LIVIA ALVARENGA SIDNEY

LABORATORY EVALUATION OF *Aphidius ervi* (Haliday) and *Praon volucre* Haliday (Hymenoptera: Braconidae) AND *Macrolophus pygmaeus* (RAMBUR) (HEMIPTERA:MIRIDAE) FOR CONTROL OF APHIDS

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia/Entomologia, área de concentração em Entomologia Agrícola, para a obtenção do título de Doutora.

Orientadora

Dr. Vanda Helena Paes Bueno

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(AVALIAÇÃO LABORATORIAL DE *Aphidius ervi* (Haliday) E *Praon volucre* Haliday (Hymenoptera: Braconidae) E *Macrolophus pygmaeus* (RAMBUR) (HEMIPTERA:MIRIDAE)PARA O CONTROLE DE PULGÕES)

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APROVADA em 1 de março de 2013.

Dr. Joop C. van Lenteren	Wageningen University
Dr. Maria del Rosario Manzano Martinez	Universidad Nacional de Colombia
Dra. Livia Mendes de Carvalho Silva	EPAMIG
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Orientadora

LAVRAS – MG

2013

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GENERAL ABSTRACT

Aphids are pests that attack both field and greenhouse crops and are frequently associated with Braconidae (Aphidiinae) parasitoids and generalists predators. Many those species are commercially used in biological control of pests. In this experiment we focused compared the quality of two strains of the parasitoid *Aphidius ervi* (Haliday) on different species of aphids; a strain commercially produced for many generations on *Acyrthosiphum pisum* (Harris), denominated STANDARD, and other strain recently collected in the field attacking *Myzus persicae* (Sulzer) and referred to as NEW. We also evaluated the biological and behavioral parameters of *Praon volucre* individuals originating from 1 and 10 founder couples in the 3rd , 6th, 9th and 12th generations on *Aulacorthum solani* (Kaltenbach) in lettuce plants in the laboratory. Next, we studied the functional response and searching behaviour of *P. volucre* parasitizing the foxglove aphid *A. solani;* and we investigated the predation capacity of two instars and adults of the predatory bug *Macrolophus pygmaeus* having as prey *M. persicae, Myzus persicae nicotianae* Blackman*, A. solani* and *Macrosiphum euphorbiae* (Thomas); and evaluate the developmental time and reproductive parameters of *M. pygmaeus* on *A. solani*, *M. persicae* and eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). The results showed that is possible the use of *A. ervi* strain (NEW) for both commercial mass rearing and as biological control agent proposals. The NEW strain presented better performance having *M.euphorbiae* or *A.solani* as aphid hosts. The results also showed that both rearing, started with 1 or 10 founder couples, can keep an acceptable quality of *P. volucre* individuals concern to the main biological and reproductive parameters and flying and searching capacities reared in *A. solani* into laboratory conditions. A significant positive linear coefficient (P1) of the logistic regression model indicates type III functional response to *P. volucre* at 4, 8, 16, and 64 densities of *A. solani.* The number of attacks hosts followed a sigmoid (S shape) curve in which the slope increases at first but then decreased. Also was possible to demonstrate *M. pygmaeus* might contribute to restrict aphid populations prior to the arrival of specialist natural enemies, and or in combination with other natural enemy and then, helping to the aphid management in greenhouse crops.

Keywords: Aphids parasitoids, quality, predator

RESUMO GERAL

Pulgões são pragas que atacam tanto em campo quanto em cultivos protegidos e estão frequentemente associados aos parasitoides da família Braconidae (Aphidiinae) e predadores generalistas, inimigos naturais que tem sido usados comercialmente no controle biológico de pragas. Neste trabalho nós focamos em comparar a qualidade de dois strains do parasitoide *Aphidius ervi* (Haliday) em diferentes espécies de pulgões como hospedeiros, um strain comercial e produzido por muitas gerações em *Acyrthosiphum pisum* (Harris) denominado STANDARD, e outro recentemente coletado em campo atacando *Myzus persicae* (Sulzer) e denominado como NEW. Também foram avaliados os parâmetros biológicos e comportamentais dos indivíduos de *Praon volucre* Haliday provenientes de 1 e 10 casais fundadores nas gerações 3ª, 6ª, 9ª e 12ª em *Aulacorthum solani* (Kaltenbach) tendo alface como planta hospedeira no laboratorio. Tambem foi determinhado a capacidade predatória de dois ínstars e adultos do inseto predador *Macrolophus pygmaeus* tendo como presas *M. persicae*, *Myzus persicae nicotianae* Blackman, *A. solani* e *Macrosiphum euphorbiae* (Thomas); tambem fo avaliado o desenvolvimento e parâmetros reprodutivos em *A. solani*, *M. persicae* e ovos de *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Os resultados demonstraram que é possível a utilização do strain *A. ervi* NEW com propositos de criação de massal e como agente de controle biologico. Este strain apresenta melhor desempenho tendo os pulgões *M.euphorbiae* ou *A.solani* como afídeos hospedeiros. Tanto a criação iniciada com 1 ou 10 casais fundadores pode manter uma qualidade aceitável dos indivíduos de *P.volucre*, considerando seus principais parâmetros biológicos, reprodutivos, capacidade de voo e resposta olfativa em condições de laboratório. Um coeficiente linear positivo e significativo (P1) do modelo de regressão logística indicou a resposta funcional do tipo III para *P. volucre* nas densidades de 4, 8, 16 e 64 *A. solani*. O número de hospedeiros atacados seguidos por uma curva sigmoide na qual declina e aumenta em primeira instância e depois declina novamente. O predador *M. pygmaeus* pode contribuir para restringir populações de pulgoes antes da chegada de inimigos naturais mais especialistas e ou em combinação com outro inimigo natural e entao, ajudar no manejo de pulgões em cultivos em casas de vegetação.

Palavras-chave: Parasitoides de pulgões, qualidade, predador

SUMMARY

FIRST PART

1 GENERAL INTRODUCTION

Protected environments provide favorable conditions for the emergence of pests and diseases, resulting in a considerable increase in the number of chemical applications. At the same time, however, this protected environment creates an opportunity to use biological control agents (BUENO, 2005; PILKINGTON et al., 2010). Among the pests frequently found in these systems, aphids are one of the most important, causing direct and indirect economic damage to the plant (BUENO, 2005; CONTI et al., 2010).

Aphids are pests that attack both field and greenhouse crops and are frequently associated with Braconidae (Aphidiinae) parasitoids, many species of which are used in the biological control of economically important crops (GASSEN; TAMBASCO, 1983). For a long time, in crops where natural enemies had been release against other pests, aphids could be effectively controlled by means of selective chemical pesticides which present very few adverse effects on the biological control. But increasing resistence to these pesticides meant that a different approach was called for, the use of natural enemies being the obvious alternative. The biological control of aphids was thus first introduced into Dutch glasshouses in 1988 (MALAIS; RAVENSBERG, 2003).

The effectiveness of such parasitoids as pest control agents has been demonstrated by various studies (LINS JÚNIOR et al., 2011, 2013; SIDNEY et al., 2010a, 2010b). Twelve species of parasitoid aphids were introduced into Brazil in the 1980s for the biological control of several aphids on wheat crops, among which were the endoparasitoids *Aphidius ervi* (Haliday) and *Praon volucre* (Haliday) (GASSEN; TAMBASCO, 1983). These two species have

considerable preference for aphids of the Macrosiphini tribe, including the species *Macrosiphum euphorbiae* (Thomas) *Myzus persicae* (Sulzer) and *Aulacorthum solani* (Kaltenbach) (MACKAUER et al., 1992; STARY´; SAMPAIO; BUENO, 2007). The potential of *A. ervi*and *P. volucre* for the control of *M. euphorbiae* has been demonstrated under experimental laboratory conditions (CONTI; BUENO; SAMPAIO, 2008; SIDNEY et al., 2010a). Currently, *A. ervi* and *P. volucre* are the only *M. euphorbiae* and *A.solani* parasitoids in Brazil and represent important tools for aphid biological control (SIDNEY et al., 2010b; STARY´ et al., 2007).

In general aphid parasitoids are the first choice to control aphids.However, the natural occurrence of hyperparasitoids often disrupts this approach (BRODEUR, 2000). Thus, the role of generalist natural enemies in biological controland their interactions with prey, of which aphids constitute one part, has also received considerable attention (EVANS, 2008) due to their importance in agroecosystems and the unsustainable nature of conventional pest control methods.

Macrolophus pygmaeus (Rambur) (Heteroptera: Miridae) is native to the Mediterranean area, and is mainly used as a biological control agent in tomato. They are polyphagous, feeding on several soft-bodied pests such as the whiteflies, aphids, thrips, leafminers, mites and Lepidoptera eggs and larvae. Several studies have shown that *M. pygmaeus* is the most commonly found predator and its ability to suppress pest populations is considered to be extremely important (PERDIKIS; LYKOURESSIS, 2002).

In order to provide important information for improvingmass rearing and the commercialization of these naturals enemies, we studied them regarding behavior and biological parameters of parasitoid aphids *A.ervi* and *P.volucre,* and also the generalist predator, *M.pygmaeus*, in order to have information in the

manner in which they could be determining their impact on the different host/prey aphid species.

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SECOND PART – ARTICLES

ARTICLE 1 Quality of two *Aphidius ervi* strains (Haliday) (Hymenoptera: Braconidae) when reared in *Macrosiphum euphorbiae* (Thomas), *Aulacorthum solani* (Kaltenbach) or *Myzus persicae nicotianae* (Blackman) (Hemiptera:Aphididae).

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RESUMO

O parasitoide *Aphidius ervi* (Haliday) é usado contra *Aulacorthum solani* (Kaltenbach), *Macrosiphum euphorbiae* (Thomas) e *Myzus persicae* (Sulzer), e a variabilidade genética pode ser um fator que afeta o desempenho dos parasitóides liberados em programas de controle biológico. O objetivo neste trabalho foi comparar a qualidade de dois strains de *A. ervi* em diferentes espécies de pulgões, sendo um strain comercial e produzido por muitas gerações em *Acyrthosiphum pisum* (STANDARD) e outro recentemente coletado no campo atacando *M. persicae*, denominado NEW. Os testes foram conduzidos em condições de laboratório a 22 ± 2 ° C, UR 70 ± 10% e fotofase de 12hs. Fêmeas de *A. ervi* strain (STANDARD e NEW) para cada especie de pulgão *A. solani, M. persicae nicotianae* and *M. euphorbiae* foram avaliadas. Fêmea acasalada e sem experiência prévia de oviposição, foi liberada em placa de Petri (9 cm) pelo período de uma hora (1ªvisita), e após, a mesma fêmea foi liberada em outra placa por um período de duas horas (2ªvisita). Todas as placas continham 50 ninfas de segundo instar de um dos pulgões testados em seção foliar sobre solução ágar/água 1%. Foram conduzidas 25 repetições para cada pulgão hospedeiro e para cada strain avaliado. O número de múmias formadas pelo strain NEW foi maior que o do strain STANDARD quando a fêmea parasitou *M. euphorbiae* em ambas as visitas. O pulgão *M. persicae nicotianae* teve menor número de múmias formadas na 1ª e 2ª visitas quando atacado pelo strain NEW. Já o strain STANDARD apresentou números de múmias formadas consistentemente mais elevados em *A. solani* quando comparado com os demais pulgões. O número de múmias formadas a partir de ambos strains, NEW e STANDARD em *M. euphorbiae*, após ter *M. euphorbiae* como hospedeiro original, foi maior em comparação a aqueles criados por uma geração em *A. pisum* antes dos testes em *M. euphorbiae*. Os resultados demonstram que é possível a utilização do strain *A. ervi* NEW com propositos de criação de massal e como agente de controle biologico. Este strain pode tambem apresentar melhor desempenho tendo os pulgões *M. euphorbiae* ou *A. solani* como afídeos hospedeiros.

Palavras-chave: Qualidade hospedeira, parasitóide de pulgões, parasitismo, linhagem.

1 INTRODUCTION

The aphid *Macrosiphum euphorbiae* (Thomas) is a very important pest in tomato crops. These aphids are pests with high reproductive potential (BUENO, 2005; CONTI et al., 2010) and intrinsic rate of increase and very difficult to control, in particular the foxglove *Aulacorthum solani* (Kaltenbach) that has become a very problematic pest on sweet pepper (SHELT; WACKERS, 2003).

The use of biological control of these aphids is common in organic crops under protected cultivation, and the parasitoid *Aphidius ervi* (Haliday) is used against *A. solani* and *M. euphorbiae* (SIDNEY et al., 2010b, 2011). But, several reasons can contribute for failure on aphid's biological control and often the parasitoids used as biocontrol agent seems not to "catch- up" with the aphid growth.

The genetic variation may have considerable influence on the parasitoids' efficiency, host recognition and acceptance (REHMAN; POWEL, 2010) and also the quality of the individuals after the mass rearing process can lead to failures in pest management (LENTEREN, 2003, 2009, 2010).

In this regard, the quality of the insects produced is one of the greatest concerns today. Loss of genetic variability in mass-reared insects may lead to the loss of wild-insect traits, preventing laboratory-reared insects from being as competitive as those found in nature (CAPINERA, 2008). Then, studies or information that provide insight to increase the quality of biological control agents through the development of new lines

of natural enemies adapted to particular pest species are important traits in biological control programs (HENRY et al., 2010). New or existing mass- reared strains must be regularly monitored for their quality allowing knowing when they should be replaced or improved (LENTEREN; NICOLI, 2004).

In this research we focused on comparing the quality of two lines of the parasitoid *A. ervi* on different aphid hosts species: one *A. ervi* strain, called STANDARD and another, NEW. The first one was collected in 1992 and reared for almost twenty years (approximately 500 generations) on the pea aphid *Acyrthosiphum pisum* (Harris); and the second one was collected in the summer of 2011 on *M. persicae* in sweet pepper crop in the Netherlands (NEW strain). This new strain was also maintained on *A. pisum*, for approximately 10 generations, and both in *Vicia faba* var. *minor*.

Currently, there were signals from the field about a low impact of *A. ervi* released especially against *M. euphorbiae* comparing to the past. Then, to set up tests comparing these two strains of *A. ervi* having the aphid hosts *M. persicae nicotianae*, *A. solani* and *M. euphorbiae* is an important tool that could help to get useful information's on their biological parameters and for their use in aphid biological control programs. Also, the knowledge on the biological parameters of the two strains of *Aphidius ervi* on different aphid host may provide opportunity to determine potential differences between the performances of the strains on these three economic important pests' aphids. According to Lenteren (2003) the correlation between values obtained from laboratory assay and field performance are important to be able to select a limited set of

laboratory criteria that give meaningful information about performance after biological control agent release.

The objectives of this study were to evaluate the parasitisation capacity, emergence rate and sex ratio of offspring of the two strains of *A. ervi* on the aphid hosts *M. euphorbiae*, *A. solani* and *M. persicae nicotianae*. Also to compare parasitism, emergence and sex ratio of these two strains on *M. euphorbiae,* in order to determine if the recognition of the host species has vital importance for parasitoids in their biology adaptation to the new host (*M. euphorbiae*).

2 MATERIALS AND METHODS

2.1 Rearing of the aphids A. solani, M. persicae nicotianae and M. euphorbiae

Aphid colonies of *A. solani* and *M. persicae nicotianae* were kept on sweet pepper (*Capsicum annuum* L.) variety 'Spider'. The aphid *M. euphorbiae* was kept on tomato leaves (*Lycopersicon esculentum* L.). Individuals from these colonies were used to start a rearing of each aphid on leaf discs in order to get standardized aphids before using them in the experiments. A rearing of *A. solani* and *M. persicae nicotianae* was started on sweet pepper leaf discs, and colonies of *M. euphorbiae* were placed out on tomatoes leaf discs (both 9 cm diameter, 1.5 cm height). The leaf discs were placed upside down during the aphid rearing process because have a preference for feeding on the abbaxial leaf plant. Five days after, the aphids individuals were transferred to fresh leaf discs placed in Petri dishes (9 cm diameter, 1.5 cm height). Aphid transfer was done by cutting the old leaf disc in two or three pieces depending on the amount of aphid on the old leaf disc and then, placed on the fresh leaf discs in order to maintain adequate nutrition of the aphids. The old leaf disc was removed from the Petri dish after a 24 h period, when all the aphids had walked to the new leaf disc. They were maintained in an environmental chamber at $22^{o} \pm 2^{o}$ C, 65% ± 10% RH, and 16 h photophase.

