Biology of *Aphaereta* sp. n (Hymenoptera: Braconidae: Alysiinae), a new larval parasitoid of *Ceratitis capitata* Wied. (Diptera: Tephritidae)

Tânia Teixeira

Ponta Delgada,
Outubro 2011

Tânia Isabel Peres Teixeira

BIOLOGY OF *APHAERETA* SP. N (HYMENOPTERA: BRACONIDAE: ALYSIINAE), A NEW LARVAL PARASITOID OF *CERATITIS CAPITATA* (WIEDEMANN) (DIPTERA: TEPHRITIDAE)

Thesis submitted in fulfillment of the requirements for the degree of

Master of Biotechnology in Biological Control

Supervisor: Doutora Luísa Oliveira

Department of Biology

Ponta Delgada

October 2011

Acknowledgments

Como nenhum trabalho pode ser realizado sem a contribuição de outras pessoas, este não foi excepção. Começo por agradecer a minha orientadora, a Doutora Luísa Oliveira, pelos seus conselhos científicos, ao Departamento de Biologia, ao Centro de Investigação de Recursos Naturais (CIRN) e a Direcção Regional da Ciência e Tecnologia pelo apoio institucional e logístico. Deixo também um agradecimento especial ao Professor Doutor José Silvino Rosa, à Doutora Isabel Borges, ao Dr. Nuno Rainha e à Dr.ª Raquel Mendes que em momentos de aflição me orientaram e ajudaram, permitindo-me deste modo finalizar este trabalho.

Agradeço também à minha família e ao meu amor todo o apoio e compreensão que sempre demonstraram em todas as minhas decisões por mais que estas provocassem as minhas ausências, porque se hoje sou o que sou, e tenho o que tenho, a vocês o devo. E como não poderia deixar de ser, deixo um agradecimento especial ao Senhor Manuel Fernando que me acompanhou em tudo, desde o inicio deste trabalho, as suas conversas, as nossas risadas e as minhas cantorias, contribuíram sempre para um bom ambiente e uma saudável amizade.

Por último, mas não menos importante a todos os meus amigos e aqueles que, directa ou indirectamente, contribuíram para a realização deste trabalho, o meu muitíssimo obrigado.

Contents

Resumo

As espécies que pertencem ao género *Aphaereta* estão distribuídas praticamente por todo o mundo, e vivem associadas a dípteros sinantrópicos e outros dípteros. Foi encontrado um total de 12 indivíduos, da população em estudo, em duas pupas de *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), estes indivíduos são endo-parasitóides gregários larva-pupa. Foram estimados pela primeira vez os parâmetros biológicos de *Aphaereta* sp., em relação ao tamanho do hospedeiro e temperatura. Foram testados três estados larvares, o primeiro (pequeno), o segundo (médio) e o terceiro (grande) estado, e cinco temperaturas (12, 15, 20, 25 e 30ºC). O tamanho do hospedeiro afecta significativamente o tempo de desenvolvimento, o número de indivíduos por pupa, a taxa de parasitismo, o sexo rácio, a fecundidade e a longevidade das fêmeas de *Aphaereta* sp. O segundo estado larvar de *C. capitata*, de todos os estados larvares testados, é o que promove uma maior taxa intrínseca de desenvolvimento (r_m). Observou-se que o parasitóide e o seu hospedeiro possuem diferentes constantes térmicas e limites de desenvolvimento. Verificou-se também que a taxa de desenvolvimento de *Aphaereta* sp. aumenta linearmente com a temperatura, e esta efeito significativo sobre os parâmetros reprodutivos (R_0, r_m, λ, DT e o T) deste parasitóide.

Palavras-Chave: *Aphaereta* sp.; *Ceratitis capitata*; desenvolvimento do parasitóide; tabelas de vida; tamanho do hospedeiro; temperatura.

Abstract

The species of the genus *Aphaereta* occurs almost everywhere around the world and lives associated to sinatropic dipterous and other Diptera. Our population was collected from two pupae of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), in a total of 12 adults, and is a gregarious larval-pupal endoparasitoid. Biological and life table parameters of the *Aphaereta* sp. were evaluated for the first time, in relation to the size of host and temperature. Three host larval instars, first (small), second (medium) and third instar (large) and five temperature levels (12, 15, 20, 25 and 30ºC) were studied. The development time, progeny per pupa, number of offspring per female parasitism, female longevity and sex ratio of the *Aphaereta* sp. are affected by host larval sizes. From all larval instars of the *C. capitata* tested, the second instar promotes a higher intrinsic rate of increase (r_m). The lower developmental threshold and thermal constant of the host and the parasitoids differ. The rate of development of the parasitoids increased with a linear trend as the temperature increased from 15ºC to 30ºC. Temperature had a significant effect on the net reproductive rate, intrinsic rate of increase, finite capacity for increase and doubling time.

Keywords: *Aphaereta* sp.; *Ceratitis capitata*; parasitoid development; life tables; host size; temperature.

CHAPTER 1

General Introduction

Introduction

The order Hymenoptera is one of the four major orders of insects, with approximately 115 000 species described. These species correspond to only 1/10 of the population of this group, which includes an impressive variety of shapes, sizes and lifestyles. Just, a few groups of insects are ecological and economically important to mankind as the Hymenoptera, but its mismanagement can cause enormous costs for ecosystem (Rasplus *et al.*, 2010). For example, Babendreier *et al.* (2003) found in laboratory study conducted to that *Trichogramma brassicae* Bezdenko parasitizes eggs of 22 out of 23 lepidopteran species tested, including some, which are listed on the Swiss red list of endangered species. Biological control is a potentially valuable control strategy against invasions of insect pests in agricultural and forest ecosystems. However, doubts about this strategy have grown due to fault of monitoring of the effects of biological control agents on target and non-target species, after his release (Delfosse, 2005).

In Azores (Portugal), we tried to search for parasitoids adapted to the conditions of the region. In recent years, we focused on capturing and recording parasitoids of the *Ceratitis capitata*. It also designated as the Mediterranean fruit fly or medfly. It was first reported in the Azores in 1829 (MacLeay, 1829), but in the last years due to the severe damages in fruit crops that have an important impact on the Azorean economic agricultural outputs. It is considered one of the most important fruit pests in the world because it develops in fruit species, most of which are of high commercial value (Medeiros, 2004). Of all parasitic Hymenoptera foraging on fruits in the field, only three, *Asobara rufescens* (Förster) (Braconidae), *Tachinaephagus zealandicus* Ashmead (Encyrtidae) and *Aphaereta* sp. n (Braconidae), parasitized medfly larvae and successfully completed a generation under laboratory conditions. The last one is a small braconid wasp of the Alysiines tribe (genus *Aphaereta*), found in October 2008 in Sao Miguel Island, inside two pupae of *C. capitata* from infected peppers, identified by K. van Achterberg (not publish).

