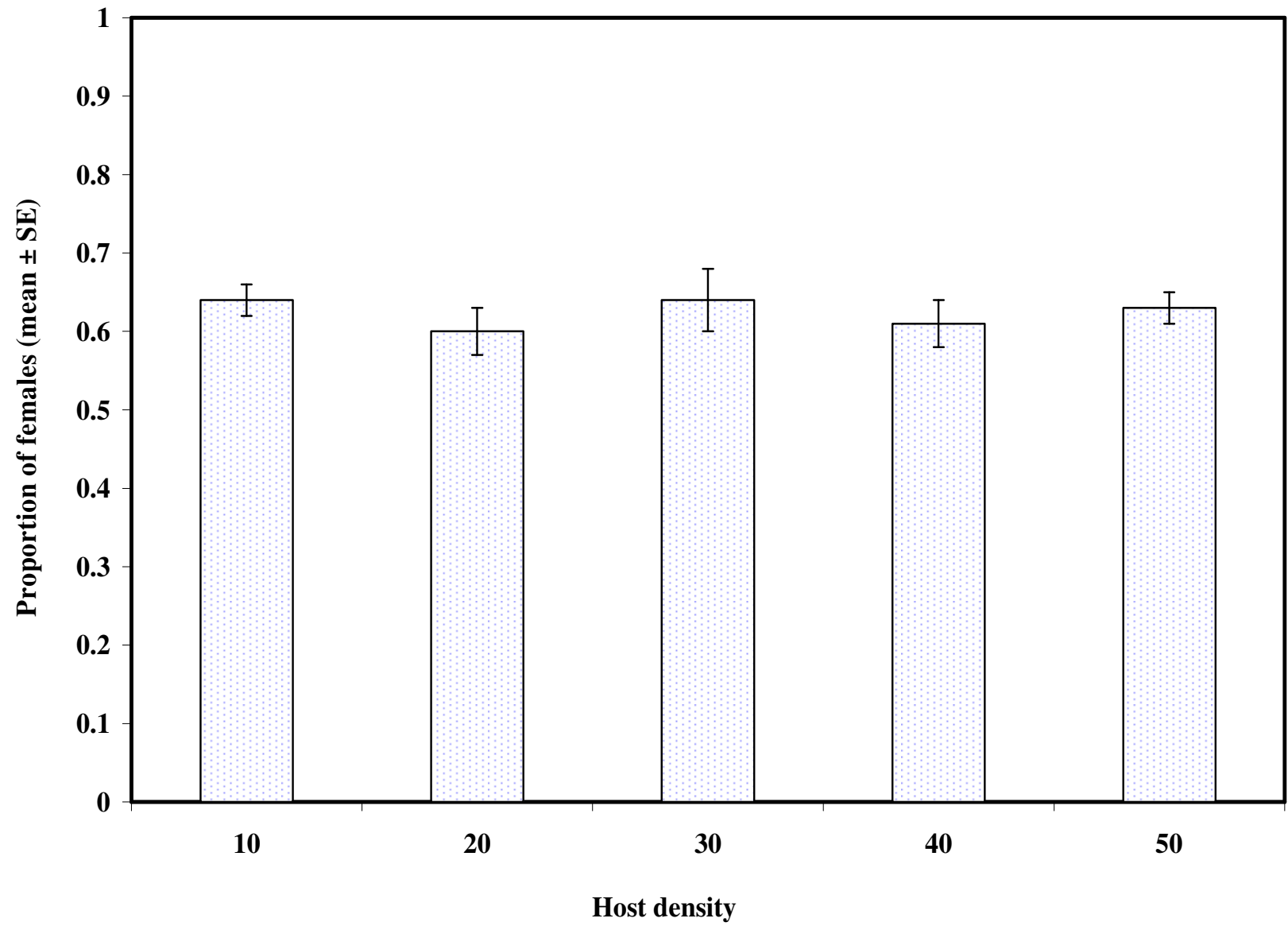
Fig. 7. Relationship between size of the rearing containers and parasitoid progeny production with a density of 50-last instar *P. interpunctella* larvae.