In order to standardize also the age of the aphid nymphs used in the experiments, approximately 50 aphid adults of each aphid species

evaluated were transferred to Petri dishes (9 cm diameter, 1.5 cm height) with a layer of 1% agar/water containing sweet pepper (*A. solani* and *M. persicae nicotianae*) and tomato leaf discs (*M. euphorbiae*). The adults were carefully removed after an 18h period and the nymphs that had been produced during this period were maintained in situ at 22 ^{o}C for more 6 hours until they developed to the second instars which were used in the experiments. This methodology was adapted according to Sidney et al. (2010a, 2010b).

2.2 Rearing of two *A. ervi* strains (STANDARD and NEW)

Stock cultures of *A. ervi* were maintained at 21 ± 2^{O}C, 65% ± 10% RH and 16 h photophase and the experiments were carried out at those conditions. The two different strains of *A. ervi* (STANDARD OR NEW) were used in the experiments. Mummies of the two strains from this stock colonies were placed individually until adult emergence in plastics tubes (10 cm x 8mm) closed off with a piece of cotton. A small drop honey and water droplets were put in the plastic tubes walls in order to provide enough nutrition to emerged adults. These adults were sexed and females and males were kept together for a 24h period for mating and female maturation. These emerged-individuals were used to set up a rearing on the aphids *A. solani*, *M. persicae nicotianae* in sweet pepper and *M. euphorbiae* on tomato plants. The two strains of *A. ervi* were reared for one generation on these aphids. The females from these rearing were used in the experiments on quality of the individuals of the two strains on these aphids.

Another test was set up in order to evaluate the influence of transferring the parasitoid direct from one aphid host to another aphid host, or being reared first from one generation on the same tested host. The two strains of *A. ervi* from the stock colony reared on the aphid *A. pisum* in sweet pepper were tested on M. *euphorbiae* in tomato. For this experiment, one group of individuals of each strain from the stock colony (n=50) was reared for one generation on *M. euphorbiae* in tomato; and other group (n=50) was transferred direct from *A. pisum* to *M. euphorbiae*. This experiment was set up in order to evaluate the host recognition by the parasitoid *A. ervi* and its biological parameters on M. *euphorbiae,* after its transference from *M. euphorbiae* or *A. pisum*. Females originated from these rearing on *A. pisum* or *M. euphorbiae* were used in the experiments.

2.3 Quality of individuals of the two Aphidius ervi strains (STANDARD and NEW) on *Aulacorthum solani, Myzus persicae nicotianae* and *Macrosiphum euphorbiae*

Twenty-five 24 h-old-females mated of each strain of *A. ervi* (STANDARD and NEW) from each aphid host *A. solani, M. persicae nicotianae* and *M. euphorbiae* were evaluated.

A female *A. ervi* strain (STANDARD or NEW), mated and without previous oviposition experience, was released in a Petri dish (9 cm diameter, 1.5 cm height) containing 50 2nd instar nymphs of one of the aphid species on a leaf disc of sweet pepper (*Capsicum annuum* L. cv Spider) or tomato (*Solanum lycopersicum* L.) on a layer of 1% solution

agar / water. The female was kept in the Petri dish for one hour period (1st visit). After, the same female was removed and released in another Petri dish, contained also 50 2nd instar nymphs of one of aphids host species previously tested in the 1st visit. The female was kept togethe with the aphid host for a two hours period (2nd visit).

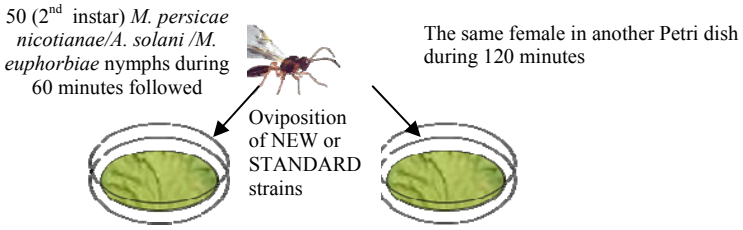

Figure 1. Figure schematic of the proposed methodology of the two *Aphidius ervi* strains (STANDARD and NEW) on *Aulacorthum solani*, *Myzus persicae nicotianae* and *Macrosiphum euphorbiae*

Those Petri dishes with the parasitized aphids (from the 1st and 2nd visits of the female parasitoid) were kept in a climatic chamber at 22 ± 2 ° C, RH 65 ± 10% and 16 h photophase. The parasitized aphids *M. persicae*, *A. solani* and *M. euphorbiae* were carefully transferred to a new leaf disc, with the aid of a paint brush (number1), four or five days after the female oviposition, due the leaf disc was decaying rapidly in response to the aphids feeding. This transference process was important for the aphids led enough nutritional supply, and not leading it to death before the mummification process. The parasitized aphids were checked daily to determine the parasitism, expressed by the number of formed mummies,

percentage of emergence and sex ratio, expressed by percentage of female.

Each aphids species was evaluated as host for the two strains of *A. ervi* in 25 replicates. Each replicate was composed by the aphids from the 1st visit and from the 2nd visit of the parasitoid female.

2.4 Parasitism, emergence and sex ratio of *Aphidius ervi* strains (NEW and STANDARD) reared for one generation on *Macrosiphum euphorbiae* or transferred direct from *A. pisum*, both before to have *M. euphorbiae* as host

The *A. ervi* female originate from rearing on *A. pisum* and from one generation on *M. euphorbiae*, was kept in Petri dish for one hour period (1st visit), and then the same female was removed and released into a new Petri dish for a 2 hours period (2nd visit). Both Petri dish contained each 50 2nd instar nymphs *M. euphorbiae.* All these Petri dishes containing the parasitized aphid (from the 1st and 2nd visits) were kept in a climatic chamber at 22 ± 2 ° C, RH 65 ± 10% and 16 h photophase. The parasitized aphids were checked daily to determine the percentage of parasitism (expressed by the number of formed mummies) or the absolute number of formed mummies, percentage of emergence and sex ratio (expressed by percentage of female).

Each repetition (n= 25) consisted of the two Petri dishes with the parasitized aphids by the same female, being composed by the parasitized aphid hosts from the 1st visit and 2nd visit of the same parasitoid female.

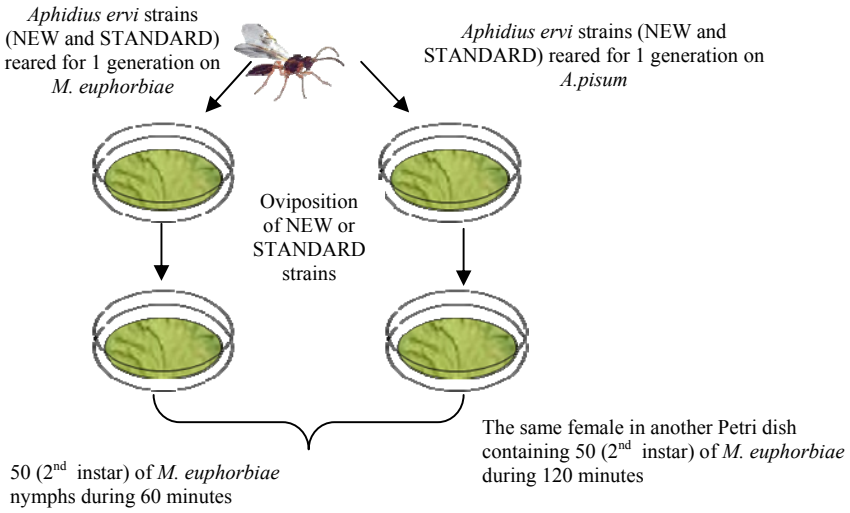

Figure 2. Figure schematic of the proposed methodology of *Aphidius ervi* strains (NEW and STANDARD) reared for one generation on *Macrosiphum euphorbiae* or *A. pisum*, both before to have *M. euphorbiae* as host

2.5 Data analyses

The experiments were conducted with three treatments (each one represented by the aphids *A. solani, M. persicae nicotianae* and *M. euphorbiae*) and twenty-five replicates of each strain of *A. ervi*. Data from number of formed mummies was evaluated by the Tukey test, (P □ 0.05), and the SAS Programme was used. Before proceeding to the variance analysis, the data were transformed into parasitism (%) and emergence (%). The number of emerged-adults was determined according to emerged-adults from formed mummies. Data were analyzed using one-way analysis of variance (ANOVA) in order to determine the significant

differences among treatment, means and regression analysis using SAS Programme (Biostat 5.3).

The means were separate using the Tukey test, (P □ 0.05). The response variable sex ratio was subjected to chi-square test ($p < 0.05$). Statistical analyzes were performed using program SigmaPlot 11.0 (SIGMAPLOT..., 2008).

3 RESULTS

3.1 Parasitism of the two *A. ervi* strains (NEW and STANDARD)

There were significant differences in the numbers of formed mummies on the two strains of *A. ervi* (NEW and STANDARD) and among the different aphid as hosts to the parasitoid. The numbers of formed mummies from the NEW strain was higher compared to the STANDARD strain when the female attacked *M. euphorbiae* during the 1st and 2nd visits. The number of formed mummies and percentage of parasitism were 34.0 ± 1.78 mummies (67.9%) (NEW, 1st visit) and 29.3 ± 1.13 mummies (58.6%) (STANDARD, 1st visit) ($df = 1F = 4.9145$; $p = 0.0295$); 28.9 ± 1.74 mummies (57.8%) (NEW, 2nd visit) and 23.6 ± 1.86 mummies (47.28%) (STANDARD, 2nd visit) ($df = 1$; $F = 4.2152$; $p = 0.043$) (Figure 3).

The numbers of formed mummies from NEW strain were in the 1st visit of 36.9 ± 1.67 mummies (73.76%) and 2nd visit of 27 ± 2.13 mummies (57.60%); 1st visit of 25.7 ± 2.24 mummies (51.44%) and 2nd visit of 17.2 ± 2.1mummies (34.32%); 1st visit of 34 ± 1.78 mummies (67.92%) and 2nd visit of 28.9 ± 1.74 mummies (57.76%), respectively, on *A. solani*, *M. persicae nicotianae* and *M. euphorbiae*.

The aphid *M. persicae nicotianae* had lower numbers of formed mummies in both visits of *A. ervi* strain NEW. Furthermore, STANDARD strain showed numbers of formed mummies consistently higher on *A. solani* with values ranging from 35.4 ± 1.18 mummies (70.80%) (1st visit: $df = 2$ $F = 6.9391$; $p = 0.0021$) to 31.5 ± 2.09

mummies (63.04%) (2nd visit: $df = 2$; $F = 7.8977$; $p = 0.0011$) (Figure 3). The numbers of formed mummies of both NEW and STANDARD strains was higher in *A. solani* compared to one in the aphids *M. persicae nicotianae* and *M. euphorbiae.*

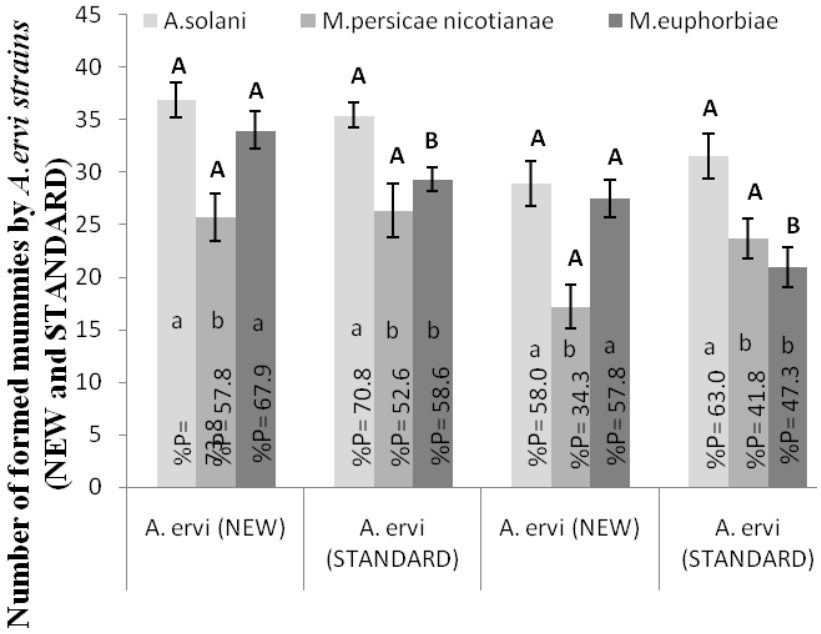

Figure 3. Number of formed mummies (± S.E.) by two *Aphidius ervi* strains (NEW and STANDARD) on the aphids hosts *Aulacorthum solani, Myzus persicae nicotianae* and *Macrosiphum euphorbiae* at 22 ± 2 ° C, RH 65 ± 10% and 16 h photophase.

Means followed by different capital letters (comparing strains in each aphid host species) and small letters (comparing aphids host species in each strain) within bars are significantly different (Tukey test, $P \leq 0.05$).
%P = values represent the percentage of parasitism (expressed by the number of formed mummies (n=50).

During the 2nd visit for oviposition of the two strains females, NEW and STANDARD, the numbers of formed mummies on the different aphids were NEW =29.9 ±2.13 mummies (57.60%) and STANDARD = 31.5 ± 2.09 mummies (63.04%) on *A. solani*, NEW = 26.3 ± 2.10 mummies (34.32%) and STANDARD = 20.9 ± 2.09 mummies (41.84%) on *M. persicae nicotianae* and NEW = 29.3 ± 1.74 mummies (57.76%) and STANDARD = 23.6 ± 1.86 mummies (47.28%) on *M. euphorbiae,* respectively (Figure 3).

3.2 Emergence of two *A. ervi* strains (NEW and STANDARD)

No significant difference was detected to the emergence rate of the two strains of *A. ervi* (NEW and STANDARD) and on the different host's aphid at the 1st visit of *A. ervi* female for oviposition. The emergence rate on *A. solani* was 91.88% ± 1.99 (NEW) and 89.94% ± 1.36 (STANDARD), on *M. persicae nicotianae* was 84.40% ± 4.55 (NEW) and 91.62% ± 1.78 (STANDARD) and on *M. euphorbiae* was 94.31% ± 2.44 (NEW) and 93.46% ± 1.4 (STANDARD). However, at the 2nd visit of the females on *M. persicae nicotianae* there was lower emergence rate (75.94% ± 5.97) when parasitized by the NEW strain compared to on the aphids *A. solani* (89.85% ± 4.11) and *M. euphorbiae* (92.45% ± 2.2). In contrast, the emergence rates of STANDARD strain were similar when the parasitoid developed on *A. solani* (92.20% ± 1.23), *M. persicae nicotianae* (94.92% ± 1.58) and *M. euphorbiae* (92.87% ± 1.55) during the 2nd visit of the female (Figure 4).

When compared both strains in the 2nd visit of female parasitoid, there was difference on the percentage of emergence between NEW and STANDARD strains, whereas STANDARD strain showed the highest emergence rate (94.9%) compared to NEW strain (75.9%) having *M. persicae nicotianae* as host ($df = 1$; $F = 16.228$; $p = 0.002$) (Figure 4).

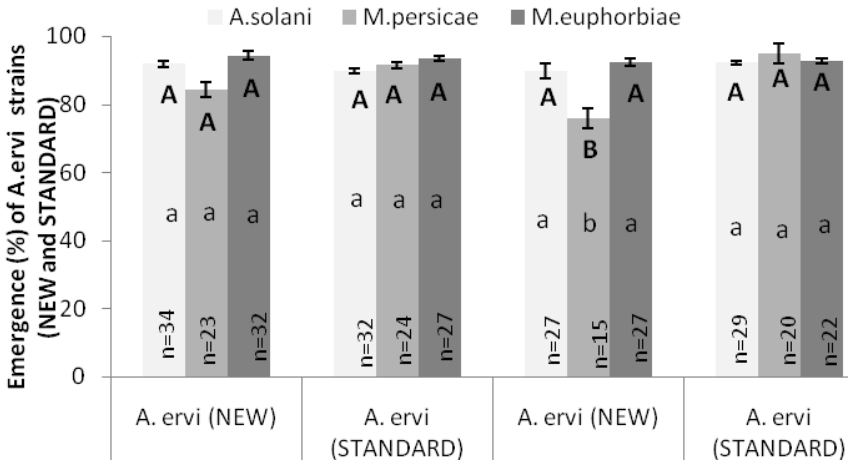

Figure 4. Emergence (%) (± S.E.) of two *Aphidius ervi* strains (NEW and STANDARD) on the aphids hosts *Aulacorthum solani, Myzus persicae nicotianae* and *Macrosiphum euphorbiae* at 22 ± 2 ° C, RH 65 ± 10% and 16h photoperiod.