All alysiines are endoparasitoids (internal parasitoids) of flies. The adult female lays her eggs keen on the egg or larva of the host fly, and her progeny emerged from the host puparium (Berry, 2007). *Aphaereta* is the only genus in its subfamily currently known to contain gregarious species (Wharton, 1980). The gregarious nature of some *Aphaereta* species is well established from rearing records, experimental studies of clutch size and developmental studies (Evans, 1933; Salkeld, 1959; Houser & Wingo, 1967; Vet *et al.*, 1993, 1994; Visser, 1994, 1996). On the other hand, few studies exist on the substantial knowledge of their biology and ecology.

The establishment of life tables is an essential constituent to know the population dynamics of a species (Southwood, 1978), and is now generally used in insect ecology to develop an understanding of age-specific mortality and reproductive rates (Carey, 1993). These life history parameters give useful information on the effectiveness of biological control agents, particularly when comparing them with the ones of the target pest. Development, reproduction and survival of parasitoid insects are life history components that depend on physiological and environmental factors (Harbison *et al.*, 2001; Ucçkan and Ergin, 2003). These factors can be either abiotic (e.g. temperature, relative humidity) or biotic (e.g. size, age, and density of the host).

For gregarious parasitoids, body size depends upon resource availability during development (Harvey *et al.*, 1998), for the reason that siblings within the same brood develop on a single host and compete for a fixed quantity of resources. Thus, to optimize progeny fitness, the female estimated to vary the allocation of eggs to a host depending on the host's size or quality (Thorne *et al.*, 2006). However, the influence of host size on koinobiont parasitoids is complex (Harvey, 2005). Larval development of koinobiont parasitoids such as *Aphaereta* sp.

subsequent to parasitization relies on the growth rate of the host (Brodeur & Boivin, 2004; Harvey, 2005; Pennacchio & Strand, 2006). The host stage selected for oviposition by a koinobiont female is primarily based on the first host evaluation. There is a host size (larval instars) threshold below which the female parasitoid rejects the host for parasitization (Brodeur & Boivin, 2004). Large hosts can exhibit defensive behavior, and this can interfere with the possibility of parasitization (Brodeur *et al.*, 1998).

It has long been recognized that temperature is the most important factor determining development rates of the immature stages and the adult maturation rates of the majority of insects (Robinson & Hooper, 1989). As a generally accepted guideline, low temperature results in a slower growth rate (Sibly & Atkinson, 1994). In host/parasitoid system, the host and the parasitoid larvae coexist and closely interact until the end of the parasitoid development, and temperature thus affects the length of time the parasitoid and the host are physiologically integrated. For example, at high temperatures, the short young development increases adult parasitoid chance for resource access, reduces the generation time (Pandey & Singh, 1999). The relationship among temperature and developmental rate has been described as negative and linear over most of the temperature range. The environmental adaptation of the introduced parasitoid is one of the factors that determine your success as a biological control agent (Kalyebi *et al.*, 2006).

Objectives of this research

This study is included within a fraction of a larger project to investigate new environment-oriented tools to control *C. capitata*. The objectives of this research were to study some biological parameters, especially the reproductive capacity of *Aphaereta* sp., with the host *C. capitata*, in order to understand their biology.

Firstly, the effect of the host larval size on biological and life table parameters of *Aphaereta* sp., such as development time, longevity, fecundity, sex ratio, and parasitization rate were investigated. Secondly, we examined the temperature effect on biological and life table parameters of *Aphaereta* sp., using the host larval instars than directly providing the best development of this parasitoid in the previous assay. Finally, we tested two models (one linear and one non-linear) to describe the relationship between temperature and the rate of development (1/D).

References

BABENDREIER, D., S. KUSKE & F. BIGLER, 2003. Non-target host acceptance and parasitism by *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) in the laboratory. *Biol Control*, 26: 128–138.

BERRY, J.A., 2007. *Alysiinae (Insecta: Hymenoptera: Braconidae): Fauna of New Zealand*. Manaaki Whenua Press, Lincoln, New Zealand.

BRODEUR, J. & G. BOIVIN, 2004. Functional ecology of immature parasitoids. *Annu Rev Entomol*, 49: 27–49.

BRODEUR, J., J.B.F. GEERVLIET & L.E.M. VET, 1998. Effect of *Pieris* host species on life history parameters in solitary specialist and gregarious generalist parasitoids (*Cotesia* species). *Entomol Exp Appl*, 86: 145–152.

CAREY, J.R., 1993. *Applied demography for biologists with special emphasis on insects*. Oxford University Press, New York, USA.

DELFOSSE, E.S., 2005. Risk and ethics in biological control. *Biol Control*, 35: 319–329.

DICKE, M., 1999. Direct and indirect effects of plants on the performance of beneficial organisms. In: Ruberson JR (ed) *Handbook of pest management*. Marcel Dekker, New York, USA.

EVANS, A.C., 1933. Comparative observations on the morphology and biology of some hymenopterous parasites of carrion-infesting Diptera. *Bull Entomol Res*, 24:385-405.

HARBISON, J.L., J.C. LEGASPI, S.L. FABRITIUS, R.R. SADAN, B.C. LEGASPI & A. ENKEGAARD, 2001. Effects of age and host number on reproductive biology of *Allorhogas pyralophagus* (Hymenoptera: Braconidae) attacking the Mexican rice borer (Lepidoptera: Pyralidae). *Environ Entomol*, 30(1): 129–135.

HARVEY, J.A., 2005. Factors affecting the evolution of development strategies in parasitoid wasps: the importance of functional constraints and incorporating complexity. *Entomol Exp Appl*, 117: 1–13.

HARVEY, J.A., L.E.M. VET, N.Q. JIANG & R. GOLS, 1998. Nutritional ecology of the interaction between larvae of the gregarious ectoparasitoid, *Muscidifurax raptorellus* (Hymenoptera: Pteromalidae), and their pupal host, *Musca domestica* (Diptera: Muscidae). *Physiol Entomol*, 23: 113–120.

HOUSER, E.C. & C.W. WINGO, 1967. *Aphaereta pallipes* as a parasite of the face fly in Missouri, with notes on laboratory culture and biology. *J Econ Entomol*, 60: 731-733.

KALYEBI, A., W.A. OVERHOLT, F. SCHULTHESS, J.M. MUEKE & S. SITHANANTHAM, 2006. The effect of temperature and humidity on the bionomics of six African egg parasitoids (Hymenoptera: Trichogrammatidae). *Bull Entomol Res*, 96: 305–314.