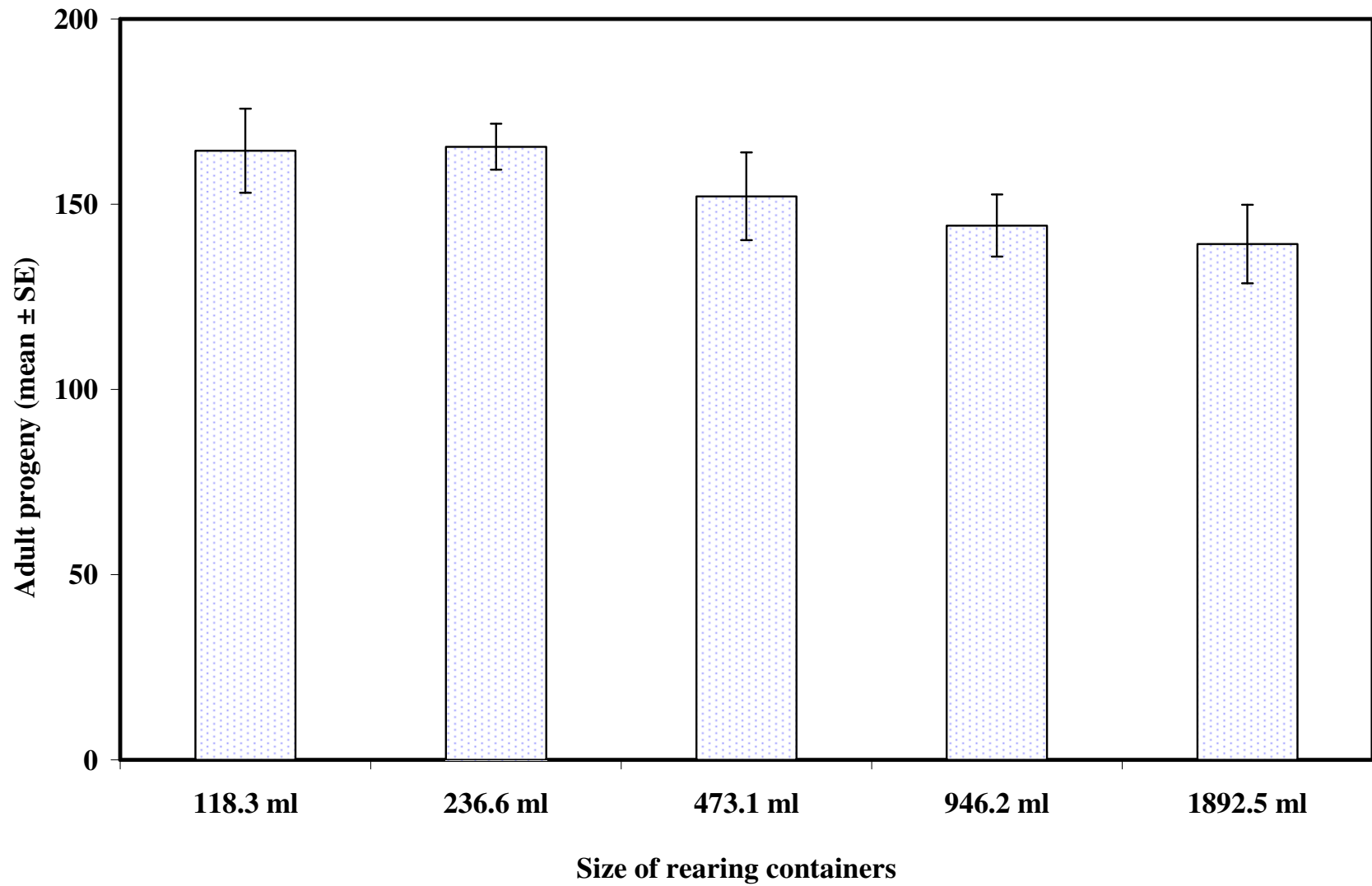
Fig. 8. *Bracon hebetor* mass rearing experiments. (A) Plastic yogurt cup 236.6 ml (8 oz). (B) Yogurt cup fitted in 473.1 ml glass jar (16 oz pin jar) and used for parasitoid density and host density experiments. (C) Size of the rearing containers (from left to right 1982.5, 946.2 473.1 236.6, and 118.3 ml) used in this experiment.