Means followed by different capital letters (comparing strains among each host aphids species) and small letters (comparing aphids host species in each strain) within bars are significantly different (Tukey test, P □ 0.05).
n = values of the absolute number of emerged adults

3.3 Sex ratio of *A. ervi* strains (NEW and STANDARD)

The sex ratio (express by the percentage of females) of the two strains NEW and STANDARD in the 1st visit of *A. ervi* female did not differ from parasitized *A. solani* (NEW: 54.61% ± 3.28 and STANDARD: 61.75% ± 5.1), *M. persicae nicotianae* (NEW: 41.06% ± 5.05 and STANDARD: 45.38% ± 5.67 %) and *M. euphorbiae* (NEW: 38.66% ± 4.3 and STANDARD: 47.51% ± 5.22 %) (x^2= 5.018 *df*=5 *P* = 0.414). At the 2nd visit, there was no difference in the sex ratio of the two strains NEW and STANDARD, and the values were 48.60% and 59.83% on *A. solani*, 44.76% and 50.96% on *M. persicae nicotianae* and NEW: 45.57% ± 5.2 and STANDARD: 55.18% ± 5.27 % on *M. euphorbiae* (x^2= 2.207 *df*=5; *P* = 0.820).

3.4 Number of formed mummies, emergence and sex ratio of two *Aphidius ervi* strains (NEW and STANDARD) on *Macrosiphum euphorbiae* after being reared by one generation on *M. euphorbiae* and or transferred direct from *A. pisum*

3.4.1 Number of formed mummies, emergence and sex ratio of two *A. ervi* strains (NEW and STANDARD) on *M. euphorbiae*

The number of formed mummies of both strains NEW and STANDARD on *M. euphorbiae*, having *M. euphorbiae* as original host, was higher compared to those that were reared before for one generation on *A. pisum*. The values were, in the 1st visit: 28.12 ± 1.69 mummies (56.2%) (NEW); 1st visit: 19.24 ± 1.67 mummies (38.5%)

(STANDARD) both from the original host: *A.pisum*) (F =13.9188 P = 0.0008); and 1st visit: 34.0 ± 1.78 mummies (56.2%) (NEW) and 1st visit: 29.28 ± 1.12 mummies (58.56%) (STANDARD) both from the original host: *M. euphorbiae*) (F = 4.9145 P = 0.0295) (Figure 5).

Also when comparing to the relationship among the original hosts *A.pisum* and *M. euphorbiae,* and the NEW and STANDARD strains on the 2nd visit of the female, still the numbers of formed mummies on *M. euphorbiae* was higher when had *M. euphorbiae* as original host (2ndvisit NEW: 24.8 ± 1.59 mummies (49.60%); 2ndvisit STANDARD: 14.3 ± 1.52 mummies (28.56%) (original host: *A.pisum*) (F = 22.7636); and 2ndvisit NEW: 28.8 ± 1.73 mummies (57.76%), 2ndvisit STANDARD: 23.6 ± 1.86 mummies (47.28%) (original host: *M. euphorbiae*) (F = 4.2152 P = 0.043). Parasitism rates were higher in NEW strain populations on *M. euphorbiae* from both original hosts and in the 1st and 2nd visits compared to STANDARD *A. ervi* strain populations (Figure 5).

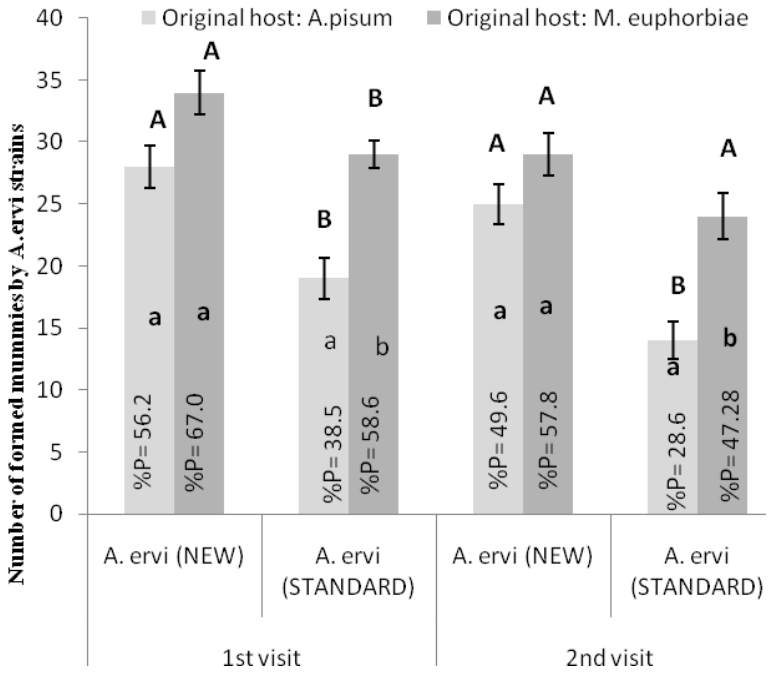

Figure 5. Number of formed mummies (± S.E.) by the two *Aphidius ervi* strains (NEW and STANDARD) on *Macrosiphum euphorbiae* after being reared before by one generation on *M. euphorbiae* and on *A. pisum*. 22±2°C, 65±10% RH and 16h photophase.

Means followed by different capital letters (comparison strains among each original host aphids species) and small letters (comparison between both original aphid host species in each strain) within bars are significantly different (Tukey test, $P \leq 0.05$).
n= values represent the percentage of parasitism (expressed by the number of formed mummies).

The emergence rate of the both strains was not affected when they had *A.pisum* and *M. euphorbiae* as originals host before the test performance on *M. euphorbiae*, even in the 1st or 2nd visits of the females (Figure 6).

No significant effect on sex ratio rates (express by the percentage of females) of the parasitoids strains having *A.pisum* and *M. euphorbiae* as originals host even on both 1st and 2nd visits. The sex ratio, of NEW and STANDARD strains did not differ when having *A.pisum* and *M. euphorbiae* as originals host during the 1st and 2nd visits of the females on *M. euphorbiae* ($x^2 = 0.0683$ *df* 1, $P = 0.794$).

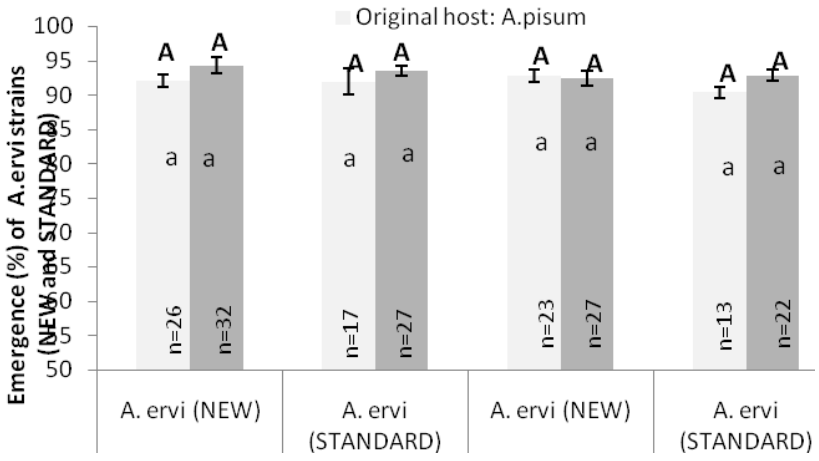

Figure 6. Emergence (%) (± S.E.) of two *Aphidius ervi* strain (NEW and STANDARD) on *Macrosiphum euphorbiae* after being reared before by one generation on *M. euphorbiae* and on *A. pisum*. 22±2°C, 65±10% RH and 16h photophase.

Means followed by different capital letters (comparison strains among each original host aphids species) and small
letters (comparison between both original aphid host species in each strain) within bars are significantly different (Tukey test, P □ 0.05).
n= values represent the absolute number of emerged adults

In the 1st visit of the female of both strains, the sex ratio were NEW = 51.76% ± 5.12 and STANDARD = 47.12% ± 5.12 having *A.pisum* as original host; and NEW = 38.66% ± 4.30 and STANDARD = 47.51% ± 4.53 having *M. euphorbiae* as originals host (Table 2). During the 2nd visit of the female of the two strains (NEW and STANDARD), the sex ratio on *M. euphorbiae* were NEW = 54.04% ± 5.45 and STANDARD = 49.09% ± 6.18 having *A.pisum* as original host; and NEW = 45.57% ± 5.22 and STANDARD = 55.18% ± 5.27 having *M. euphorbiae* as originals host, respectively.

4 DISCUSSION

The parasitism rate or the absolute numbers of formed mummies of the two strains of *A. ervi* (STANDARD and NEW) were significantly different, and these rates of both strains in the first visit of the female to the hosts was above 50%, which ensures the suitability of the three aphids as host to the parasitoid *A. ervi*.

The NEW strain has potential for a successful parasitisation on *M. euphorbiae* and *A. solani* as this strain showed highest parasitism compared to STANDARD strain on *M. euphorbiae* in the laboratory. This result was somewhat unexpected, because the STANDARD strain is currently commercially produced and used as a biological control agent against *M. euphorbiae* and *A. solani* in Europe (LENTEREN, 2012) and the different genetic variation between STANDARD and NEW strains might be an explanation for those finding, due the NEW was more recently collected in the field, and may have a higher genetic variability. Secondly, the STANDARD strain was originated from a stock culture maintained on *A.pisum*, and this strain may need some time to adapt to a new host species, being necessary a minimum of 4-5 generations (CAMERON; POWEL; LOXDALE, 1984) to increase the performance of STANDARD strain on *M. euphorbiae*.

Laboratory populations are often kept at constant environments with stable abiotic factors like light, temperature wind, humidity and constant biotic factors such as absence of competition or intraguild predation. Also there is no selection to overcome unexpected stresses, and the result is a change of the criteria that determine fitness and

modification of the whole genetic system (LENTEREN, 2003). Also genetically different strains of the same natural enemy species may react in very different ways to the same set of chemical stimuli from hosts and theirs host plants. To choose the best strain of the natural enemy for a particular task, it is important to have knowledge of these inherited preferences for particular environments and to match such inherited preferences with the stimuli present in the environment where natural enemy strains will be release (LENTEREN; NICOLI, 2004).

M.persicae represented the less suitable hosts for both studied strains in comparison to *M. euphorbiae* and *A. solani*, showing lower number of formed mummies. However, the parasitism of the *P.volucre* female on *M. euphorbiae, A. solani* and also on *M. persicae* was higher than 50% in the first visit. Nevertheless several studies have demonstrated that the parasitoid *Aphidius colemani* is a more efficient natural enemy to attack *M.persicae* comparing to *A. ervi* (PERDIKIS et al., 2004; SAMPAIO; BUENO; CONTI, 2008; SAMPAIO; BUENO; LENTEREN, 2001; SAMPAIO; BUENO; PÉREZ-MALUF, 2001). Also, the relationship between host and parasitoid can directly influence the parasitism rate (BUENO; SAMPAIO, 2009).

Takada and Tada (2000) compared two strains of *A. ervi*, one of them originating from The Netherlands (EVP), and the other one from Northern Japan (SPR) and the values of parasitism rate of both were lower than the ones on this current work, when attacking two similar aphid species: 13% and 30% on *M. euphorbiae* on tomato plants and 10% and 42% on *A. solani* on sweet pepper plants, respectively. Studies conducted in south-eastern Brazil showed that the *A. ervi* Brazilian strain

has potential as a biological control agent of *M. euphorbiae* and *A. solani*. Sidney et al. (2010a) showed high percentage of formed mummies on *A. ervi* Brazilian strain on *M. euphorbiae* (74.1%) and *A. solani* (72.1%) on lettuce (*Lactuca sativa*) as a host plant.

The host *A. solani* was well accepted by the parasitoid *A. ervi* considering that the rate of parasitism was high for both strains. This aphid is readily attacked by *A. ervi* in both natural and agricultural settings but produces few offspring (TAKADA; TADA, 2000) and also the use of *A. ervi* for controlling *A. solani* still not have had always the expected success (SCHWORER; VOLKL, 2001; SIDNEY et al., 2010b).

Some biological parameters showed differences on quality between both strains tested in this study. The differences in quality of the parasitoid strains can be also due genetic variability. Genetic problems associated with the initial stock colony size of parasitoids and selections during mass rearing have been found by several authors (LENTEREN, 2003, 2009; REHMAN; POWELL, 2010). Unruh et al. (1983) showed that heterozygosity of an *A. ervi* population decreased during laboratory-rearing over several generations. Wratten and Powell (1991) demonstrated by electrophoretic techniques that there are genetic differences between laboratory populations of *A. ervi* from different hosts. Thus, mass rearing on alternative host species than the target or preferential species may alter the genetic composition of the aphidiid parasitoids.

Another interesting point that could interfere is the evolutionary interactions between bacteria and aphids, as have been demonstrated that *Hamiltonella defensa*, an endosymbiont of aphids, protects its aphid host

from attack by parasitoid wasps. This bacterium provides aphid host resistance to parasitoid attack (DEGNAN et al., 2009). These authors found *H. defensa* in *A. pisum*, and this bacterium blocked the larval development of the solitary endoparasitoid *A. ervi.* Although, was not evaluated in this study the presence of the bacteria *H. defensa* in the host aphids evaluated, this could be also an explanation for the lower parasitism of the STANDARD strain on *M.persicae*. However there are still a few studies that prove the relationship between *H. defensa* and *M.persicae*.

The high percentage of emergence for the NEW and STANDARD strains on all host aphids tested in this study, might ensure the success of the dispersion and parasitism of *A. ervi* in the next formed-generation in case of its release in greenhouse against *M. persicae nicotianae* and *A. solani* on sweet pepper crops and *Macrosiphum euphorbiae* on tomato crops. The high percentage of females of both strains developed in *A. solani* can be related to the suitability and size of this aphid host. Sex ratio may be unaffected by host size at parasitisation, but a higher proportion of female offspring emerge from larger hosts. The sex ratio of the emerging parasitoids may also be influenced by the parental sex ratio (REHMAN; POWELL, 2010).

The parasitism rate of NEW and STANDARD strains on *M. euphorbiae* was higher when they were reared one generation before on *M. euphorbiae* compared to those reared before on *A.pisum*. Both strains parasitoids populations were reared on standard host population *A.pisum*, and then transferred to *M. euphorbiae* as host and they also was transferred direct from *A. pisum* to *M. euphorbiae* in order to evaluate the

host recognition by the parasitoid *A. ervi* and its biological parameters on *M. euphorbiae*. This result was expected due the "previous experience" of the parasitoid related to it host, i.e., the experience accumulated by parasitoids. The parasitoid *A. ervi* may need some time to adapt to a new host species. A reduced reproductive performance in particular when both strains were tested directly on *M. euphorbiae*, in this study, without previous experience in this host may be assume that the parasitoid strains are still attempting to select the highest quality host for their offspring. Also we can infer that natal host influences a female's host assessment criteria in that it changes the ranking of perceived host quality with relation to parasitism.

Many studies have showed that parasitoids may be conditioned from previous experience, and this can affect the results on host species preference. Rehman and Powel (2010) stated that the parasitoid *A. ervi* collected from the pea aphid *A.pisum* also parasitized *Sitobion avenae* (F.) and *M.persicae*, whereas *A. ervi* collected from *S.avenae* would not parasitize *A.pisum* and *M. persicae*. However the field populations are more genetically diverse than from the laboratory populations and may show different behaviour if genotype influences host acceptance and host suitability. Cameron, Powel and Loxdale (1984) demonstrated that a slow adaptation to new aphid hosts occurred in *A. ervi*, accompanied by changes in the enzyme systems detected by electrophoresis. Similar results were found by Takada and Tada (2000), demonstrating that the a Japanese strain has the potential for a successful parasitisation of *M. euphorbiae* and *A. solani*, but these aphids represent less suitable hosts for both studied strains (SPR and EVP) in comparison to *A.pisum*, due to

a lower rate of parasitism. The explanation for these findings could be that *A.pisum* may generally represent a host of higher quality. Secondly, it was used parasitoids that originated from a stock culture maintained on *A.pisum*. This present result assumption would be in line with Cameron, Powel and Loxdale (1984) who found that 4-5 generations were necessary for *A. ervi* to increase the performance on *Sitobion avenae* (F). Highlighting that behavioural variation may exist because individuals differ genetically; our current research showed that parasitism rates were generally higher in NEW strain populations for both generations than to the STANDARD strain.