MACLEAY, W.S., 1829 Notice of *Ceratitis citriperda*, an insect very destructive to orange. *Zool J*, 4: 475-482.

MEDEIROS, A.M.C., 2004. Bioecologia e controlo com nematodes entomopatogénicos de *Ceratitis capitata* (Diptera: Tephritidae) em S. Miguel. M.S. thesis, Universidade dos Açores, Portugal.

PANDEY, S. & R. SINGH, 1999. Host size induced variation in sex ratio of an aphid parasitoid *Lysiphlebia mirizai*. *Entomol Exp Appl*, 90:61– 67.

PENNACCHIO, F. & M.R. STRAND, 2006. Evolution of developmental strategies in parasitic Hymenoptera. *Annu Rev Entomol*, 51:233–258.

RASPLUS, J.-Y., C. VILLEMANT, M.R. PAIVA, G. DELVARE, A. ROQUES, 2010. Hymenoptera. Chapter 12. In: Roques A et al. (Eds) *Arthropod invasions in Europe*. BioRisk 4(2):669–776

ROBINSON, A.S. & HOOPER, G., 1989. *Fruit flies their biology, natural enemies and control*, vol 3B. Elsevier science publishers, Amsterdam, Netherlands.

SALKELD, E.H., 1959. Notes on anatomy, life-history, and behavior of *Aphaereta pallipes* (Say) (Hymenoptera: Braconidae), a parasite of the onion maggot, *Hylemia antiqua* (Meig.). *Can Entomol*, 91:93-97.

SIBLY, R.M. & D. ATKINSON, 1994. How rearing temperature affects optimal adult size in ectotherms. *Funct Ecol*, 8: 486–493.

SOUTHWOOD, T.R.E., 1978. *Ecological Methods*. Wiley, New York, USA.

THORNE, A.D., J.J. PEXTON, C. DYTHAM & P.J. MAYHEW, 2006. Small body size in an insect shifts development, prior to adult eclosion, towards early reproduction. *Proc. R. Soc. B*, 273: 1099-1103.

UÇKAN, F. & E. ERGIN, 2003. Temperature and food source effects on adult longevity of *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae). *Environ Entomol* 32(3): 441–446.

VET, L.E.M., A. DATEMA, A. JANSSEN & H. SNELLEN, 1994. Clutch size in a larval-pupal endoparasitoid: Consequences for fitness. *J Anim Ecol*, 63 (4):807-815.

VET, L.E.M., A. DATEMA, K. VAN WELZEN, H. SNELLEN, 1993. Clutch size in a larval-pupal endoparasitoid. *Oecologia*, 95: 410-415.

VISSER, M.E., 1994. The relationship between size and fitness in females of the parasitoid *Aphaereta minuta* (Hymenoptera: Braconidae). *J Anim Ecol*, 63: 963-978.

VISSER, M.E., 1996. The influence of competition between foragers on clutch size decisions in an insect parasitoid with scramble larval competition. *Behav Ecol*, 7: 109-114.

WHARTON, R.A., 1980. *Review of the nearctic Alysiini species* (Hymenoptera, Braconidae) *with discussion of generic relationships within the tribe*. University of California publications in entomology, Berkeley, California, USA.

CHAPTER 2

Life history of Aphaereta *sp. n, a newly discovered larval parasitoid of* Ceratitis capitata

Life history of *Aphaereta* sp. n, a newly discovered larval parasitoid of *Ceratitis capitata*

Tânia Teixeira* and Luísa Oliveira

Abstract

Given the economic importance of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) in fruit growing and the few non-evasive means to combat this pest, we pretend with this work explore the ability of a new braconid wasp of the Alysiines tribe (genus *Aphaereta*), found in the Azores in 2008. We evaluation the biological and life table parameters of this parasitoid, in relation to the size of the host and temperature. Larvae of different sizes (first, second and third instar larvae) of *C. capitata* and five temperature levels (12, 15, 20, 25 and 30ºC) were used. Of all the biological parameters evaluated, only development time, progeny per pupa, number of offspring female per female, female longevity, and sex ratio of the adults of the *Aphaereta* sp differed significantly between the first instar and the other two instars tested. The combination of these results with those obtained with the life table parameters tell us that the second instar to directly providing higher fitness of the adults of the parasitoid. Temperature had a significant effect on the net reproductive rate, intrinsic rate of increase, finite capacity for increase and doubling time. Data were adjusted to the linear degree-day model and the nonlinear Gaussian function model. According to the linear model the lower developmental threshold and thermal constant of the host and the parasitoids differ. The rate of development of the parasitoids increased with a linear trend as the temperature increased from 15ºC to 30ºC.

Keywords: *Aphaereta* sp.; *Ceratitis capitata*; parasitoid development; life tables; host size; temperature.

Introduction

Ceratitis capitata Wiedemann (Diptera: Tephritidae) is a pest that causes substantial economic losses in the Mediterranean fruit due to their high dispersal ability and ecological plasticity (Liquido *et al.*, 1991; Gillani *et al.*, 2002). Traditionally, the management of the fruit fly has been based primarily on organophosphate insecticides, such as malathion, fenthion and trichlorphon witch cause serious environmental problems like pest resurgence and secondary pest outbreaks, insecticide resistance, environmental pollution, and increased human health risks (Dantas *et al.*, 1999; Ekesi, 1999). This situation originates a need to find alternative methods to control this pest. Headrick & Goeden (1996) consider that the parasitic Hymenoptera are the most promising candidates for Medfly biological control.

In Azores (Portugal), several studies have addressed the distribution of the *C. capitata* in S. Miguel Island (Medeiros 2004), as well as their control, biology and ecology (Medeiros, 2004; Lopes *et al.*, 2006; Oliveira *et al.*, 2008). In recent years, we have been focusing on capturing and recording parasitoids of the *C. capitata*, in order to prevent the introduction of exotic species that can cause adverse effects on the parasitoids native and non-target species. Of all parasitic Hymenoptera foraging on fruits in the field only three present a sufficient number for parasitized Medfly larvae and successfully completed a generation under laboratory conditions: *Asobara rufescens* (Förster) (Braconidae), *Tachinaephagus zealandicus* Ashmead (Encyrtidae) and *Aphaereta* sp. n (Braconidae).

Aphaereta sp. n were found in São Miguel Island, during autumn 2008, in two pupae of *C. capitata* collected in red sweet pepper, and identified by K. van Achterberg. Is the first time that an individual from this genus, is found to parasitize *C. capitata*. Like another species of this genus, it is a small gregarious larval-pupal endoparasitoid. As such, it is necessary to know the main phases of its biology, before thinking about using it in classical biological control strategy. Life history factors can be used to calculate approximately the possible impact of the parasitoid on host populations (Bernal & González, 1993). These parameters depend on physiological and environmental factors (Harbison *et al.*, 2001; Uçkan & Ergin, 2003). These factors can be either abiotic (e.g. temperature, relative humidity) or biotic (e.g. size, age, and density of the host).