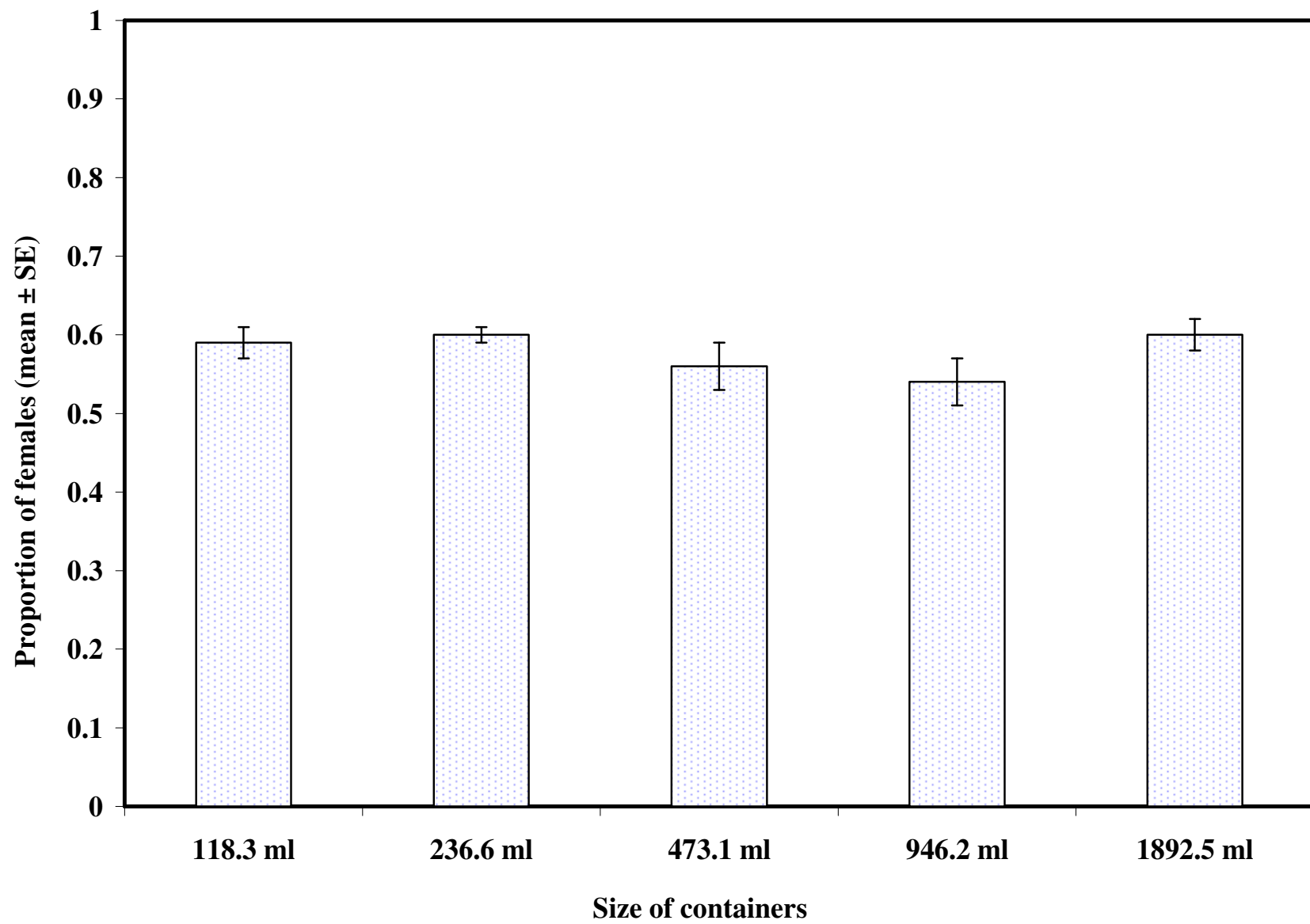


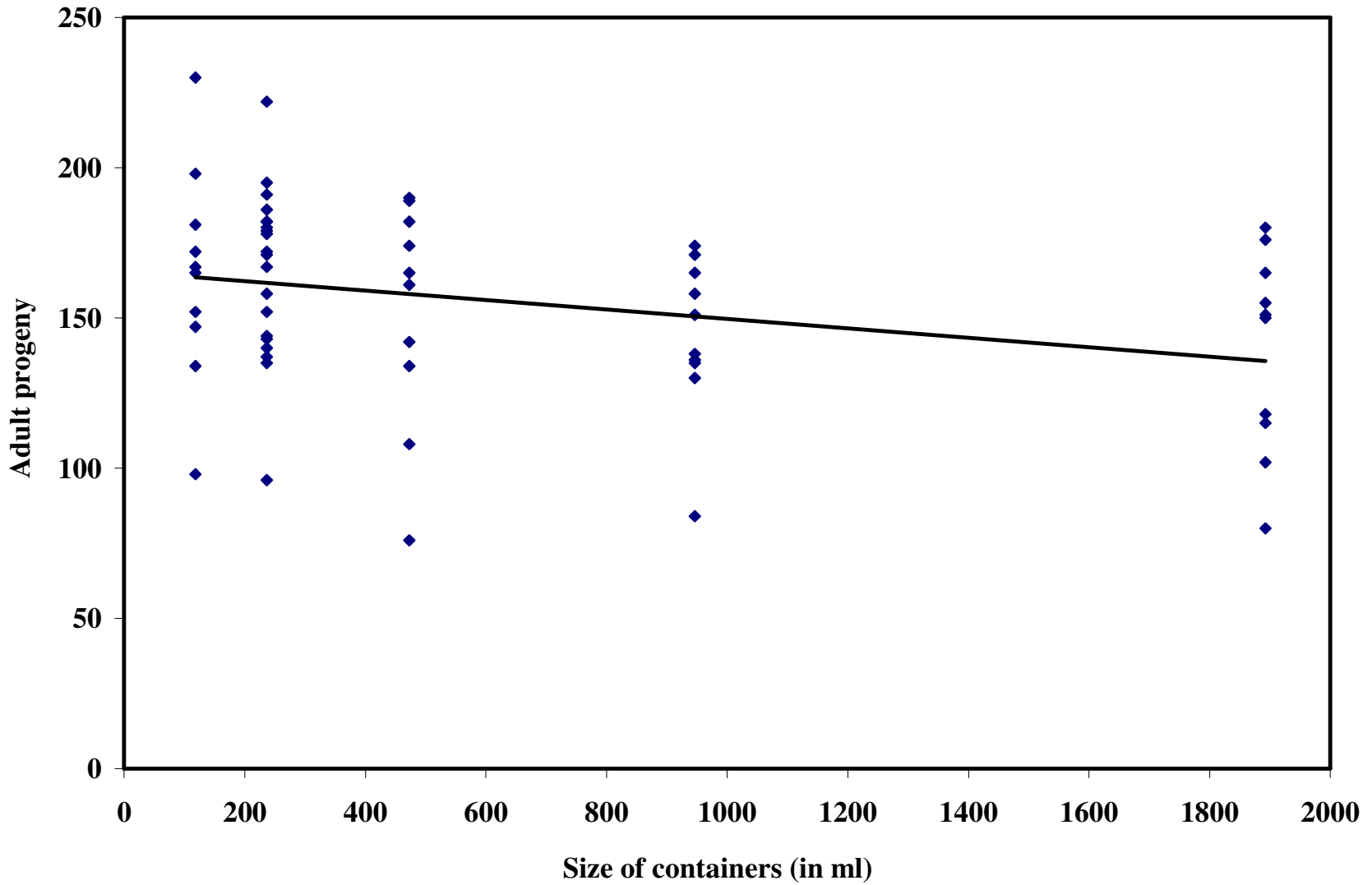


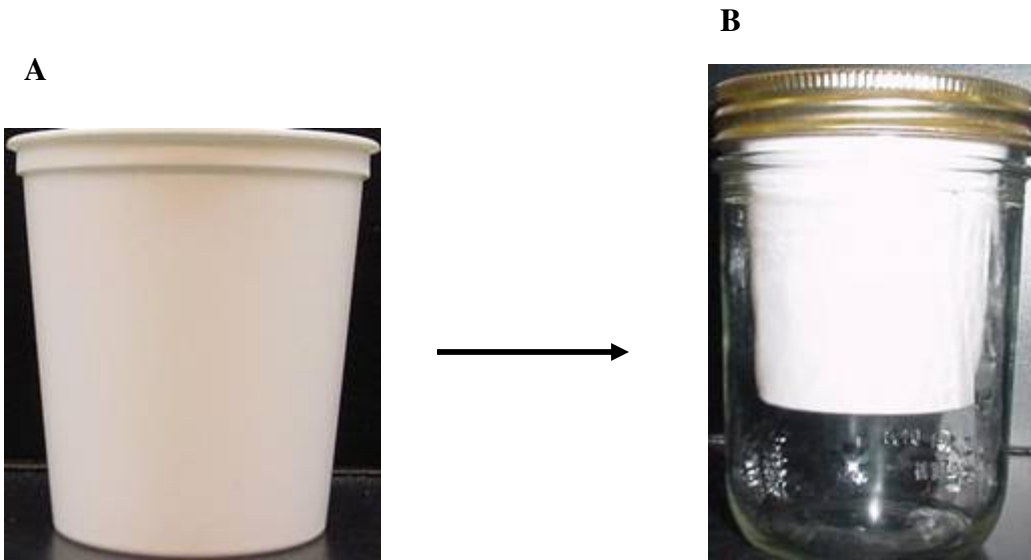












CHAPTER V
SUMMARY AND CONCLUSIONS

The research described herein provides valuable information on effects of lepidopteran host species on reproductive performance of *Bracon hebetor* Say (Hymenoptera: Braconidae). *B. hebetor* is a cosmopolitan gregarious ectoparasitoid of several species of Lepidoptera particularly stored-products pyralid moths. *B. hebetor* could become an efficient and environmentally friendly biological control agent for the management of stored-product moths in granaries, warehouses, feed-mills, and food processing facilities. There is potential for combining *B. hebetor* with other control measures, including chemical control as well as combining with egg parasitoid and predators, which could make treatment more effective and facilitate better integrated pest management of stored-products insects (Press et al. 1974, 1977, 1982, Grieshop et al. 2006, Baker et al. 1995).

In Chapter II, the results on the suitability of various lepidopteran hosts for development of *B. hebetor* was presented. The results of this study indicated that *B. hebetor* females were able to paralyze and oviposit on most or all individuals of each host species that were presented and they reproduced to some degree on all hosts except for *Tineola bisselliella* (Hummel) (Lepidoptera: Tineidae). The cumulative fecundity in the five-day period was highest on *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) (106.42 ± 5.19 eggs) and lowest on *T. bisselliella* (9.64 ± 1.28 eggs). The highest percentage of parasitoid survival to the adult stage was