5 CONCLUSION

It is possible the use of the *A. ervi* strain NEW for both mass rearing and commercial proposals, and this strain could be improved by rearing it on *M. euphorbiae* or *A. solani* due it great performance on these host aphids.

ABSTRACT

The parasitoid *Aphidius ervi* (Haliday) is used against *Aulacorthum solani* (Kaltenbach), *Macrosiphum euphorbiae* (Thomas) and *Myzus persicae nicotianae* (Blackman), and the genetic variability is a factor affecting the performance of parasitoids in the aphid biological control programs. In this research we focused in comparing the quality of two strains of the parasitoid *A. ervi* on different aphid hosts species: a strain commercially produced for many generations on *Acyrthosiphum pisum* (Harris), denominated STANDARD, and other strain recently collected in the field attacking *M. persicae* and referred to as NEW. The tests were carried out under laboratory conditions at 22 ± 2°C, RH 70 ± 10% and 12h photophase. 24 h-old-females mated of the two *A. ervi* strain (STANDARD and NEW) from each aphid host *A. solani, M. persicae nicotianae* and *M. euphorbiae* were evaluated. A female *A. ervi* (STANDARD or NEW), mated and without previous oviposition experience, was released in a Petri dish (9 cm) for one hour period (1st visit), and then, the same female was released in another Petri dish for a two hours period (2nd visit). These Petri dishes contained 50 2nd instar nymphs of one of the aphid species on a leaf discs on a layer of 1% agar / water solution. Twenty-five replicates were done for each *A. ervi* strain. There were significant differences in the numbers of formed mummies of the two strains of *A. ervi* and among the different aphid as hosts. The number of formed mummies from the NEW strain was higher compared to the number from STANDARD strain when the female attacked *M. euphorbiae* during the 1st and 2nd visits. The aphid *M. persicae nicotianae* had lower numbers of formed mummies in both visits of strain NEW. However, the STANDARD strain showed numbers of formed mummies consistently higher on *A. solani.* The number of formed mummies of both strains NEW and STANDARD on *M. euphorbiae,* after having *M. euphorbiae* as original host, was higher compared to those that were reared before for one generation on *A. pisum*. The results showed that is possible to use the *A. ervi* strain (NEW) for both commercial mass rearing and as biological control agent proposals. Also this strain presented better performance having *M. euphorbiae* or *A. solani* as aphid hosts.

Key-words: Host quality. Aphid parasitoid. Strain.

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ARTICLE 2 Laboratory evaluation of several biological characteristics of *Praon volucre* Haliday (Hymenoptera: Braconidae: Aphidiinae) originating from 1 or 10 founder couples in *Aulacorthum solani* (Kaltenbach) (Hemiptera: Aphididae) over generations

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RESUMO

O conhecimento do efeito do número de casais fundadores ao longo das gerações é uma ferramenta importante para a criação massal em condições de laboratório e por mudanças comportamentais, como na capacidade de voo e no forrageamento pelo hospedeiro. No entanto, este efeito ainda não é bem conhecido e, geralmente pouco estudado, em particular para os parasitóides de pulgões que são criados por vários produtores comerciais e utilizados como agentes de controle biológico. O objetivo deste trabalho foi avaliar os parâmetros biológicos, reprodutivos e comportamentais dos indivíduos de *P. volucre* provenientes de 1 e 10 casais fundadores nas 3ª, 6ª, 9ª e 12ª gerações em *A. solani* tendo alface como planta hospedeira no laboratorio. Os indivíduos de *P. volucre* originados da criação iniciada com 10 casais fundadores apresentaram maior taxa de parasitismo na 3ª (63,6%) e na 9ª (55,5%) gerações quando comparados aos indivíduos originados de 1 casal fundador na 3ª (46,5%) e 9ª (32,5 %) gerações. A emergência, razão sexual e o número de ovos nos ovariolos não diferiram entre as gerações em ambas as criações. A capacidade de voo de *P. volucre* permaneceu elevada mesmo ao longo das gerações, tanto na criação iniciada com 1 ou 10 casais fundadores. Indivíduos provenientes de ambas as criações não diferiam em sua reação na busca por planta de alface infestada por pulgões (1 casal fundador: 61,6% e 10 casais fundadores: 53,3%) ou para plantas não infestadas (1 casal fundador: 20,0% e 10 casais fundadores: 16,7%). A maioria das femeas de ambas as criações se dirigiram para plantas infestadas com pulgoes em relaçao as plantas nao infestadas. Os resultados indicam que tanto a criação iniciada com 1 ou 10 casais fundadores pode manter uma qualidade aceitável dos indivíduos de *P. volucre*, considerando seus principais parâmetros biológicos, reprodutivos, capacidade de voo e resposta olfativa até a 6 ª geração em *A. solani* em condições de laboratório.

Palavras chave: Geração. Parasitoides. Número de ovos. Capacidade de voo. Capacidade de busca.

1 INTRODUCTION

The complex interactions pest - host plant - natural enemies, together with several environmental factors and crop management practices make not so easy to provide timing recommendations for the use of natural enemies in biological control. In this way, obstacles must be overcome for the continued adoption of biological control in greenhouses (MAHR et al., 2001) and quality control programmes has to be developed to released mass-reared organisms (LENTEREN, 2003).

The main issue of the quality control programmes is to check whether the quality of naturals enemies is maintained after mass rearing. So, characteristics that affect overall quality have to be identified (LENTEREN, 2009), when aimed release of the mass-produced natural enemies to control the pest. Lenteren (2003) claimed that is not necessary to consider maximal or optimal quality, but rather acceptable quality concern to their main characteristics.

Several Aphidiinae parasitoids are reared by different commercial producers (LENTEREN, 2012) under artificial conditions. Studies have demonstrated that, the parasitoid, *Praon volucre* Haliday is able to parasitize several aphid species in laboratory (LINS JÚNIOR et al., 2011; SIDNEY et al., 2010, 2011; SILVA et al., 2009), in greenhouses and in the field (STARY´; SAMPAIO; BUENO, 2007). Also, studies on developmental time and parasitism rate indicated that *P. volucre* might be a promising candidate for biological control of *Macrosiphum euphorbiae*, *A. solani,* and *Myzus persicae* (CONTI et al., 2011; SIDNEY et al., 2010a; SILVA et al., 2009; STARY´; SAMPAIO; BUENO, 2007).

However, the effects of the number of founder couples over the generations in the quality of the population of parasitoids is no known. Also the number of founder couples to started a rearing or the size of the initial sample is crucial and this number has been controversial by several authors (LENTEREN, 2003; LEPPLA, 2002; NUNNEY, 2002; PREZOTTI et al., 2002). Among the differents challenges to mass rearing biological control agents, are the development of an adequate mass rearing methodology and procedures that could optimize this rearing and minimize the effects from the mass rearing under artificial conditions, and then, maximize the efficacy in the field (LEPPLA, 2002). Again, relevant information and studies on those procedures on the parasitoid *P. volucre*, mainly concern to different numbers of founder couples and how these are affecting over several generations, are still poorly studied. Also in this parasitoid, the genetic variability in rearings started with different number of founder couples maybe does not reflect on its biological and behavioral parameters.

So, the objectives of this work were to evaluate the biological, reproductive and behavior parameters of *P. volucre* individuals originating from 1 and 10 founder couples in the 3rd , 6th, 9th and 12th generations in *A. solani*in lettuce plants in the laboratory conditions.

2 MATERIAL AND METHODS

2.1 Collection and rearing of *A. solani*

A. solani aphids were collected in greenhouse lettuce crops (*Lactuca sativa* L., cv Verônica) on the campus of the Federal University of Lavras (UFLA). After identification (PEÑA-MARTINEZ, 1992), the aphids were transferred to Petri dishes (15 cm diameter) containing a lettuce leaf disc (14 cm diameter), of the same cultivar on a layer of 1% water-agar, and kept in a climatic chamber at 22 ± 1°C, 70 ± 10% RH and 12h photophase. The offspring from the 3rd laboratory generation was used to start a stock colony of *A. solani* on the potted lettuce plants into acrylic cages (60x30x30 cm) in the laboratory. This rearing was according to the methodology proposed by Conti et al. (2010) and Sidney et al. (2010a). Aphids' individuals from the stock colony were used for the parasitoid *P. volucre* rearing and in the experiments.

2.2 Ollection and rearing of *P. Volucre*: Parasitoid mummies were collected and reared according to the methodology proposed by Lins Júnior et al. (2011) and Sidney et al. (2010b)

Sowthistle plants, *Sonchus oleraceus* L., infested with *Uroleucon* sp. (Hemiptera: Aphididae) and containing mummies and non-mummified aphids were collected from the field in 2010 in several areas to avoid obtaining parental parasitoids individuals on the municipality of Lavras, Minas Gerais state (Brazil). *Praon* sp. mummies were recognized based in the characteristic colour pale with silky filaments under the

cocoon and pupation occurred outside the host (BUENO; SAMPAIO, 2009). Those mummies were placed individually in glass tubes (10 cm x 8mm) sealed with organza until adult emergence. Small honey and water drop were put on the glass tubes walls to provide enough food to emerged adults. Non - mummified aphids were kept on the same plant with the petiole in glass tubes with water, into an acrylic cage (60x30x30 cm). These plants were examined regularly during a 7 days period until the mummy formation and adult emergence, and kept in an climate room at 22 ± 3 º C, RH 60 ± 10 and 12 h photophase. Thereafter, a sample of parasitoids individuals were identified by the use of the key of Tomanovic et al. (2003), and also with help of the *P. volucre* collection reference kept on the Department of Entomology of UFLA. Fifth individuals emerged of the mummies collected in the field, were separated by sexes and females and males were kept together for a 24h period for mating and female maturation, and a stock colony was started in the laboratory. This first generation from the stock colony collected were taken to start the rearing's with 1 or 10 founder couples over the generations in the laboratory conditions.

2.3 Biological parametes and flying and searching capacities of *P. volucre* originating from 1 and 10 founder couples in *A. solani* over the generations in the laboratory

Two initial rearing's were started with 1 and 10 founder couples of *P. volucre*, being the individuals released in each acrylic cages (60x30x30 cm) containing potted lettuce plants infested with nymphs of

A. solani. The rearing's were kept in acclimatized room at 22 ± 3 º C, RH 70 ± 10% and 12 h photophase. To minimize the defense behaviour and overstressing of the *A. solani*nymphs when confronted with the parasitoid individuals, the infested potted lettuce plants were introduced into the acrylic cages 24 h before the release of the parasitoids. After five days, new greenhouse-grown disease-free lettuces plants were introduced into the cages. At this time, the nearly-mummified aphids abandoned the original infested plants and moved to the new plants, where the mummification was completed.

After emergence and mating of adults parasitoids, *A. solani*-infested potted lettuce plants were replaced by new ones to continue the parasitoid *P. volucre* rearing. The individuals from these rearing were evaluated in their biological and reproductive parameters and on its flying and searching capacities. The maintenance - rearing of 1 or 10 founders couples were according to the methodology by Lins Júnior et al. (2011) and Sidney et al. (2010a, 2010b). The biological parameters of *P. volucre* individual's originating from these founder couple were evaluated in the 3rd, 6th, 9th and 12th generations in the laboratory. This procedure followed the reports of several authors (HOPPER; ROUSH, 1993; LENTEREN, 2003; NUNNEY, 2003) concern to that adaptive changes were seen in the first ten generations of the rearing insects in the laboratory.

2.3.1 Development time, percentage of parasitism and emergence, sex ratio, longevity and size

This experiment was carried out for each generation tested (3rd, 6th, 9th and 12th).

Fifty mummies from the previous generation to be tested were isolated in glass tubes (10 cm x 8mm) with a small honey and water droplets to provide enough food to emerged adults, to ensure that would be obtained 10 females and 10 males for the tests (n=10). After adult emergence, each previously mated parasitoid female of *P. volucre* less than 24 h old and without previous oviposition experience was released in a Petri dish (5 cm diameter) containing 20 2nd and 3rd instars nymphs (about 38 to 60 hours old) of the aphid *A. solani*on a lettuce leaf disc (5 cm diameter) on a layer of 1% water-agar.

The female parasitoid was kept in the Petri dish for 90 minutes period to contact and oviposit on the host. The Petri dishes containing the parasitized aphid nymphs were sealed with paper towel, turned upside down, and kept in climatic chamber at 22 ± 1 º C, RH 70 ± 10% and 12 h photophase until the mummy's formations (± 7 days). The parasitized aphids were periodically transferred to new Petri dish containing fresh lettuce disk as needed. Upon mummification, the aphids were individualized in glass vials (100 mm x 8mm) until adult parasitoid emergence, which were fed daily with honey and distilled water, both deposited in the form of small droplets on the vial's inner walls.

Daily observations were made in order to evaluate the developmental time (from oviposition to adult emergence); percentage of

parasitism (based on number of mummies formed) and emergence; sex ratio and female and male longevities. To measure the longivety of *P. volucre* over generations (3rd, 6th, 9th and 12th) for both rearings tested, 15 males and 15 females were maintained individually in glass vials (100 mm × 8 mm) without hosts and fed with honey and water daily until they died. In order to determine parasitoid size, measurements of the right hind tibia of 15 male and 15 female *P. volucre* adults were made. The parasitoid size was measured by the right hind tibia from 15 females and 15 males of *P. volucre* adults. Size measures were made using an ocular micrometer under the optical microscope (100 x magnification) by properly removing the tibia and mounting it under a coverslip on a slice with a droplet of 70% alcohol.

2.3.2 Potential fecundity

Ten 24h-old *P. volucre* emerged-females originated from each parasitoid generation (3rd, 6th, 9th and 12th) from each rearing started with 1 and 10 founders couples were killed in 70% ethanol and dissected to count the number of eggs in their ovarioles. First, the ovarioles were separated from the remainder of the gaster under a stereomicroscope and stained with lactophenol blue solution to facilitate the seeing of the eggs. Next, the eggs in the two ovarioles were counted under a stereomicroscope (Olympus-SZ40, Olympus Corporation, Japan) according to methodology proposed by Lins Júnior et al. (2011) and Sampaio, Bueno and Conti (2008).

2.3.3 Flight capacity of *P. volucre*

One day old males and females of *P. volucre* previously fed with honey and originated from each generation from each rearing started with 1 or 10 founders couples were placed into an acrylic tube (3.5x2 cm) in an open Petri dish (15 cm diameter) which formed the bottom of a cylinder. The cylinder had opaque walls, with 20 cm high and diameter of 10 cm. The top of the cylinder had a transparent glass cover with a layer of glue at the underside (LINS JÚNIOR et al., 2011). A lamp (9 W white lights) was placed above of the cylinder to attract parasitoids. To prevent that parasitoids could walk to the top of the cylinder, water was placed in the Petri dish surrounding the acrylic tube with parasitoids. The number of parasitoids glued to the top of the cylinder was recorded after 4 h period. Tests were conducted out in a acclimatized room (22 ± 3° C, 70% RH) with 10 parasitoids *P. volucre* (5 females and 5 males) per test and replicated five times for each generation (3^{rd}, 6^{th}, 9^{th} and 12^{th}) on each rearing started with 1 and 10 founders couples. The flight capacity of the parasitoid *P. volucre* was evaluated according to methodology described by Langer, Boivin and Hance (2004), Lenteren (2003) and Lins Júnior et al. (2011).

2.3.4 *P. volucre* response to establish in olfactometer

For this test, we isolated 70 mummies from the previous generation to be tested to ensure that we would be obtained 30 newly emerged females from each generation (3^{rd}, 6^{th}, 9^{th} and 12^{th}) from each

rearing started with 1 and 10 founder couples. The choice between aphids-infested and non-infested plants was offered to the females. Lettuce plants were cultivated in plastic pots until they reached approximately 10 cm high and had their primary leaves completely opened. These plants were transferred to 2 liters glass pots and infested with aphids (35 *A. solani* adults per plant) 48 h before the olfactometer tests were performed. Control plants were grown in the same way, but were not infested with aphids.

The attractiveness of *P. volucre* to volatiles was evaluated using a glass Y-tube olfactometer. The 'Y tube' olfactometer consisted of a central glass tube (15 cm long and 3.5 cm internal diameter) and two arms (17.5 cm long, 3.5 cm internal diameter) mounted at an angle of 75^{o} to each other which carried out the volatile stimuli emanating from the 2 liters glass pots containing the aphids-infested or non infested plants, to the base of the central glass tube. The olfactometer was held in a vertical position, as described by Carvalho, Bueno and Castañé (2011) and Venzon, Janssen and Sabelis (1999). The flow of air through the olfactometer was measured using a fluxometer and was maintained at a 0.2 m/s with the aid of a vacuum pump. *P. volucre* females were placed individually at the base of the central tube and their movement towards one of the arms of the Y tube was timed using a chronometer. Timing was stopped when the female reached the middle of one of the arms.