Among the biotic factors, host size (= quality) is one of the most important signals to the female parasitoid, because it is based on this size that the female estimates the amount of egg laying (clutch size), especially the females gregarious species (Omwega & Overholt, 1997; Seko & Nakasuji, 2004). The mechanism of estimating host size previous to allocating the progeny is without doubt a strategy to avoid competition between the developing of the offspring. It is reported that host size influences parasitoid's preference, fecundity, and developmental time (Opp & Luck, 1986; Bai *et al.*, 1992; Smith, 1993) and be positively correlated to fitness parameters, such as fecundity and survival of parasitoids (Mackauer & Sequeira, 1993; Godfray, 1994).

Temperature is one of the main abiotic factors influencing the physiological performance of ectothermic organisms (Krenek *et al.*, 2011). In host/parasitoid system, the host and the parasitoid larvae coexist and closely interact until the end of the parasitoid development and temperature thus affects the length of time the parasitoid and the host are physiologically integrated (Colinet *et al.*, 2007). For example, at high temperatures, the short immature development increases adult parasitoid opportunities for resource access (food, hosts, mating), reduces the generation time (Pandey & Singh, 1999). These influences in the life history parameters of a parasitoid affect their efficiency as biological control agent.

This work is included within a fraction of a larger project to investigate new environment-oriented tools to control *C. capitata*. The effect of environmental factors on the biological characteristics of *Aphaereta* sp. has never been studied using its host *C. capitata*.

In the present study, we investigated the host larval size effect: first instar (small), second instar (medium) and third instar (large), on biological and life table parameters of *Aphaereta* sp. in laboratory conditions. Another set of laboratory experiments investigated the temperature effect on biological and life table parameters of *Aphaereta* sp., using the host size than directly providing the best development of this parasitoid in the previous assay. We tested two models (one linear and one non-linear) to describe the relationship between temperature and the rate of development (1/D). The lower developmental threshold and thermal constant of the parasitoids were also estimated.

Material and Methods

Parasitoid and Host Cultures

Laboratory cultures of *Aphaereta* sp. were established in October of 2008 using specimens found on pupae *C. capitata* in S. Miguel (Azores, Portugal), by the approach of Falcó *et al.* (2006). Since that date, this specie has been reared on *C. capitata*. The adults of *Aphaereta* sp. were kept into cylindrical plastic containers (5 cm height × 8 cm diameter), with a source of diluted honey (30 %), for 72 h to mate. After this period, the containers are closed with a lace cloth, and it is on this cloth are placed different sized Medfly larvae with artificial diet.

Under the larvae, is placed a lid which keeps the humidity needed for proper development of the larvae. After 48 h, the larvae were removed and placed in another container (2.5 cm height × 5 cm diameter), previously prepared with artificial diet for larvae. Then slap the container with silver paper and place it inside a new cylindrical plastic container with a meshed lid, until the emergence of the new generation.

The host larvae were maintained on an artificial diet, according to Albajes & Santiago-Álvarez (1980) with some modifications, in the acrylic container

(2 L volume). Adults were fed with a mixture of sugar and hydrolysed yeast (3:1, w:w) and water. The cultures were sustained at 25 ± 1ºC, 70 ± 10% rh and 14:10 h (L: D) photo period.

Influence of host larval size on biological and life table parameters of Aphaereta *sp.*

To test the influence of host size on biological characteristics of *Aphaereta* sp., host larvae were selected by instars and size (1.1 ± 0.2, 1.8 ± 0.3, 5.0 ± 0.4 cm length for first, second and third instars, respectively). The parasitoid females used to have between 48 and 72 h old, in order to ensure they mated. A caged cohort of parasitoids was established by enclosing five female and one male into cylindrical plastic containers (2.5 cm height × 5 cm diameter) with a solution of 30 % of honey in a cotton wick. Larval hosts were presented to the cohort in groups of fifteen larvae with a little artificial diet, as described before. Larvae was removed and replaced by fifteen new larvae daily, as explained previous, during four days. The parasitized host pupae were checked daily until the emerged of the parasitoid. After emergence, the adults are placed in cylindrical plastic containers with honey and water in a cotton wick, until his death. Development time, the number and the longevity of adults, number of parasitoids dead before emergence, parasitism rate (number of parasitized pupae/total of pupae), the sex-ratio and the fecundity (number of females offspring/female) were recorded. After parasitoid emergence, we had the attention of dissecting all the pupae, except those giving rise to medfly, to verify the existence of the parasitoid, since, this is gregarious parasitoids and dead insects stay in the pupae. In this study, a total of 20 caged cohort of parasitoid for each of the larval hosts instars were set up and held at 25 ± 1ºC, 70 ± 10% rh and 14:10 h (L: D) photo period.

Temperature effect on biological and life table parameters of Aphaereta *sp.*

This experience was built the same way as the previous experiment. Larval hosts, in the second instar, were presented to cohort in groups of thirty larvae with a little artificial diet, as described before. After 48 h, the larvae were removed and placed thirty new larvae for over 48 h. A total of 14 caged cohort of parasitoid were set up and held in one of five constant temperature conditions (12, 15, 20, 25 and 30°C), 70 ± 10% rh and 14:10 h (L: D) photo period. The parasitized host pupae were checked daily until the emerged of the parasitoid. Development time, the number and the longevity of adults, number of parasitoids dead before emergence, parasitism rate (number of parasitized pupae/total of pupae), the sex-ratio and the fecundity (number of females offspring/female) were recorded. As in the previous experiment, we were careful to dissect the pupae.

Statistics Analysis

The biological parameters such as development time, percent parasitism, mortality, number of parasitoid emerged per pupa of each host, longevity of descendants and sex ratio were analyzed by one-way Anova followed by Fisher's least significant difference (LSD) range test ($P \leq 0.05$). Except for the percentage-based data (mortality and sex ratio), which used the arcsine transformation, the transformation used was the square root transformation.