on *A. transitella* (84.07 ± 2.26) and no adults were produced on *T. bisselliella*. Egg to adult development time was shortest on *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) (9.75 ± 0.25 days) and longest on *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) (12.63 ± 0.28 days). Based on reproductive fitness parameters such as mean daily fecundity, parasitoid survival to adulthood, development time, and parasitoid secondary sex ratio, this study

revealed that *A. transtitella* was the most suitable host followed by other pyralid species, except for *G. mellonella*, which appeared to be a marginally suitable host, and *T. bisselliella*, which was the most unsuitable host of those tested. Of the noctuid species, *Spodoptera exigua* (Hübner) appeared to be a marginally suitable host and two heliothine species tested were very low suitability hosts. Although *B. hebetor* can be considered relatively polyphagous because it could parasitize and successfully develop on moth larvae from several families of Lepidoptera, this research suggests that *B. hebetor* is a relative host specialist on stored-product pyralid moths in the sub-family Phycitinae.

In Chapter III the results on the reproductive performance of *B. hebetor* on six different pyralid hosts was presented. The results of this study indicate that host species can have a significant effect on several aspects of the parasitoid's reproductive performance, such as developmental time, oviposition period, lifetime fecundity, longevity, progeny production, and egg-to-adult survivorship. The duration of egg-to-adult development period was longest on *G. mellonella* (12.6 d) and shortest on *E. cautella* (9.7 d) and *Plodia interpunctella* (Hübner) (9.9 d), indicating that *B. hebetor* immatures respond differently to different host resources, both qualitatively and quantitatively, by either developing slowly and utilizing host resources with maximum efficiency or by developing quickly and utilizing host resources with lower efficiency (Godfray, 1994). The duration of the oviposition period was longest on *E. kuehniella* (Zeller) (48.7 d) and *E. cautella* (49.2 d) and shortest on *Corcyra cephalonica* (Stainton) (33.7 d) and *P. interpunctella* (34.7 d). Results from Chapter II reveal that, although *B. hebetor* can paralyze and lay eggs on larvae of several pyralid species, it can not necessarily develop and reproduce optimally on those species.

Chapter IV presents results on mass rearing methods for *B. hebetor* using *P. interpunctella* larvae. The results of this study indicate that commercial-scale rearing of *B. hebetor* can be maximized with a host density of 50-last instar *P. interpunctella* larvae in a relatively small container of about 250 ml. (e.g., an 8 oz. glass jelly jar or plastic yogurt cup) and with eight male-female pairs of *B. hebetor* to allow adequate host utilization and wasp development over a five-day period in a controlled environment. Economic costs of mass rearing biological control agents should be minimized for the benefits of such activities to be profitable and effective for the pest management activity (Schoeller et al. 2006). The maximum production of *B. hebetor* adults in a 5-day cycle using small rearing containers reported here could facilitate more cost-effective mass-rearing of this important parasitoid for biological control of stored product moths.