The maximum observation period was 10 minutes, and females which did not choose an arm within this interval were scored as having made no choice (when there was not orientation for aphids-infested or non-infested plants). After five observations, the Y-tube was cleaned with

current water e dried on the heater to remove any residual material (such as faeces, etc.) left by previous females evaluated. Tests were performed at climatized room at 21 ± 3 ^{o}C; 70% ± 10% RH and 12 h photophase.

Each experimental group consisted of 30 parasitoids female (n=10) originated from 1 or 10 founders couples in the 3rd, 6th, 9th and 12th generations. Each female was tested individually and only once. This methodology has been described and adapted according to the methodology proposed by Carvalho, Bueno and Castañé (2011).

3 DATA ANALYSES

Were compared all generation tested in each rearing separately and also was compared each generatio between the between both rearings started with 1 and 10 founders couples. The effect over the generations of parasitoids originate from rearing started with 1 and 10 couples founders on parasitism (expressed by the number of formed mummies), emergence (%), development (from oviposition to adult) (days), female and male longevity (days), flight activity (%), the number of eggs in the ovarioles were used the generalized linear models constructed by using generalized linear models (GLM) and the appropriate distribution of errors analyzed of each response variable (CRAWLEY, 2005). The means were compared by Tukey test ($p < 0.05$). For significance of effects of the models, the sex ratio was subjected to chi-square test ($p < 0.05$). The statistical analyzes were performed using program (R DEVELOPMENT CORE TEAM, 2005) and using the package multcomp (HOTHORN; BRETZ; WESTFALL, 2008).

The length of tibia of females and males of *Praon volucre* were subjected to analysis of variance and the means were compared by Tukey test ($p < 0.05$). Statistical analyzes were performed with the program R (R DEVELOPMENT CORE TEAM, 2005) and using the package agricolae (MENDIBURU, 2012).

The olfactory response of the *P. volucre* were compared by chi-square adherence ($p < 0.05$), and statistical analyzes were performed with the use of the program R (R DEVELOPMENT CORE TEAM, 2005).

4 RESULTS

4.1 Parasitism

The percentage of parasitism, expressed according the number of formed mummies, in *A. solani* from individuals *P. volucre* originate from 1 founder couple was similar in the 3rd and 9th generations and showing significant differences compared to the number in the 6th and 12th generations. The number of formed mummies in the 6th (76.5 %/15.3 mummies) and 12th (65.8 %/13.2 mummies) generations were higher compared to the number on the 3rd (46.5 %/9.3 mummies) and 9th (32.5%/6.5mummies) generations (*d.f.* =3; *F*= 12,043; *P* <0.001) (Table 1) (Figures 1a and 1b).

However, no significant differences were found in the number of formed mummies on *A. solani*by *P. volucre* among 3rd, 6th, 9th and 12th generations from individuals originating from rearing started with 10 founder couples (*d.f.* = 3; *F*= *1*.805; *P*= 0.170) (Table 2) (Figure 2).

The number of formed mummies on *A. solani*by *P. volucre* in the 3rd and 9th generations was significantly different for 1 or 10 founder couples. The individuals from the rearing started with 10 founder couples showed higher rate parasitism or formed mummies in both generations, 3rd (10 couples = 63.6%/ 12.7 mummies; 1 couple = 46.5%/9.3 mummies *d.f.*= 19 *F*= 0.01801) and 9th (10 couples = 55.5%/ 11.1 mummies; 1 couple = 32.5%/ 6.5 mummies *d.f.*= 18 *F*= 0.0005 (Figures 1a and 1b).

4.2 Development and emergence

P. volucre individuals from the 9th (17.4 days) and 12th (17.3 days) generations showed developmental time (from oviposition to adult emergence) longer than the individuals from the 3rd (14.1 days) and 6th (14.2 days) generations when the rearing started with 1 founder couple (Table 1). However individuals originated from rearing started with 10 founder couples under 3rd (16.0 days), 6th (16.1 days) and 9th (16.6 days) generations demonstrated shorter developmental time than the individuals from 12th (23. 0 ± 1.48 days) generation (Table 2). There was no difference in the developmental time of individuals originating from 10 and 1 founder couples over the generations, showing the following values in the 3rd (10 = 16.04; 1 = 14.14 days), 6th (10 = 16.14; 1 = 14.21 days) and 9th (10 = 16.62; 1 = 17.37 days) generations. However, *P. volucre* individuals in the 12th generation from 10 couples founder showed longer developmental time (23.04 days) when compared to individuals from 1 couple founder (17.27 days) (*d.f.*= 20; *F*= 0.0026) (Figure 2).

The emergence rate of all individuals originating from rearing started with 10 and 1 founder couples from each generation tested (3rd, 6th, 9th and 12th) were similar in the 3rd (10 =81.0/ 1= 91.1%/ *d.f.* = 18; *F*= 0.1133), 6th (10 = 68.3/ 1= 78.7%/ *d.f.*= 18; *F*= 0.2813), 9th (10 = 72.9/ 1= 86.2%/ *d.f.*= 18; *F*= 0.1648) and 12th (10= 77.2 %/ 1= 83.0%/ *d.f.*= 20; *F*= 0.3272) generations (Table1) (Table 2) (Figure 3).

4.3 Sex ratio and number of eggs in the ovarioles

The sex ratio and the number of eggs of parasitoid's females did not differ among all generations tested when the rearing started with 1 or 10 founder couples. The sex ratios were 3rd (10 = 0.41/ 1= 0.33), 6th (10 = 0.41/ 1= 0.35), 9th (10 = 0.33/ 1= 0.36) and 12th (10= 0.39/ 1= 0.50) generations (Tables 1, 2).

The number of eggs in the ovarioles of parasitoid's females considering the generations and the founder couples were 3rd (10 =100.2 eggs / 1= 95.0 eggs / *d.f.* = 18; *F*= 0.1133), 6th (10 = 88.9 eggs / 1= 78.7 eggs / *d.f.*= 18; *F*= 0.2813), 9th (10 = 90.7 eggs / 1= 97.8 eggs / *d.f.*= 18; *F*= 0.1648) and 12th (10= 98.6 / 1= 94.4 eggs / *d.f.*= 20; *F*= 0.3272) (Figure 4).

4.4 Longevity and flight activity

There were differences in the longevity of females of *P. volucre* from the 3rd, 6th, 9th and 12th generations originated from rearing started with 1 founder couple. The longevity of females was longer in the 6th generation (19.2 days) than on the 3rd (14.8 days) and 9th (16.0 days) and 12th (14.8 days) generations. The male longevity in the 9th generation was shorter (13.1 days) than on the others generations evaluated (Table 1).

No effect over the generations originating from rearing started with 10 founder couples was found for the female and males longevities, and the values ranged from 13.8 to 17.4 days for females and 12.4 to 14.1 days for males (Table 2). Also no effect was found comparing over the

generations between 10 and 1 founder couples in the male longevity (Figure 5), but in the 6th generation, the female longevity originating from 10 founder couples (13.8 days) was shorter than to females from 1 founder couple (19.2 days) (*d.f.*= 17; *F*= 0.00038) (Figure 6).

The flight activity, express by percentage of adults flying, originating from rearing started with 1founder couple , was higher (88.0 %) in the 9th generation when compared to individuals on the 6th generation (72.0 %) (Table 1). However, adults originating from rearing started with 10 founders couples had the flight activity higher in the 3rd and 6th generations (82.0 and 84.0 % respectively) than in the 9th and 12th generations (66.0 and 66.0% respectively) (Table 2). And, when compared both individuals from rearing started with 1 and 10 founder couples over the generations, the flight activity by parasitoid's adults was different only in the 9th generation, in which the percentage of adults flying from rearing started with 10 founder couples (66%) was shorter than the number individuals from 1 founder couple (88%) (*d.f.*= 8; *F*= 0.0218) (Figure 7).

Table 1. Biological parameters of *Praon volucre* (± S.E.) in *Aulacorthum solani* over the 3rd, 6th, 9th and 12th generations originating from rearing started with 1 founder couple. 22 ± 2 ° C, RH 70 ± 10% and 12 h photophase.

Biological parameters of *P. volucre*[1]	Generations[1]			
	3rd	6th	9th	12th
Parasitism (%)	46.5±4.15b (93)	76.5±6.46a (153)	32.5±5.28b (65)	65.8±5.22a (158)
Development (oviposition-adult) (days)	14.1±0.12b (84)	14.2±0.07b (120)	17.4±1.10a (56)	17.3±0.77a (131)
Emergence (%)	91.2±3.76a (84)	78.7±7.14b (120)	86.3±6.63ab (56)	83.0±4.30ab (131)
Sex ratio[2]	0.33±0.11a (30)	0.35±0.55a (53)	0.36±0.09a (23)	0.50±0.08a (79)
The number of eggs in the ovarioles	95.0±8.37a (100)	87.2±11.89a (100)	97.8±12.34a (100)	94.4±6.53a (100)
Female longevity (days)	14.8±1.02b (30)	19.2±1.49a (30)	16.0±0.84ab (30)	14.8±0.48b (30)
Male longevity (days)	15.5±0.70a (30)	16.4±1.04a (30)	13.1±1.02b (30)	14.9±0.57ab (30)
Flight activity (%)	86.0±3.60ab (43)	72.0±5.66b (36)	88.0±1.41a (44)	84.0±3.29ab (42)

[1]Means followed by the same small letter in the row, do not differ by Tukey test (P<0,05).
[2]Means followed by the same small letter in the row, do not differ by chi-square test (P<0,05).
n= number of individuals

Table 2. Biological parameters of *Praon volucre* (± S.E.) in *Aulacorthum solani* over the 3rd, 6th, 9th and 12th generations originating from rearing started with 10 founder couples. 22 ± 2 ° C, RH 70 ± 10% and 12 h photophase.

Biological parameters of *P. volucre* [1]	Generations[1]			
	3rd	6th	9th	12th
Parasitism (%)	63.6±6.50a (126)	73.5±5.53a (147)	55.5±5.65a (111)	62.0±6.63a (124)
Development (oviposition-adult) (days)	16.0±0.84b (102)	16.1±0.69b (100)	16.6±0.56b (80)	23.0±1.48a (95)
Emergence (%)	81.0±4.73a (102)	68.3±6.04a (100)	72.9±6.42a (80)	77.2±3.70a (95)
Sex ratio[2]	0.41±0.07a (41)	0.41±0.06a (41)	0.33±0.89a (26)	0.39±0.89a (37)
The number of eggs in the ovarioles	100.2±15.26a (100)	88.9±7.99a (100)	90.7±9.34a (100)	98.6±8.32a (100)
Female longevity (days)	13.8±1.60a (30)	13.8±1.46a (30)	17.4±1.72a (30)	14.2±1.26a (30)
Male longevity (days)	14.1±1.24a (30)	13.7±1.28a (30)	13.0±0.94a (30)	12.4±1.27a (30)
Flight activity (%)	82.0±3.30a (41)	84.0±2.83a (42)	66.0±5.29b (33)	66.0±6.56b (33)

[1]Means followed by the same small letter in the row, do not differ by Tukey test (P<0.05).
[2]Means followed by the same small letter in the row, do not differ by chi-square test (P<0.05).
n= number of individuals

4.5 Size

The length of the hind tibiae of individuals in the 3rd, 6th, 9th and 12th generations originating from rearing started with 1 founder couple was significant different. The values of the female and male tibias' size in the 3rd and 6th generations were larger than the size in the 9th and 12th generations (Table 3).

When compared the tibiae size among all generations tested and from individuals originating from rearing started with 10 founder couples, the smaller size of the female and male tibias' was found in 12th generation (Table 3). The size of the adult females tibias was larger on individuals originated from rearing started with 1 founder couple in the 3rd (0.789 mm♀) and 12th generations than on individuals from 10 founder couples in the 3rd and 12th generations (Table 3).

Table 3. The length (± S.E.) of tibias ♀ and ♂ adult *Praon volucre* (± S.E.) originating from 1 or 10 founder couples over the 3rd, 6th, 9th and 12th generations in *Aulacorthum solani.* 22 ± 2 ° C, RH 70 ± 10% and 12 h photophase.

G	Tibia length of parasitoids (mm)[1] (n=15)			
	Female ♀		Male ♂	
	1 couple	10 couples	1 couple	10 couples
3rd	0.789±0.0086aA	0.725±0.0142bA	0.676±0.0095aA	0.628±0.0085bA
6th	0.747±0.0117aAB	0.707±0.0193aAB	0.667±0.0101aAB	0.611±0.0161bAB
9th	0.718±0.0153aB	0.746±0.0201aA	0.617±0.0122aB	0.641±0.0170aA
12th	0.729±0.0120aB	0.684±0.0136bB	0.621±0.0130aAB	0.574±0.0160bB

[1]Means followed by the same small letter in the row for the same sex and capital letter in the column for different generations, do not differ by Tukey test

4.6 Response to volatiles by *P. volucre* to aphid-infested and non infested lettuce plants

The individuals originating from rearing started with 1 or 10 founder couples did not diffe in their incidence when searching for aphid-infested - lettuce plant (individuals from 1 founder couple: 61.6 % and individuals from 10 founder couples: 53.3 %) or for non - infested lettuce plant (individuals from 1 founder couple: 20.0 % and individuals from 10 founder couples: 16.7 %) (Table 4). Also no difference when the females did not choose between the two odors, *i.e.* they did not move or react to both plant odors (individuals from 1 founder couple: 13.3 %; individuals from 10 founder couples: 30.0 %) (Table 4).

However, in both rearing started with 1 and 10 founder couples there were significant differences among the numbers of females parasitoids that moved towards the odors from aphids-infested lettuce plant comparing to females which moved to non-infested lettuce plant and to females that did not respond to the odors within the 10 min period (no choice to any odor) (Table 4).

Table 4. Response to volatiles by *Praon volucre* (± S.E.) originated from 1 or 10 founder couples to odors from aphid-infested lettuce plant (*Aulacorthum solani*) and non-infested- lettuce plant at 22 ± 2° C, RH 70 ± 10% and 12 h photophase.

Odors	Incidence of the parasitoid (%)[1] (n=30)	
	1 couple	10 couple
Aphid-infested lettuce plant	61.6±11.34aA	53.3±13.40aA
Non-infested lettuce plant	20.0±6.45aB	16.7±7.58aB
No choice by the female to odors	13.3±7.07aB	30.0±9.31aB

[1]Means followed by the same small letter in the row and capital letter in the column, do not differ by chi-square

5 DISCUSSION

The parasitism rate of *P. volucre* individuals originating from rearing started with 1 founder couple was high until the 6th generation, however in the 9th generation there was a decrease, and increased again in the 12th generation. Maybe this was reflected by the origin of the individuals (only one founder couple) and the influence of genetic varialbilty over generations. When were compared individuals from 10 or 1 founder couples, the individuals from rearing started with 10 founder couples showed higher parasitism rate in the 3rd and 9th generations than individuals originated from 1 founder couple in the same generations. Individuals of *P. volucre* from the 3rd generation showed a parasitism rate of 46.5% (3rd /1 founder couple) and 63.6% (3rd /10 founder couples) on *A. solani*. These values were similar to the parasitism rate on *A. solani* (58.5%) (SILVA et al., 2009) and on *M. persicae* (57.5%) (SIDNEY et al., 2010a).

The developmental time of *P. volucre* increases over generations when the rearings started with 1 and 10 parasitoids founder couples. Host quality for parasitoid growth and development is often assumed to be associated with host size (WAAGE, 1986). Furthermore, host-plant effects can be cumulative, as in some aphids species reared for consecutive generations on nutrient-deprived host-plant, this can be reflect in a longer time to become adults and than in the increased mortality of parasitoids developing in such hosts (REHMAN; POWEL, 2010). The genetic deterioration can happen (GURR; WRATTEN, 2000) from the initial stock rearing in a laboratory colony over several

generations and then may increasing the developmental time of the parasitoids larvae. In this study, from 3rd to 9th generations there was no difference between individuals from 10 and 1 founder couples over the generations on the developmental time. However, individuals *P. volucre* from the 12th generation and originated from rearing started with 10 founder couples showed longer developmental time than ones from 1 founder couple. Even the rearing started with 10 founder couples contained more individuals than the rearing started with one founder couple over the 12th generation and showing long developmental time. However, the parasitoid population formed by 10 founder couples may provide individuals with a low genetic adaptation on the laboratory environment. One hypothesis for this result would be that the one founder couple initially had a genetic variability aceitable and better skills in behavior due the extrinsic factors, such as climatic conditions and host density in the laboratory conditions. Lewis et al. (2003) described that strains of parasitoids that occupy different regions with different climatic conditions are inherently more suited for their respective ecological conditions.