Population growth rates were calculated by constructing life tables according to Birch (1948). To construct the life tables, age-specific survival rates (l_x) and number of female offspring (m_x) for each age interval (x) per day were used. From these data, the net reproductive rate ($R_0 = \Sigma\, l_x m_x$) which is the number of female progeny per female per generation, the intrinsic rate of increase ($r_m = 1 = \Sigma\, e^{-rmx} l_x m_x$), the finite capacity for increase ($\lambda = e^{rm}$) expressed as multiplication per female per unit time, the doubling time ($DT = (\ln 2)/r_m$) and mean generation time ($T = \ln(R_0/r_m)$, in days) were calculated. After r was computed for the original data (r_{au}), the differences in r_m values were tested for significance by estimating the variance using the jackknife method, which facilitated calculation of the standard errors of r_j estimates (Sokal & Rohlf, 1981; Meyer *et al.*, 1986). The jackknife pseudovalue r_j was calculated for *n* samples using the following equation: $r_j = nr_{au}-(n-1)r_i$. The mean values of (n-l) jackknife pseudo-values for the mean growth rate in each treatment were subjected to independent one-sample *t*-test and to one-way Anova followed by LSD range test ($P \leq 0.05$) for host larval size and temperature effects, respectively.

The effect of temperature on the developmental rate (1/D) of *Aphaereta* sp. was examined using mathematic models. We selected the linear regression and simple *Gaussian* function models to describe the relationship between temperature and developmental rate of this parasitoid. Equations of these models are as follows: i) Linear model: 1/D = a+bT, where 1/D is the developmental rate, a and b are regression coefficients, T is the temperature in ºC; ii) $1/D\ (T) = 1/D_{max}\ \exp[-0.5((T-T_{opt})/\sigma)^2]$, where $1/D_{max}$ is the maximal developmental rate at optimum temperature (T_{opt}) and parameter σ is related to the full curve width at $1/D_{max_{1/2}}$.

The linear model used for describing the developmental rate enables the calculation of the lower developmental threshold (t_l) and thermal constant (K), a kind of physiological time defined as the amount of heat units required for development. K is the reciprocal of the slope b of the straight line so b = 1/K; $t_l = -a/b$ (when 1/D = 0 then $T = t_l$) so $a = -b\ t_l = t_l/K$; $D = K/(T- t_l)$. Linear Regression equations were calculated, and Anova was performed. All statistical analyses for this study were performed using SPSS software version 15.0 for Windows (SPSS, 2006), except the non-linear model (simple *Gaussian* function) was performed using TableCurve 2D software (version 5.01, Systat Software Inc).

Results

Influence of host larval instar (size) on biological and life table parameters of Aphaereta *sp.*

From the three host larval instar tested only two (second and third) were successfully parasitized by *Aphaereta* sp., but sometimes the first instar can be parasitize. This difficulty is reflected in the values obtained for the parasitoid life-history parameters, as shown in Table 1.

The proportion of successfully parasitized larvae was significantly lower for the first and the third instar compared to second instar (F = 64.495, df = 59, P = 0.000). Depending on the larval instar, there were significant differences in development time (F = 89.039, df = 59, P = 0.000), progeny per pupa (F = 65.293, df = 59, P = 0.000), number of offspring female per female (F = 54.194, df = 59, P = 0.000), female longevity (F = 5.437, df = 303, P = 0.05), and sex ratio of the adults (F = 29.263, df = 59, P = 0.000) of the *Aphaereta* sp. On the other hand, there are no significant differences in male longevity (F = 0.288, df = 178, P = 0.750) or mortality rate (F = 0.767, df = 59, P = 0.469).

All the life table parameters differ significantly ($p<0.05$) between hosts instars, as R_0, r_m, T, and λ are higher, while DT are lower in second than third instar (Table 2), revealing that second instar is the more desirable for the development of the parasitoid.

Table 1 Reproductive characteristic (Mean ± SE) of *Aphaereta* sp. when reared on three different larval instars of the *Ceratitis capitata*

Parasitoid life-history parameters	**Host larval instar**		
	First instars	Second instars	Third instars
Development time (days)	21.3 ± 0.2 a	19.4 ± 0.6 b	18.3 ± 0.4 b
Progeny per pupa	1.3 ± 0.4 b	5.7 ± 0.2 a	6.3 ± 0.4 a
Longevity of Females (days)	4.2 ± 1.0 a	2.7 ± 0.1 b	3.1 ± 0.1 a
Longevity of Males (days)	2.8 ± 0.6 a	2.7 ± 0.1 a	2.8 ± 0.1 a
Mortality rate of parasitoid (%)	97.5 ± 0.1 a	36.1 ± 0.0 a	45.8 ± 0.1 a
Parasitization rate (%)	1.7 ± 0.8 c	30.8 ± 2.7 a	12.7 ± 1.3 b
Sex ratio (% of females in the population)	13.3 ± 6.5 a	66.4 ± 1.7 a	70.7 ± 4.1 a
Female fecundity (number of female per female)	0.06 ± 0.0 c	7.5 ± 1.0 a	3.7 ± 0.6 b

Means within each row followed by the same letters were not significantly different between temperature $P \leq 0.05$ (LSD range test)

Table 2 Life table parameters (Mean ± SE) of the parasitoid *Aphaereta* sp. ovipositing in different host larval sizes of the *Ceratitis capitata*. Legend: R_0 - net reproductive rate, r_m - intrinsic rate of increase, T - mean generation time, λ - finite capacity for increase, DT - doubling time

Parameters	Jackknife estimation	
	Second instars	Third instars
Net reproductive rate (R_0)	7.46 ± 0.00 a	3.62 ± 0.00 b
Intrinsic rate of increase (r_m)	0.45 ± 0.01 a	0.34 ± 0.01 b
Generation time (T)	4.86 ± 0.09 a	4.41 ± 0.13 b
Finite capacity for increase (λ)	1.54 ± 0.01 a	1.37 ± 0.01 b
Doubling time (DT)	1.67 ± 0.03 b	2.37 ± 0.07 a

Means within each row followed by the same letters were not significantly different *t*-test at 5% for comparison between larvae of the second and third instars.

Temperature effect on biological and life table parameters of Aphaereta *sp.*

Of the five temperatures tested, only in three of them, 15, 20 and 25ºC, it confirmed the emergence of adults of this parasitoid. The development time of *Aphaereta* sp. decreased significantly (F = 37.592, df = 41, P = 0.000) with an increase in temperature from 15ºC to 25ºC (Table 3). The progeny per pupa was significantly differed depending on the temperature (F = 3.559, df = 41, P = 0.038), similar at 15 and 20ºC (54.1 and 59.8, respectively) and significantly reduced at 25ºC. The mortality rate of parasitoids (F = 0,877, df = 41, P = 0.424), the parasitization rate (F = 1.783, df = 41, P = 0.182), the sex ratio of the adults (F = 0.243, df = 41, P = 0.785) and the female fecundity (F = 2.150, df = 41, P = 0.130), did not differed significantly when *Aphaereta* sp. developed at study temperatures (Table 3). Life table parameters are presented in Table 4. In general, the life table parameters predictable by the method of "Jackknife estimation" are higher at 20ºC, except for the DT that is higher at 25ºC.