References

- Baker, J. E., D. K. Weaver, J. E. Throne, and J. L. Zettler. 1995.** Resistance to protectant insecticides in two field strains of the stored-product insect parasitoid *Bracon hebetor* (Hymenoptera: Braconidae). *J. Econ. Entomol.* 88: 512-519.
- Grieshop, M. J., P. W. Flinn, and J. R. Nechols. 2006.** Biological control of Indianmeal moth (Lepidoptera: Pyralidae) on finished stored products using egg and larval parasitoids. *J. Econ. Entomol.* 99: 1080-1084
- Godfray, H. C. J. 1994.** Parasitoids. Behavioral and evolutionary ecology. Princeton University Press, NJ, USA
- Press, J. W., B. R. Flaherty, and R. T. Arbogast. 1974.** Interactions among *Plodia interpunctella*, *Bracon hebetor*, and *Xylocoris flavipes*. *Environ. Entomol.* 3: 183-184.
- Press, J. W., B. R. Flaherty, and R. T. Arbogast. 1977.** Interactions among *Nemeritis canescens* (Hymenoptera: Ichneumonidae), *Bracon hebetor* (Hymenoptera: Braconidae), and *Ephestia cautella* (Lepidoptera: Pyralidae). *J. Kans. Entomol. Soc.* 50:259-262.
- Press, J. W., L.D. Cline, and B. R. Flaherty. 1982.** A comparison of two parasitoids, *Bracon hebetor* (Hymenoptera: Braconidae) and *Venturia canescens* (Hymenoptera: Ichneumonidae), and a predator *Xylocoris flavipes* (Hemiptera: Anthocoridae) in suppressing residual population of the almond moth, *Ephestia cautella* (Lepidoptera: Pyralidae). *Ibid.* 55: 725-728.

Scholler, M., P.W. Flinn, M. Grieshop, and E. Zdarkova. 2006. Biological Control of Stored Product Pests. In: J.W. Heaps, Editor, Insect Management for Food Storage and Processing. St. Paul, MN. AACC International. p. 67-87.

VITA

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DISSERTATION: REPRODUCTIVE PERFORMANCE OF THE PARASITOID
BRACON HEBETOR SAY (HYMENOPTERA: BRACONIDAE) ON VARIOUS
HOST SPECIES OF LEPIDOPTERA.

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Pages in Study: 116

Candidate for the Degree of Doctor of Philosophy

Major Field: Entomology

Scope and Method of Study: The objective of this study was to investigate the reproductive performance of *Bracon hebetor*, a larval ecto-parasitoid of stored product moth species, on several species of lepidopteran insects, including *Plodia interpunctella*, *Ephestia kuehniella*, *E. cautella*, *Corcyra cephalonica*, *Amyelois transitella*, and *Galleria mellonella*. *B. hebetor* females were introduced singly into experimental arenas and allowed to sting and oviposit for five days with a fresh host given daily. Experiments were conducted using Petri dishes (100 × 15 mm). In life history studies, *B. hebetor* females were introduced singly into Petri dishes and given a single host larva every day throughout their life time. I also investigated the effect of parasitoid and host density, and size of rearing containers on progeny production and sex ratio of *B. hebetor*. Statistical analyses were done using PC SAS Version 9.1.

Findings and Conclusions: The mean cumulative fecundity in five days was highest on *A. transitella* (106.42 ± 5.19) and lowest on *T. bisselliella* (9.64 ± 1.28). The egg-to-adult survivorship and progeny sex ratio were also significantly affected by the host species. The highest percentage of parasitoid survival was on *A. transitella* (84.07 ± 2.26) and zero on *T. bisselliella*. Daily fecundity was highest on *G. mellonella* (22.09 ± 0.42) and *C. cephalonica* (21.64 ± 0.35) and lowest on *E. cautella* (13.39 ± 0.24). The egg-to-adult survivorship and progeny sex ratio were also significantly affected by the host species. The highest percentage of parasitoid survival was on *A. transitella* (75.69 ± 1.99) and *C. cephalonica* (75.42 ± 2.47) and lowest on *G. mellonella* (49.71 ± 1.74). Although *B. hebetor* can paralyze and lay eggs on several moth species, it can develop and reproduce optimally on only a few species of pyralid hosts. Maximum progeny production in mass-rearing (188.89 ± 12.28 adults) was achieved with eight female wasps on a density of 50 host larvae in glass jars of approximately 0.25 L. The possible application of these results for biological control of stored product insects is discussed.

ADVISER'S APPROVAL: Kristopher L. Giles