The emergence of *P. volucre* on *A. solani* was higher and there is no influence over the generations in both rearing started with 1 and 10 founder couples, even when compared to both founder couples. The sex ratio of the emerged parasitoids may also be influenced by the parental sex ratio (REHMAN; POWELL, 2010). Maybe in this study, the lower percentage of emerged females was related to the small number of original founder couple. Part of an "open population", which gene's migration can occur and environmental diversity is big, it is broken in the

laboratory, making it a “closed population” and all genetic changes will come from a limited variation of the original founders (LENTEREN, 2009).

The number of eggs in the ovarioles (egg load) at the emerged female is widely used as an indicator of potential reproductive performance and normally directly related to parasitoid size (CHAU; MACKAUER, 2001; SAMPAIO; BUENO; CONTI, 2008). Our results show that *P. volucre* in *A. solani*, even over the different generations tested in rearing started with 1 or 10 founder couples, have a stable reproductive potential or no variation on the number of eggs on the ovarioles. Lins Júnior et al. (2011) demonstrated that emerged females from mummies stored for 20 days had half of the number of eggs (84.8 eggs) than females from the control treatment (no storage) (169.8eggs). However, the higher number of eggs in the control treatment found by Lins Júnior et al. (2011) may be explained by the use of *M. euphorbiae*, a larger aphid host (SIDNEY et al., 2010a) than *A. solani*, used in this study. Host quality is in general associated with host size, with larger hosts having more resources, resulting in a greater parasitoid fitness (CHAU; MACKAUER, 2001; NICOL; MACKAUER, 1999).

The males and females from rearing started with 1 founder couple had the longest longevity in the 6th generation. This result supports that in the 6th generation the females can oviposit on more numbers of aphids host and contributing for aphid biological control.

The importance of flight tests has been discussed (LENTEREN, 2003, 2009; LINS JÚNIOR et al., 2011; PREZOTTI et al., 2002), but evaluations on flight capacity of aphid parasitoids are still rare.

Interestingly, this study shows that the flight capacity of *P. volucre* remains high even over the generations tested in both rearing started with 1 or 10 founder couples. Thus, the females' parasitoid has the ability to disperse more in the crop and consequently increases the encounter with aphid's hosts and contributing to aphid biological control.

Several braconid aphid parasitoid species have been reported as responding to a variety of both contact and olfactory cues associated with the host or with the host's habitat (CARVER; FRANZMANN, 2001; JANG et al., 2000). It is assumed that the release of plant volatiles due to herbivore-feeding damage serves as a mechanism that first evolved as a direct defense against herbivores and pathogens, and the function of attracting natural enemies evolved secondarily (LO PINTO et al., 2004). In this study, the attraction of *P. volucre* by odors derived from plants shows that parasitoid females are able to locate the host *A. solani*and is also able to discriminate between non-infested and infested lettuce plants by flying upwind toward odors emitted by the *A. solani*or lettuce plant–host complex. Similar studies with *Aphidius colemani* Viereck on *Myzus persicae* Sulzer as a host and bell pepper (*Capsicum nnuum* L.) as host plant, showed that experienced females responded significantly more to infested-plants than to non-infested ones (GRASSWITZ, 1998). The ability of parasitoids to locate their host is expected to vary with different host and plant species, because they release different blends of volatiles (REDDY, 2012) and several braconid aphid parasitoid species have been reported as responding to a variety of olfactory cues linked with the host or with the host's habitat.

Orientation response and search of individual's *P. volucre* from rearing's started with 1 or 10 founder couples in the aphid host *A. solani* on lettuce plant was determined by the effects of the plant's volatiles on the host-searching behaviour of *P. volucre*, and by examining the olfactory response of this parasitoid species to *A. solani*on lettuce plants. Odors from host food plants seem particularly important in habitat location.

6 CONCLUSION

The orientation response and search of *P. volucre* didn't show influence by relationship between initial sample of rearing, if started with 1 and 10 founder couples of *P. volucre*. However the biological parameters can be modified depending on the numbers of founder couples.

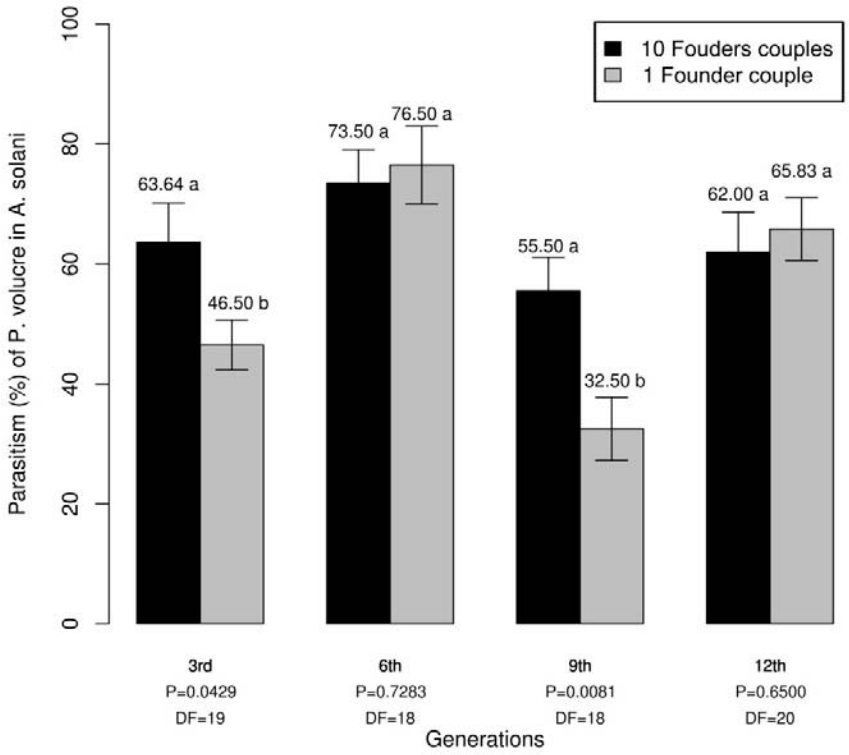

Figure 1a. Parasitism (%) in *Aulacorthum solani* by *Praon volucre* (± S.E.) originating from 1 and 10 founder couples in the 3rd, 6th, 9th and 12th generations in the laboratory. 22 ± 2 ° C, RH 70 ± 10% and 12 h photophase.

[1]Means followed by the same small letter in the row, do not differ by Tukey test ($P<0.05$).

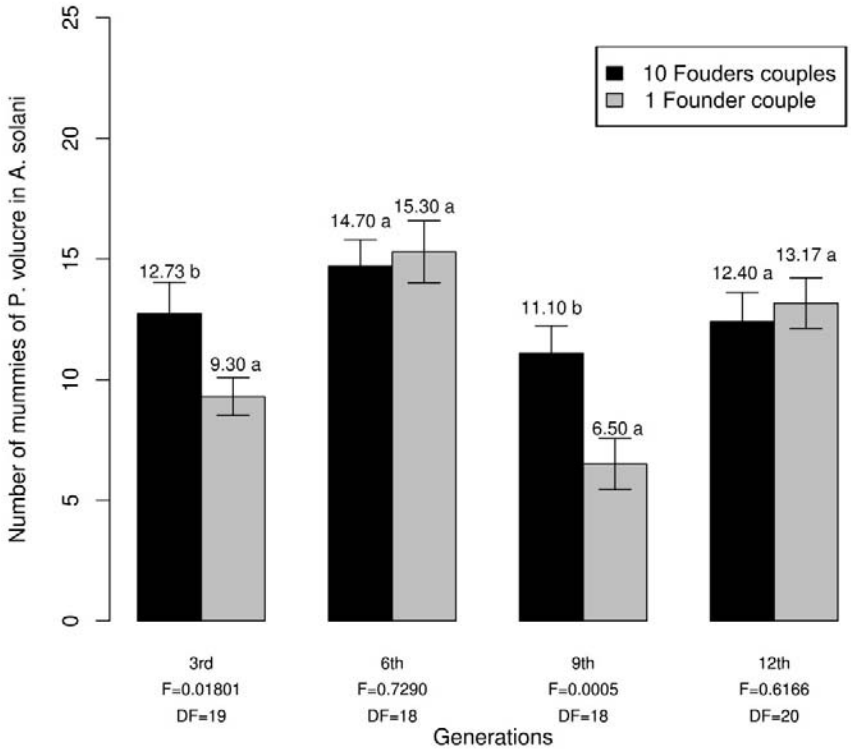

Figure 1b: Number of formed mummies of *Praon volucre* in *Aulacorthum solani* (± S.E.) originating from 1 and 10 founder couples in the 3rd, 6th, 9th and 12th generations in the laboratory. 22 ± 2 ° C, RH 70 ± 10% and 12 h photophase.

[1]Means followed by the same small letter in the row, do not differ by Tukey test ($P<0.05$).

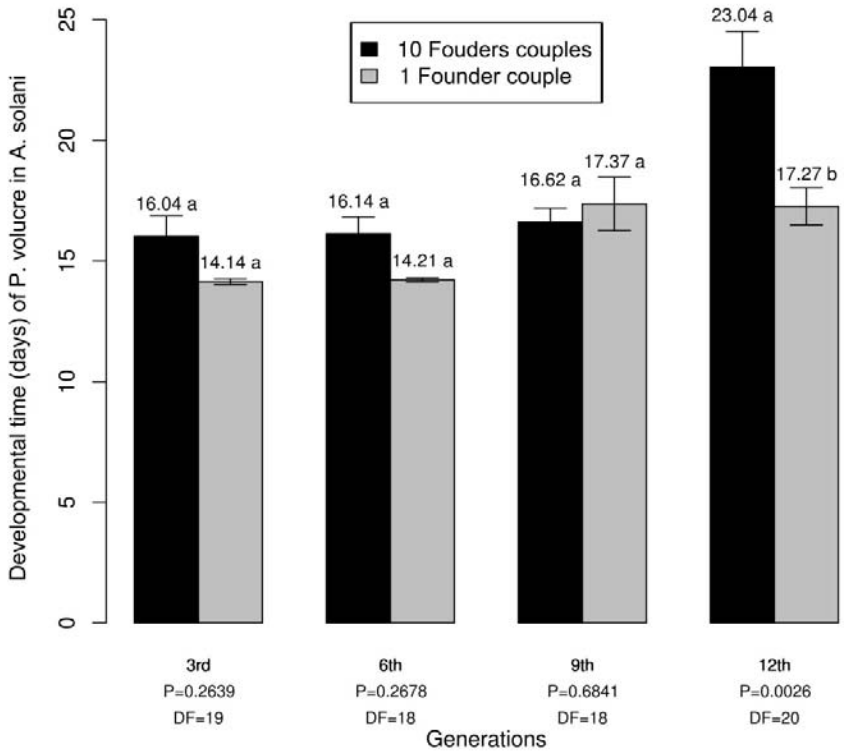

Figure 2. Developmental time (days) of *Praon volucre* in *Aulacorthum solani* (± S.E.) originating from 1 and 10 founder couples in the 3rd, 6th, 9th and 12th generations in the laboratory. 22 ± 2 ° C, RH 70 ± 10% and 12 h photophase.

[1]Means followed by the same small letter in the row, do not differ by Tukey test ($P<0.05$).

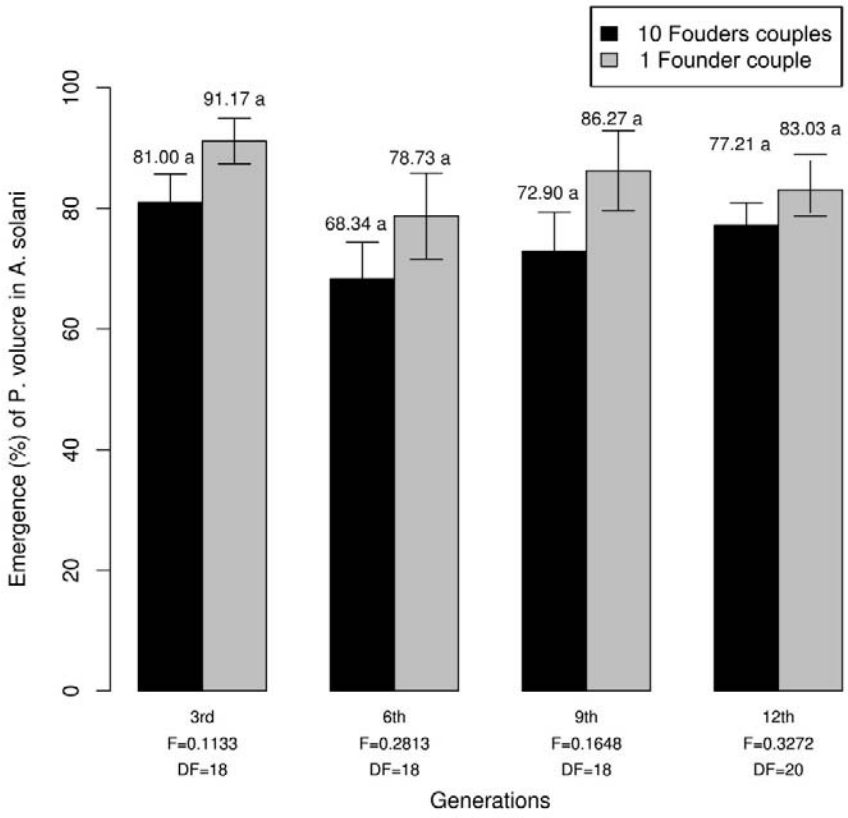

Figure 3. Emergence (± S.E.) of *Praon volucre* in *Aulacorthum solani* originating from 1 and 10 founder couples in the 3rd, 6th, 9th and 12th generations in the laboratory. 22 ± 2 ° C, RH 70 ± 10% and 12 h photophase.

[1]Means followed by the same small letter in the row, do not differ by Tukey test ($P<0.05$).

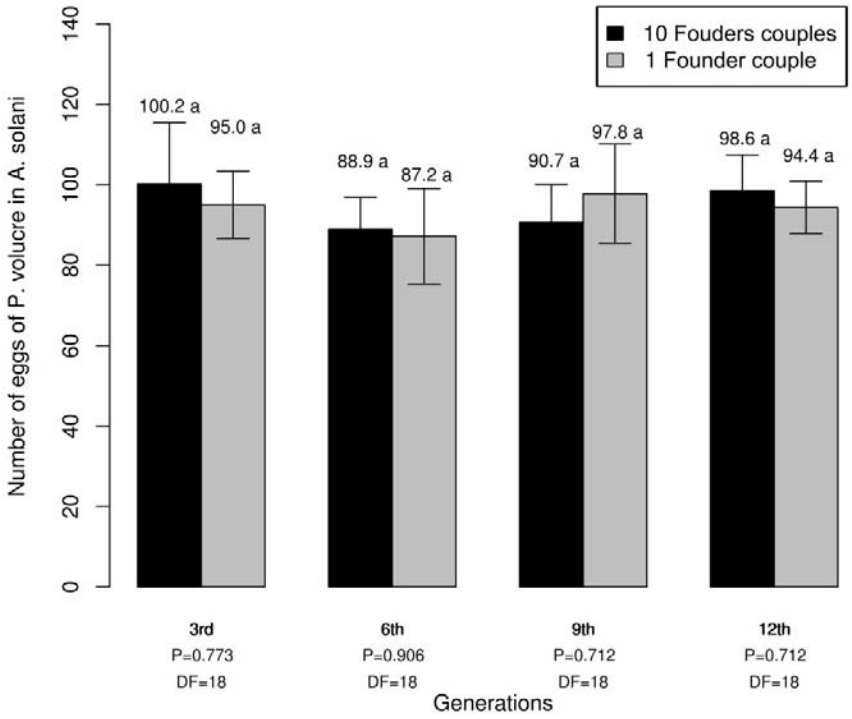

Figure 4. Number of eggs in the ovarioles (± S.E.) of *Praon volucre* in *Aulacorthum solani* originating from 1 and 10 founder couples in the 3rd, 6th, 9th and 12th generations in the laboratory. t 22 ± 2 ° C, RH 70 ± 10% and 12 h photophase.