Table 3 Reproductive characteristics (Mean ± SE) of *Aphaereta* sp. when reared on second instar larvae of the *Ceratitis capitata* at three different temperatures

Parasitoid life-history parameters	**Temperatures regimes**		
	15ºC	20ºC	25ºC
Development time (days)	41.0 ± 1.2 a	25.5 ± 0.5 b	19.2 ± 1.6 c
Progeny per pupa	3.8 ± 0.3 a	4.0 ± 0.4 a	2.9 ± 0.4 b
Mortality rate of parasitoid (%)	49.6 ± 3.3 a	42.6 ± 3.7 a	49.5 ± 6.9 a
Parasitization rate (%)	23.0 ± 2.9 a	28.3 ± 3.3 a	33.9 ± 5.5 a
Sex ratio (% of females in the population)	54.0 ± 5.1 a	54.1 ± 2.5 a	56.7 ± 6.3 a
Female fecundity (number of females per female)	2.8 ± 0.4 a	3.6 ± 0.4 a	2.4 ± 0.7 a

Means within each row followed by the same letters were not significantly different between temperature $P \leq 0.05$ (LSD range test)

Table 4 Life table parameters (Mean ± SE) of *Aphaereta sp.* at different temperature. Legend: R_0 - net reproductive rate, r_m - intrinsic rate of increase, T - mean generation time, λ - finite capacity for increase, DT - doubling time

Parameters	**Jackknife estimation**		
	15º C	20 ºC	25 ºC
Net reproductive rate (R_0)	2.79 ± 0.00 b	3.66 ± 0.00 a	2.33 ± 0.00 c
Intrinsic rate of increase (r_m)	0.29 ± 0.01 b	0.35 ± 0.01 a	0.28 ± 0.02 c
Generation time (T)	3.75 ± 0.10 b	3.87 ± 0.09 a	3.76 ± 0.20 b
Finite capacity for increase (λ)	1.33 ± 0.01 c	1.42 ± 0.01 a	1.35 ± 0.03 b
Doubling time (DT)	2.54 ± 0.06 b	2.06 ± 0.05 c	3.07 ± 0.16 a

Means within each row followed by the same letters were not significantly different between temperature $P \leq 0.05$ (LSD range test)

The theoretical development threshold was estimated to be 7.0 and 12.3ºC for *Aphaereta* sp. and *C. capitata*, respectively (Fig. 1). Based on the development threshold, completed development required 336 and 253 DD for the parasitoid and their host, respectively. Linear regression analysis revealed a negative correlation no significant between the temperature and development time for parasitoid (R^2 = 0.992; F = 65.130; df = 2; P=0.078) and host (R^2 = 0.932; F = 13.267; df = 3; P = 0.068).

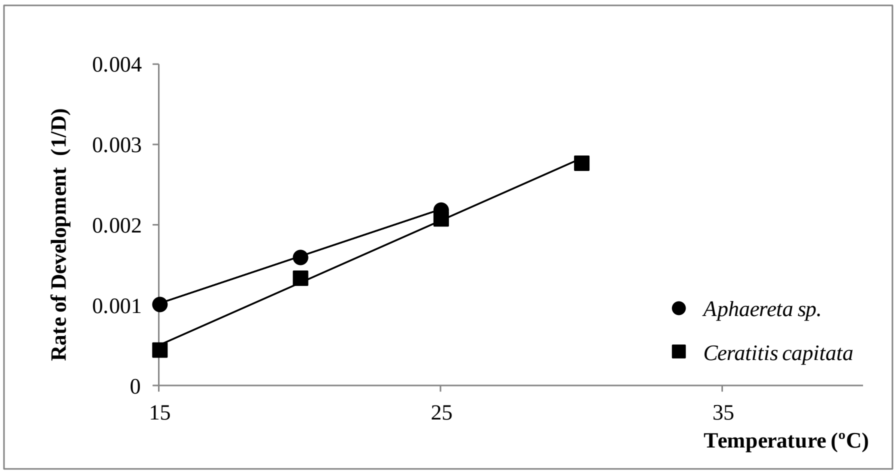

Fig. 1 Lower developmental threshold (ºC) of the host *Ceratitis capitata* and the parasitoid *Aphaereta* sp.

The non-linear models describe the developmental rate over a wider range of temperatures and provide estimates of maximum and optimum temperatures for development of the insects. The simple *Gaussian* function (Fig. 2) acceptably described the relationship between the development rate and the rearing temperature (R^2 = 0.81). The maximal developmental rate we obtain in estimated optimum temperature (22.3ºC) is 0.053.

Discussion

The success of the descendants of survival depends on the selection of a suitable host. This selection is based on the choice of a host that provides a good quality and quantity of nutrients for healthy offspring. Necessarily, the size of the host is related to its good nutrition, that is, older host larvae supply more nutrients to the parasitoids than younger larvae resulting in faster growth and development (Pascua & Pascua, 2004).

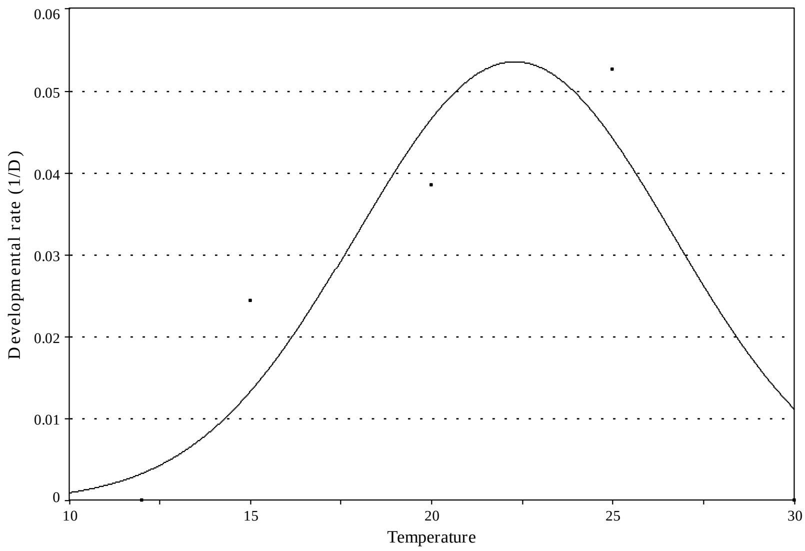

Fig. 2 Relationship between the temperature and the developmental rate of *Aphaereta* sp. as described by the *Gaussian* function.

This study reveals that the females of *Aphaereta* sp. can parasitize all instars of *C. capitata* larvae, but it was observed that this parasitoid has more difficulty to parasitize the first larval instar (< 2%). Such as result associated with low immature survival, which may be due to limited nutritional resources available in such early instar larvae (Harvey *et al.*, 2004). Hence, the parasitoid took longer to complete its development on younger larvae. As it was observed, the host size at oviposition was reported to affect the development time of koinobiont parasitoids (Colinet *et al.*, 2005; Vet *et al.*, 1993, 1994).

In the most gregarious parasitoids, the number and the size of offspring they produce is influenced to a large degree by the size of the host (= quality) (Bezemer & Mills, 2003; Lacoume *et al.*, 2006). The offspring sex ratio of this parasitoid was similar when developing on second and third instar. The percentage of female that emerged from larvae parasitized at the second instar was higher, but the longevity of these females is significantly lower compared with the females developed in the third instar. This indicates that this females offspring requires more nutritional resources to complete their development (Colinet *et al.*, 2005), and thus to be able to convert this energy gained to increase their longevity. The intrinsic rate (r_m) of increase is the most important parameter for estimating population dynamics (Southwood, 1978). The r_m of *Aphaereta* sp. in the small (second instar) and large (third instar) host is 0.45 and 0.34 female eggs/female/day, respectively. These results associated with a higher R_0, λ revealed that second instar of the *C. capitata* are a more acceptable host for *Aphaereta* sp. than third instar.

The founding of biological control agents depends upon a countless of factors, such as climatic conditions in the area of release favorable to survival and growth of the insects (Dent, 1991). To this end, the studies under controlled laboratory conditions may provide valuable insight into insect population dynamics, although temperatures are not constant in nature (Uygun & Athhan, 2000). The results obtained in this study show the effect of temperature on development time, progeny per pupa, mortality, parasitization rate, sex ratio and fecundity of this parasitoid. This parasitoid follows the typical pattern where development time is negatively correlated with temperature during development. The temperature of 20ºC was the optimum for development of *Aphaereta* sp. among those tested because it yielded a shorter development time in combination with a higher fecundity. The net reproductive rate and the intrinsic rate of increase at 25ºC was lower than at 15ºC and 20ºC, the final parameter is a good indicator of the combined effect of temperature on development, survival and reproduction. Short development time and high fecundity indicate that the *Aphaereta* sp. population has the potential for rapid population growth at 20ºC.

Temperature requirements for development (thermal constant) of the host are lower than is required for the parasitoid. At 12°C and 30°C there was no parasitism, due to the minimum temperature of development of the host and the fact that this parasitoid does not parasitize at 30°C. The t_l values of *Aphaereta* sp. are relatively lower than the host’s so that development of the gregarious parasitoid can start before the temperatures allow the population of the host to grow in high numbers. However, the probability of a destabilizing effect on the parasitoids/pest system should be considered (Campbell *et al.*, 1974).

Conclusions

The present study provides basic information on the influence of larval host size and temperature on some biological parameters of *Aphaereta* sp. exploiting its host *C. capitata*, and is an essential step in the development of biological control approaches against this pest. These results indicate that *Aphaereta* sp. has the potential to be an agent of biological control of *C. capitata*, within an Integrated Pest Management Strategies (IPM). However, and taking in the history of precipitation of researchers in the use of biological control agents and the negative consequences of these agents in the ecosystem in which are inserted, as mentioned earlier, further studies are needed to verify the behavior of this parasitoid. For this it is necessary to build a conscious plan, composed of more laboratory studies (assessment of clutch size, preferably of different host species, production optimization, etc.), passing later to semi-field studies and only after the completion of this testing and careful consideration of their results, carried out releases of this parasitoid, but always taking care to perform monitoring studies.

Acknowledgments

We would like to thank M. Fernando Almeida, from the University of Azores for the insect production. We are also grateful to K. van Achterberg for the identification of this parasitoid. The research work was supported by funds from Regional Secretariat for Science, Technology and Equipment (SRCTE) and ProEmprego in the course of the research grant M316/F/041/2009.

References

ALBAJES, R. & C. SANTIAGO-ÁLVAREZ, 1980. Efectos de la densidad larvaria y de la alimentación en la proporción de sexos de *Ceratitis capitata* (Diptera: Tephritidae). *Anales INIA/Serie Agrícola*, 13: 175–18.

BAI, B., R.F. LUCK, L. FORSTER, B. STEPHENS & J.M. JANSSEN, 1992. The effect of host size on quality attributes of the egg parasitoid, *Trichogramma pretiosum*. *Entomol Exp Appl*, 64: 37–48.

BERNAL, J. & D. GONZÁLEZ, 1993. Temperature requirements of four parasites of the Russian wheat aphid *Diuraphis noxia*. *Entomol Exp App*, 69: 173-182.

BEZEMER, T.M. & N.J. MILLS, 2003. Clutch size decisions of a gregarious parasitoid under laboratory and field conditions. *Anim Behav*, 66: 1119–1128.

BIRCH, L.C., 1948. The intrinsic rate of natural increase of an insect population. *J Anim Ecol*, 17(1): 15–26.

CAMPBELL, A., B.D. FRAZER, N. GILBERT, A.P. GUTIERREZ & M. MACKAUER, 1974. Temperature requirements of some Aphids and their parasites. *J Appl Ecol*, 11(2): 431–438.

COLINET, H., C. SALIN, G. BOIVIN & T.H. HANCE, 2005. Host age and fitness-related traits in a koinobiont aphid parasitoid. *Ecol Entomol*, 30: 473–479.

COLINET, H., G. BOIVIN & T.H. HANCE, 2007. Manipulation of parasitoid size using the temperature-size rule: fitness consequences. *Oecologia*, 152: 425–433.

DANTAS, L., R. PEREIRA, A. BARBOSA, N. SILVA, J. CALDEIRA & J. PACHECO, 1999. Madeira-Med, a sterile insect technique program for control of the Mediterranean fruit fly. *Sup. n° 6 Bull Spen*, 185-192.

DENT, D., 1991. *Insect Pest Management*. CAB International, London.

EKESI, S., 1999. Insecticide resistance in filed populations of the legume pod borer *Maruca vitrata* Fabricius (Lepidoptera: Pyralidae), on cowpea, *Vigna unguiculata* (L.) Walp. in Nigeria. *Int J Pest Manag*, 45(1): 57–59.

FALCÓ, J.V., I. TARAZONA, M. PÉREZ-HINAREJOS, E. GARZÓN-LUQUE, J. MALAGÓN & F. BEITIA, 2006. Two Native Pupal Parasitoids of *Ceratitis capitata* (Diptera, Tephritidae) Found in Spain. *Bulletin IOBC/WPRS* 29: 71-74.