[1]Means followed by the same small letter in the row, do not differ by Tukey test ($P<0.05$).

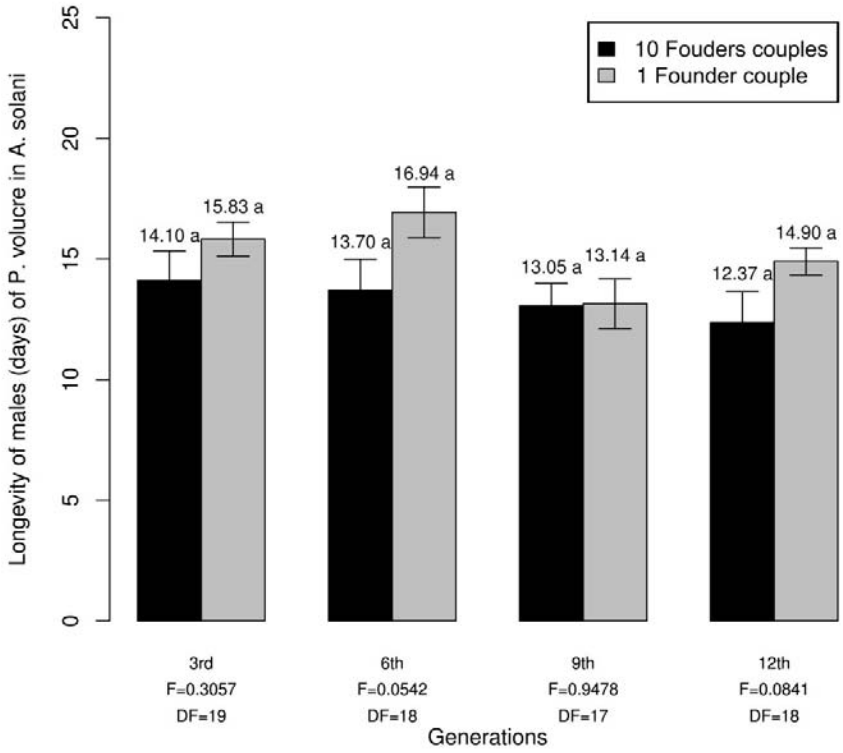

Figure 5. Male longevity (days) (± S.E.) of *Praon volucre* in *Aulacorthum solani* originating from 1 and 10 founder couples in the 3rd, 6th, 9th and 12th generations in the laboratory. 22 ± 2 ° C, RH 70 ± 10% and 12 h photophase.

[1]Means followed by the same small letter in the row, do not differ by Tukey test ($P<0.05$).

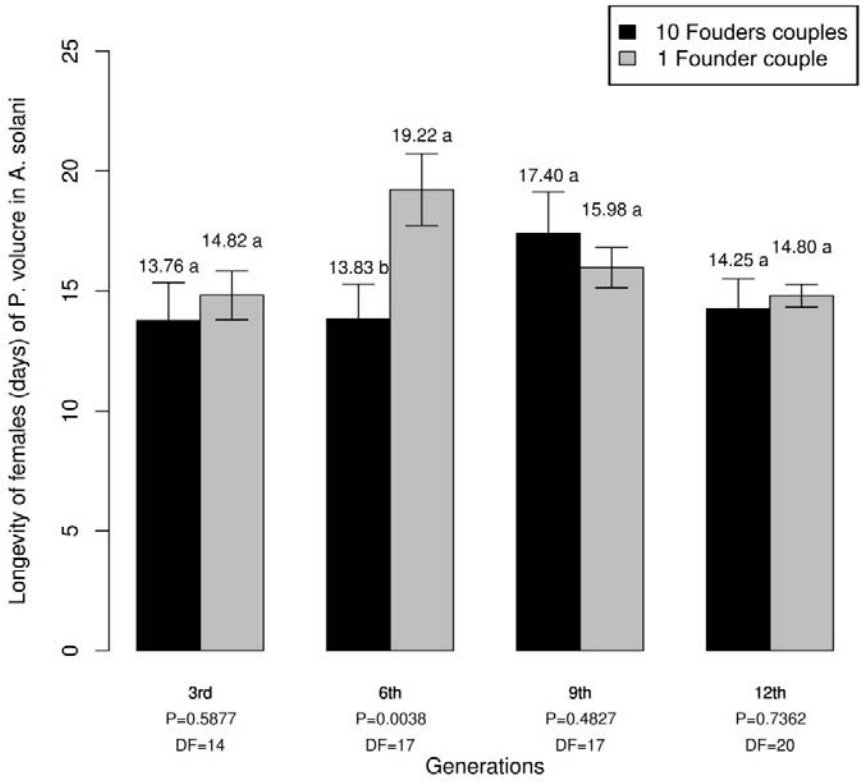

Figure 6. Female longevity (days) (± S.E.) of *Praon volucre* in *Aulacorthum solani* originating from 1 and 10 founder couples in the 3rd, 6th, 9th and 12th generations in the laboratory. 22 ± 2 ° C, RH 70 ± 10% and 12 h photophase.

[1]Means followed by the same small letter in the row, do not differ by Tukey test ($P<0.05$).

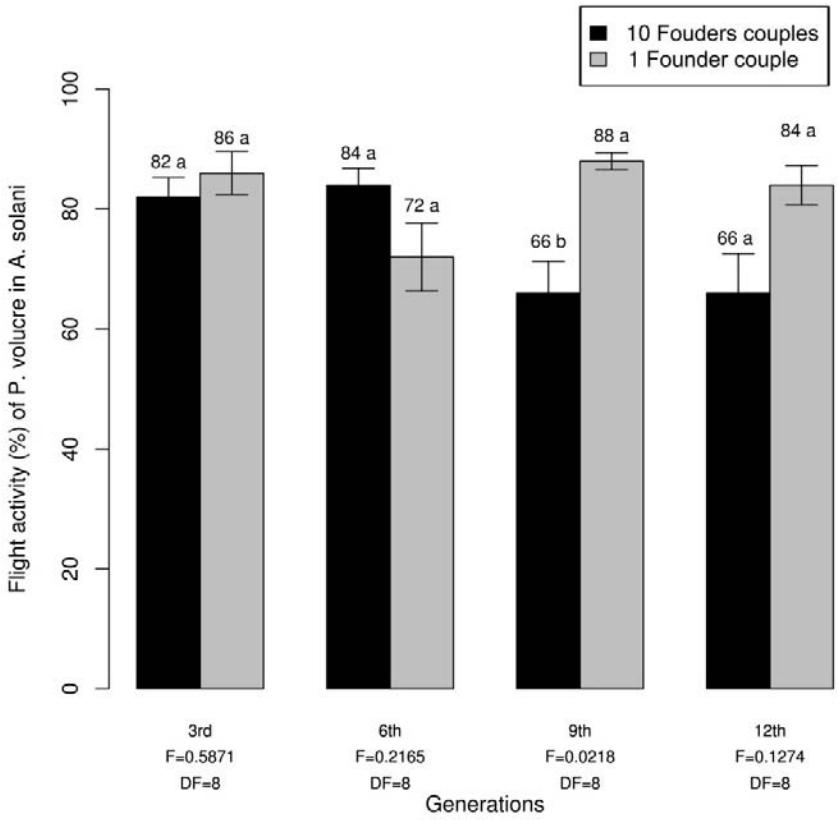

Figure 7. Flight activity (%) (± S.E.) of *Praon volucre* in *Aulacorthum solani* originate from 1 and 10 founder couples in the 3rd, 6th, 9th and 12th generations in the laboratory. t 22 ± 2 ° C, RH 70 ± 10% and 12 h photophase.

[1]Means followed by the same small letter in the row, do not differ by Tukey test

(P<0.05).

ABSTRACT

The knowledge of the effect of the number of founder couples of a parasitoid over the generations is important tool for mass rearing at laboratory conditions and also for some behavioural parameters, as flying and searching capacities for the hosts in the field. However this effect is not well known and usually is lacking, particularly to several aphid parasitoids those are reared by different commercial producers and used as biological control agents. The objectives of this work were to evaluate the biological, reproductive and behavioral parameters of *P. volucre* individuals originating from 1 and 10 founders couples in the 3rd , 6th, 9th and 12th generations in *Aulacorthum solani* in lettuce plants in the laboratory. Two initial rearing's started with 1 and 10 founder couples of *P. volucre* from individuals collected in the field. The *P. volucre* individuals originating from the rearing started with 10 founders couples showed higher parasitism rate in the 3rd (63.6%) and 9th (55.5%) generations than individuals from 1 founder couple in the same 3rd (46.5%) and 9th (32.5%) generations. The emergence, sex ratio and the number of eggs in the ovarioles did not differ from individuals from both rearing among the generations. The flight capacity of *P. volucre* remains high even over the generations in both rearing. Females originating from rearing started with 1 or 10 founders couples did not differ in their reaction when searching for aphid-infested-lettuce plant (individuals from 1 founder couple: 61.6 % and from 10 founder couples: 53.3 %); or for non-infested lettuce plant (individuals from1 founder couple: 20.0 % and from 10 founder couples: 16.7 %). Most females from the both rearings walked toward the odors containing aphids-infested lettuce plant comparing to non-infested plant. The results showed that both rearing, started with 1 or 10 founder couples, can keep an acceptable quality of *P. volucre* individuals concern to the main biological and reproductive parameters and also in their flying and searching capacities when reared in *A. solani* up to 6 ª generation into laboratory conditions.

Key-words: Generation. Parasitoids. Number of eggs. Flight capacity. Searching capacity.

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ARTICLE 3 **Functional Response and Searching Behaviour of *Praon volucre* Haliday (Hymenoptera: Braconidae) at Different Densities of *Aulacorthum solani* (Kaltenbach) (Hemiptera: Aphididae).**

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RESUMO

Respostas comportamentais são uma das ferramentas importantes na seleção de inimigos naturais para o controle biológico. A resposta funcional e o comportamento de busca de *Praon volucre* Haliday (Hym.: Braconidae) parasitando o pulgão *Aulacorthum solani* (Kaltenbach) (Hemiptera: Aphididae), foram determinados a diferentes densidades de *A. solani* a 22 ± 2 ° C, UR 70 ± 10% e fotofase de 12 h. Um coeficiente linear positivo e significativo (P1) do modelo de regressão logística indicou a resposta funcional do tipo III para *P. volucre* nas densidades de 4, 8, 16 e 64 *A. solani*. O tempo de manipulação (*Th*) e eficiência de busca (*a*) investigados pela equação de Rogers, mostrou uma estimativa média do tempo de manipulação (*Th*) de 0,23 e eficiência de busca (*a*) de 0.05h-1. A estimativa máxima de parasitismo (*T* / *Th*) foi 8,71 ninfas parasitadas / female/2h. O tempo e o número de encontros e ataques de fêmeas com os pulgões aumentaram com o aumento da densidade do hospedeiro. O tempo da prova com o ovipositor por fêmeas foi maior quando a arena de forrageamento continha maior número de pulgões (densidade de 64 pulgoes= 8,8 segundos) do que em quatro (2,2 segundos) e 16 (2.9 segundos). Fêmeas expostas a densidade de quatro pulgoes, permaneceram mais tempo inativas no disco foliar (13,6 min.) do que as fêmeas expostas à densidade de 64 hospedeiros (5,0 min.). Os resultados sugerem que *P.volucre* tem potential para ser um agente de controle biológico de *A.solani* e que o comportamento de busca de *P.volucre* foi afetado de acordo com as diferentes densidades de pulgões as quais o parasitoide foi exposto. Estas informacoes implicam em diferentes efeitos dos padroes de exploracao em agrupamento de hospedeiros para o controle biologico e condicoes de campo por este parasitoide.

Palavras chave: Parasitoides de pulgões. Afídeos. Parasitismo e resposta comportamental.

1 INTRODUCTION

Several aphid parasitoid species (Hymenoptera: Braconidae: Aphidiinae) have been tested for their usefulness as biological control agents, and based on populations growth rates and searching capacity, *P.volucre* has potential to be promising natural enemy against *A.solani* (SIDNEY et al., 2011). Currently it can be found in many localities, and in the State of Minas Gerais (southeast region) *P.volucre* develops on at least ten different species of aphids (STARÝ; SAMPAIO; BUENO, 2007).

Behavioral responses are one of the most important tools in selecting natural enemies for biological control programs (WAJNBERG; BERNSTEIN; ALPHEN, 2007). Understanding the behaviour of parasitoids and the relationship with their hosts is useful for determining how they influence the population dynamics of their hosts and the role that they play in the structure and function of the insect communities (ARAJ et al., 2011). Functional response is the number of successfully attacked hosts as a function of host density (SOLOMON, 1949), and it describes how a predator or parasitoid responds to the changing density of the prey/ host, and measuring it helps to determine the expected effectiveness of natural enemies (BAZYAR; HODJAT; ALICHI, 2011).

When analyzing the interaction between parasitoid and host by using quantitative models, descriptive parameters of such models can be used for predicting the parasitoid – host relations (HASSELL, 1978). The basic element in these models is the functional response which was first defined by Solomon (1949) as the relation between the number of

attacked hosts by a predator and the host densities. The reason for applying the name “functional response” is that the number of attacked hosts by a parasitoid will be a function of the host densities (HOLLING, 1959). This researcher found 3 types of functional responses and their curves. In the first type the number of attacked hosts increases in a linear manner as do the host densities to reach the maximum level and then remains stable. In this case the percentage of attacked hosts is constant (independent from the densities) and then decreases. In the second type of functional response, the number of attacked hosts increases as the host densities decrease, but not in a linear manner, and the curve grade decreases gradually till it reaches a steady state. In this case, the percentage of attacked hosts decreases gradually (depending on the inverse densities).

However, the functional response type is affected by the different experimental conditions, origin of the populations, age of parasitoids, time of exposure, host species and temperatures (FARHAD; TALEBI; FATHIPOUR, 2011).

The functional response depends on handling time ($T_{h:}$ the time that a natural enemy needs to parasitize a single host) and searching efficiency (*a*: the rate at which a parasitoid searches). Also, various interactions like learning and conditioning factors, which play an important role in host selection behaviour of foraging parasitoids, have been discussed (REHMAN; POWEL, 2010; STEENIS; EL-KHAWASS, 1995) and the studies about behaviour of aphid parasitoids create opportunities to manipulate their behaviour for better control of aphids populations. In this sense, understanding the searching strategy of *P.*

volucre, might gain insight into the mechanisms by which the control of *A. solani* is obtained in greenhouses crops, and provide information on how to improve the biological control of aphids.

The goal of this study was to describe functional response of *P. volucre* on *A. solani* infesting lettuce plants in laboratory tests. We evaluated the functional response on different densities of *A. solani*, and also the behaviour of the female parasitoid by direct observations on the handling, searching, resting and preening times, those could interfere on its functional response on the host *A. solani*.

2 MATERIALS AND METHODS

2.1 Origin and rearing of *A.solani*:

Colonies of *A.solani* were collected from lettuce plants (*Lactuca sativa* L.) in the field. Lettuce leaves infested with aphids were transported to the laboratory and placed in Petri dishes (15 cm diameter) sealed with organza. After identification according to Peña-Martinez (1992), the aphids were reared on potted lettuce plants placed in acrylic cages (60 × 30 × 30 cm) maintained in a climatic room at 22 ± 2°C, RH 70 ± 10% and 12h photophase. To standardize nymph age, adults of *A.solani* were kept for 24h in Petri dishes (15 cm diameter) containing a leaf disc of lettuce on a 1% agar/water solution, after which, the adult aphids were removed. Nymphs of the second instar were used in the experiments.

2.2 Origin and rearing of *P.volucre*

Sowthistle plants, *Sonchus oleraceus* L., infested with *Uroleucon sonchi* (L.) and containing parasitoid mummies were collected in the field and transported to the laboratory inside paper bags. The plants were placed in a pot containing water, inside an acrylic cage (30 × 30 × 60 cm), and the mummies developing on these plants were collected during 1 week period and were individually placed in glass tubes (100 mm × 8 mm) maintained in a climate room (22 ± 2°C, RH 70 ± 10% and 12 h photophase) until emergence of adults. A small honey and water droplets

were put in the glass tubes walls to provide enough food to emerged adults. The adults of *P. volucre* were identified using the characteristics provided by Tomanovic et al. (2003). Individuals from 6th generation of the laboratory-reared populations were used in the experiments.

2.3 Functional response and foraging behavior of *P. volucre* in different densities of *A. solani*: These experiments were set up according to the methodology proposed by Farhad, Talebi and Fathipour (2011) and Sampaio, Bueno and Pérez-Maluf (2001).