GILLANI, W.A., T. BASHIR & I. MOHAMMAD, 2002. Studies on population dynamics of fruit flies (Diptera: Tephritidae) in guava and nectrin orchards in Islamabad. *Pak J Biol Sc*, 5(4): 452-454.

GODFRAY, H.C.J., 1994. *Parasitoids: behavioral and evolutionary ecology*. New Jersey: Princeton University Press.

HARBISON, J.L., J.C. LEGASPI, S.L. FABRITIUS, R.R. SADAN, B.C. LEGASPI & A. ENKEGAARD, 2001. Effects of age and host number on reproductive biology of *Allorhogas pyralophagus* (Hymenoptera: Braconidae) attacking the Mexican rice borer (Lepidoptera: Pyralidae). *Environ Entomol*, 30(1): 129–135.

HARVEY, J.A., T.M. BEZEMBER, J.A. ELZINGA & M.R. STRAND, 2004. Development of the solitary endoparasitoid *Microplitis demolitor*: host quality does not increase with host age and size. *Ecol Entomol*, 29: 35–43.

HEADRICK, D.H. & R.D. GOEDEN, 1996. Commentary: issues concerning the eradication or establishment and biological control of the mediterranean fruit fly, *Ceratitis capitata* (Widemann) (Diptera: Tephritidae), in California. *Biol Control*, 6: 412-421.

KRENEKA, S., T.U. BERENDONKA & T. PETZOLDT, 2011. Thermal performance curves of *Paramecium caudatum*: A model selection approach. *Eur J Protistol*, 47: 124–137.

LACOUME, S., C. BRESSAC & C. CHREVRIER, 2006. Effect of host size on male fitness in the parasitoid wasp *Dinarmus basalis*. *J Insect Physiol*, 52: 249–254.

LIQUIDO, N.J., L.A. SHINODA & R.T. CUNNINGHAM, 1991. Host plants of mediterranean fruit fly (Diptera: Tephritidae): An Annotated World Review. *Misc Publ Entomol Soc Am*, 77: 1-52.

LIU, S., 1985. Development, adult size and fecundity of *Apidius sonchi* in two instars of its aphid host, *Hyperomyzus lactucae*. *Entomol Exp Appl*, 37: 41–48.

LOPES, J.D.H., C.P. RAIMUNDO, D. AGUIN-POMBO, L. DANTAS, M.A. PEREIRA, O.R. RODRIGUES, D.A. FIGUEIREDO, M.S.R. PIMENTEL, M. ZORMAN, C.F.N. MACEDO, C.A.P. MEDEIROS, M.C.F. CARVALHO, M.L.O. SILVA, J.T.O. MARTINS, J.D. MUMFORD & A.M.M. MEXIA, 2006. O Projecto INTERFRUTA II e o estudo dos problemas fitossanitários das fruteiras dos Açores. I Simpósio Nacional de Fruticultura, Alcobaça, Portugal.

MACKAUER, M., R. SEQUEIRA, 1993. Patterns of development in insect parasites. In: Beckage NE, Thompson SN, eds. *Parasites and Pathogens of Insects*, Vol. 1, Academic Press USA.

MEDEIROS, A.M.C., 2004. Bioecologia e controlo com nematodes entomopatogénicos de *Ceratitis capitata* (Diptera: Tephritidae) em S. Miguel, M.S. thesis, Universidade dos Açores, Açores, Portugal.

MEYER, J.S., C.G. INGERSOLL, L.L. MCDONALD & M.S. BOYCE, 1986. Estimating uncertainty in population growth rates: jackknife vs. bootstrap techniques. *Ecology*, 67: 1156-1166.

OLIVEIRA, L., A.MEDEIROS, J.V. FALCÓ, F. BEITIA, M.J. VERDÚ, P. GARCIA (2008) Parasitoids from Azores (Hymenoptera: Encyrtidae, Pteromalidae, Braconidae): potential use in integrated pest management against *Ceratitis capitata* (Diptera: Tephritidae). *Biocontrol Sci Tech*, 18: 741-744.

OMWEGA, C.O. & W.A. OVERHOLT (1997) Progeny production and sex ratios of field populations of the parasitoids *Cotesia flavipes* and *Cotesia sesamiae* reared from Gramineous stem borers in Coastal Kenya. *Insect Sci Appl*, 17(1): 137–142.

OPP, S.B. & R.F. LUCK, 1986. Effects of host size on selected fitness components of *Aphytis melinus* and *A. lingnanensis* (Hymenoptera: Aphelinidae). *Ann Entomol Soc Am*, 79: 700–704.

PANDEY, S. & R. SINGH, 1999. Host size induced variation in sex ratio of an aphid parasitoid *Lysiphlebia mirizai*. *Entomol Exp Appl,* 90: 61– 67.

PASCUA, L.T. & M.E. PASCUA (2004) The preference, acceptability and suitability of ichneumonid wasp, *Eriborus argenteopilosus* Cameron (Hymenoptera: Ichneumonidae) on the different larval stages of cotton bollworm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae). *Philippine J of Sci*, 133 (2): 103-108.

SEKO, T. & F. NAKASUJI, 2004. Effect of egg size variation on survival rate, development and fecundity of offspring in a migrant skipper, *Parnara guttata* (Lepidoptera: Hesperiidae). *Appl Entomol Zool*, 39(10): 171–176.

SMITH, L., 1993. Host size preference of the parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) on *Sitophilus zeamais* (Col: curculionidae) larvae with a uniform age distribution. *Entomophaga*, 38: 225–233.

SOKAL, R.R. & E.J. ROHLF, 1981. *Biometry*. Freeman, New York, USA.

SOUTHWOOD, T.R.E., 1978. *Ecological Methods*. Wiley, New York, USA.

SPSS, 2006. *SPSS Base 15.0 for Windows User's Guide*. SPSS Inc., Chicago, IL, USA.

UÇKAN, F. & E. ERGIN, 2003. Temperature and food source effects on adult longevity of *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae). *Environ Entomol* 32(3): 441–446.

UYGUN, N. & ATHHAN, R., 2000. The effect of temperature on development and fecundity of *Scymnus levaillanti*. *BioControl*, 45: 453-462.

VET, L.E.M., A. DATEMA, A. JANSSEN & H. SNELLEN, 1994. Clutch size in a larval-pupal endoparasitoid: Consequences for fitness. *J Anim Ecol*, 63 (4):807-815.

VET, L.E.M., A. DATEMA, K. VAN WELZEN, H. SNELLEN, 1993. Clutch size in a larval-pupal endoparasitoid. *Oecologia*, 95: 410-415.