2.3.1 Functional response

Second-instar nymphs of *A. solani* in each 4, 8, 16, 32, or 64 densities, were placed into a Petri dish (5 cm diameter) arena containing a lettuce leaf disk on a 1% agar/water solution. The top of the Petri dishes were covered with fine nylon mesh. The nymphs were exposed to a single *P.volucre* female, 1-day-old previously mated and without oviposition experience for 2h period to allow oviposition.

After exposition to the hosts, the females were removed and the Petri dishes containing the parasitized nymphs were stored in a climatic chamber (22 ± 1 º C, RH 70 ± 10% and 12 h photophase) and observed daily until the mummies formation and the emerged-adult. Before mummification, the parasitized aphids were periodically transferred to new Petri dish containing fresh lettuce leaf disks as needed. The number of parasitized aphids was counted and the percentage of mummification was calculated in each *A. solani* density

The nymphs that died before the formation of the mummies were ignored in the analysis data, according to the methodology used by Steenis (1993), and starting from the hypothesis that parasitism does not affect the natural mortality of nymphs before mummification. But when that mortality exceeds 10% of the number of insects initially offered to parasitoids per density, the replicate was excluded and redone.

Ten females of each aphids density tested (4, 8, 16, 32, or 64) were evaluated under 10 replicates from each aphid density.

To determine the type of functional response, the data were fitted to the logistic regression following Farhad, Talebi and Fathipour (2011) and Juliano (2001), and express by:

$$N_a / N_0 = \frac{\exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}{1 + \exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}, \text{ where} \qquad (1)$$

P_0, P_1, P_2 and P_3 are the intercept, linear, quadratic and cubic coefficients, respectively. N_a is the number of hosts parasitized, N_0 is the initial host density, and N_a / N_0 is the proportion of total aphids parasitized. A significant negative or positive linear coefficient (P_1) of the logistic regression model indicates type II or III, respectively (JULIANO, 2001). After defining the type of functional response, the handling time (T_h) and searching efficiency (a) of a type II response were estimated using Rogers equation (ROGERS, 1972), as follow:

$$N_a = N_0[1 - \exp(-\frac{aT\mathrm{P_t}}{1 + aT_h N_0})], \qquad (2)$$

N_a is the number of host parasitized, N_0 is the initial host density, T is the duration of the experiment (= 2h), a is searching efficiency, T_h is handling time and P_t is the number of parasitoids. Handling time (T_h) and searching efficiency (a) were estimated using non-linear regression and SAS software (STATISTICAL ANALYSIS SYSTEM INSTITUTE - SAS INSTITUTE, 2003).

2.4 Foraging behavior of *P. volucre* in different densities of *A. solani*

Second instar of *A.solani* were placed into a Petri dish (5 cm diameter) containing a lettuce disk leaf on a 1% agar/water solution. The amount of agar adhered to the bottom surface of the leaf disc did not allow the aphids be hidden in this region, and allowed the observations on foraging of *P.volucre* besides to added the maintenance of the turgidity of the leaf. The top of the Petri dishes were covered and sealed with perforated PVC film. Three hours after the aphids were placed on the Petri dishes, a female 1-day-old previously mated and without oviposition experience prior to the experiment was released on the arena. The parasitoid female was allowed to remain in the Petri dish arena for 2h period.

During this period, the behavior of each female was visualized and observed with a help of a stereomicroscope, on each Petri dish with different densities of aphids (4, 16 and 64) in a climatized room (22 ± 1 º C, 70 ± 10% RH and a 12 h photophase) (Table 1):

Ten replicates of each host density (4, 16, and 64) were done, and the following parameters were recorded, according to events proposed by Rasekh et al. (2010):

a) Time of encounters of *P.volucre* with *A.solani*;
b) Number of encounters of *P.volucre* with *A.solani*;
c) Number of probes with the ovipositor on the host;
d) Time of probes with the ovipositor on the host *A.solani*;
e) Number of attacks of *P.volucre* on the host *A.solani*;
f) Attack time of the *P.volucre* on the host *A.solani*;
g) Time the female spent walking on the leaf area;
h) The time the female remained stopped in the leaf;
i) Time cleaning of the female;
j) Time that remained on the leaf disc during the first visit;
k) Total time that the female remained on Petri dish and total time that the female remained on the leaf.

During the 2h period timed display, all steps about parasitoid´s behaviour were observed and recorded (Table 1), and according to Rasekh et al. (2010) and Rehaman and Powel (2010).

Table 1. Activity of the female parasitoid on behavioral test

Activity	Definition
Probes	Insertion of the ovipositor, making short touches on the host with the ovipositor
Parasitism	Parasitoid attacks on hosts (host acceptance, viewed by long touches with ovipositor)
Searching	Walking along the leaf area
Resting	Inactivity, permanently fixed in leaf area or on the cover of the Petri dish
Cleaning	The female is smooth and clean their tarsi scrubbing against each other or against the wings or antennae. The female cleans and grooms her ovipositor and antennae.

2.5 Test of permanence in the foraging arena

Specifically 3 aphids densities (4, 16 and 64) were evaluated concern to the permanence of female *P.volucre* in the foraging arena containing the number of aphids (2^{nd} instar) corresponding to the densities evaluate. Each female remained in the arena for 12h period and every 30 minutes it was recorded if the parasitoid was on the leaf disc or on the cover top of the Petri dish.

This experiment was conducted in a completely randomized design, consisting of three densities (4, 16 and 64 aphids) and 10 replicates.

3 STATISTICAL ANALYSIS

To test the effect of the density of host about the percentage of parasitism was constructed generalized linear model (GLM), using the appropriate error distribution of each response variable analyzed (CRAWLEY, 2005). Statistical analyzes were performed using program (R DEVELOPMENT CORE TEAM, 2005) and using the package multcomp (HOTHORN; BRETZ; WESTFALL, 2008).

The foraging behaviour of *P.volucre* was transformed to *log(x+1)* and submitted to analysis of variance and the means were compared by Tukey test ($p < 0.05$).

4 RESULTS

A significant positive linear coefficient (P1) of the logistic regression model indicates type III functional response to *P. volucre* at different densities of *A. solani* (Table 2). The number of attacks hosts followed a sigmoid (S shape) curve in which the slope increases at first but then decreased. In this case the percentage of attacked hosts increased until the host density 16 and then it decreased (Figure 1).

The mean estimate of handling time (*Th*) was 0.23 and the searching efficiency (*b*) was $0.05h^{-1}$. The maximum estimate of parasitism (*T/Th*) was 8.71 nymphs parasitized/ female/2h (Table 3).

Table 2. Maximum likelihood estimates from logistic regression of proportion of different densities of *A. solani* by *P. volucre*.

Parameter	Estimates
Constant (P_0)	-0.8399±0.01010
Linear (P_1)	0.2688±0.00152
Quadratic (P_2)	-0,0105±0.00005
Cubic (P_3)	0.0013±0.00001

Figure 1. Type III- functional response of *Praon volucre* on different densities of second-instar nymphs of *Aulacorthum solani* at 22 ± 2 ° C, RH 70 ± 10% and 12 h photophase.

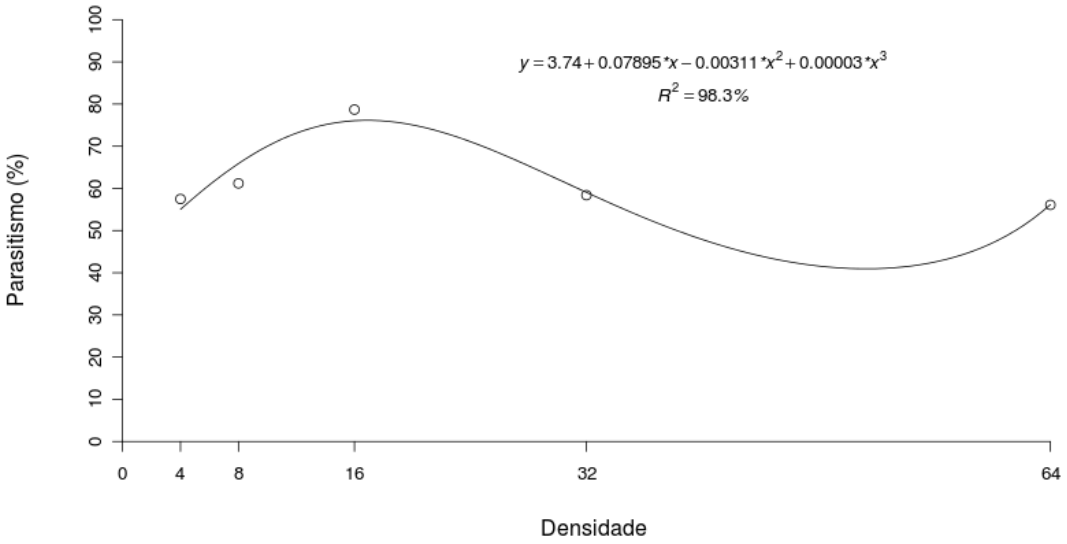

Table 3. Mean estimates of handling time (T_h), searching efficiency (b) and maximum rate of parasitism (T/T_h) of *P. volucre* on *A. solani*.

Parameters	Mean
Handling time (*Th*)	0.23±0.022
Searching efficiency (*b*)	0.05±0.002
Maximum rate of parasitism (*T/Th*)	8.71
Coefficient of determination (r^2)	0.93

The time of encounters of females parasitoid with the hosts increased with an increase in the host densities (4 = 1.3mins; 16 = 2.7mins and 64 = 7.1mins) consequently, the number of encounters of parasitoid with hosts also increase from host densities 4 (12.8 encounters), 16 (23.2 encounters) until 64 (63.1 encounters) (Table 4).

The number of probes with the ovipositor by female's *P.volucre*, was higher in the host density 64 (12.4 probes) than 4 (4.3 probes) and 16 host densities (4.0 probes). The time of probes with the ovipositor by females was longer when the arena forage contained higher number of aphids (64 host densities = 8.8 seconds) than on 4 (2.2 seconds) and 16 (2.9 seconds) host densities. A significantly difference was found in the number of attacks by female's parasitoids among the three hosts densities. The number of attacks increased over the densities as following on 4 (4.2 attacks) □ 16 (12.2 attacks) □ 64 host densities (33.5 attacks). There were no significant differences in the time that the female spent walking on the lettuce leaf disk and the time values on the 4, 16 and 64 host densities were 95.2; 88.0 and 83.2 minutes, respectively. Nevertheless, female's exposure to the 4 host density remained inactive in the leaf disk more time (13.6 minutes) than to females exposed to the 64 host density (5.0 minutes). Females exposed to the three host densities evaluated showed a similar time cleaning (4= 16.6; 16= 15.2 and 64= 22.1 minutes). No significant difference was detected to the time that the parasitoid's female remained on the leaf disk during the first visit before leave the arena in the 4, 16 and 64 host densities and the time values ranged from 37.6 to 59.7 minutes (Table 3).

Females exposure to 4 host density (0.2 minutes) showed shorter total time remained on the Petri dish than females exposed to 16 (4.4minutes) and 64 hosts densities (1.8 minutes). However, when the females remained on leaf disk, the total time was shorter for females exposed to the host density 16 (113.4 minutes) than 4 (119.5 minutes) and 64 (118.5 minutes) host densities (Table 4).

When was tested if the female parasitoid was remained in leaf disc or in the top of cover of the Petri dish during half day, the percentage that the female parasitoid remained in leaf disc was 97.1%; 97.5% and 100.0% for 4, 16 and 64 hosts densities, respectively (Table 4).

Table 4. Behavioral data (means ± SE) for *Praon volucre* exposure to different *A.solani* host densities in a Petri dish arena with lettuce leaf disk during 2h period (22 ± 2 ° C, RH 70 ± 10% and 12 h photophase).

Behaviour of *P.volucre* [1] on the Petri dish arena with *A.solani*	Density[2]		
	4	16	64
Time of encounters of *P.volucre* with hosts (minutes)	1.3±0.22c	2.7±0.39b	7.1±0.51a
Number of encounters of *P.volucre* with hosts (average)	12.8±1.81c	23.2±2.56b	63.1±3.66a
Number of probes with the ovipositor	4.3±0.86b	4.0±0.83b	12.4±1.51a
Time of probes with the ovipositor (seconds)	2.2±0.41b	2.9±0.64b	8.8±1.11a
Number of attacks of *P.volucre* on the host	4.2±0.47c	12.2±1.05b	33.5±2.30a
Attack time of the *P.volucre* on the host (seconds)	9.8±1.29c	38.1±2.90b	83.5±7.32a
The time of the female spent walking on the leaf area (minutes)	95.2±8.54[a]	88.0±6.66a	83.2±3.88a
The time of the female remained stopped in the leaf (minutes)	13.6±3.96[a]	9.7±6.34ab	5.0±1.32b
The time cleaning of the female (minutes)	16.6±2.62[a]	15.2±3.60a	22.1±3.58a
Time that female remained on the leaf disc during the first visit (minutes)	55.1±15.22[a]	37.6±8.67a	59.7±12.53a
Total time of the female remained on Petri dish (minutes)	0.2±0.08b	4.4±1.34a	1.8±0.96[a]
Total time of the female remained on the leaf discs (minutes)	119.5±0.30[a]	113.4±2.35b	118.5±0.83a
Test remained on the leaf female[3] (%)	97.1±1.08[a]	97.5±1.27a	100.0±0.00a

[1] Original data transformed to *log(x+1)*.
[2] Means followed by the same letter in the row, are not different by Tukey test ($p < 0.05$).
[3] Test evaluated during 12 h period

5 DISCUSSION

The parasitoid *P.volucre* exhibited a type III functional response and this suggest that direct density dependence up to some threshold host density, which may contribute to stability if average densities fall below this threshold (HASSELL; LAWTON; BEDDINGTON, 1977). The type III has also been reported to aphid parasitoids as *P. volucre*, *Aphidius rhopalosiphi* De Stefani-Perez and *Aphidius ervi* Haliday on *Sitobion avenae* (Fabricius) (STILMANT, 1996); *Aphidius colemani* Viereck and *Lysiphlebus testaceipes* Cresson against *Schizaphis graminum* (Rondani) (JONES et al., 2003), *Ibalia leucospoides* Horchenwarth against *Chromaphis juglandicola* Kaltenbach (VALERIA; CORLEY, 2005) and *Trioxys pallidus* Haliday against *Sirex noctilio* Fabricius (RAKHSHANI et al., 2004). However, *A. colemani* had functional response type II, when using as parameter the number of mummies formed on *Myzus persicae* (Sulzer) on sweet-pepper leaves (SAMPAIO; BUENO; PÉREZ-MALUF, 2001). Farhad, Talebi and Fathipour (2011) reported a type II for *P. volucre* parasitizing *S. avenae* in different temperatures (10, 15, 20, 25, and 30^{o} C).

According to Farhad, Talebi and Fathipour (2011) and Hassel, Lawton and Beddington (1977), the functional response is affected by the different experimental conditions, origin of the populations, age of parasitoids, time of exposure, and temperatures. Our results supports those affirmation because the experimental conditions used, such Petri dish as arena, time of exposure, host *A.solani* and lettuce plant may underestimate or overestimate the results in the laboratory conditions if

compared to the field conditions and may modify the functional response type. In fact, *Aphidius uzbekistanicus* Luzhetzki showed a type III response when parasitizing third-instar nymphs of *Hyalopteroides humulis* Walker but a type II response when parasitizing *Metopolophium dirhodum* (Walker, 1849) (DRANSFIELD, 1979). Functional response of *A. ervi* on *Sitobion avenae* were obtained from the logistic regression, and indicated types II (Holling's model) and III (Roger's model) on the host aphid plant Alvand as a resistant cultivar and Sardary as a sensitive cultivar of wheat crop, respectively (BAZYAR; HODJAT; ALICHI, 2011). Hofsvang and Hagvar (1983) showed for *Ephedrus cerasicola* Stary type II, type I, and type II functional responses when exposed to aphid hosts, *M.persicae*, for 1, 6 and 24h periods, respectively. The parasitoid *L.testaceipes* against *Aphis gossypii* (Cresson) showed type II and type III functional responses at 20◦C and 28◦ C, respectively (BAZZOCCHI; BURGIO, 2001).

Laboratory studies do not take into account alternative host/prey as it occurs in the field. Therefore, the prevalence of Type II functional responses found under restricted conditions may overestimate their occurrence and importance in nature (SCHENK; BACHER, 2002). It is recognized that the functional response derived from laboratory studies may bear little resemblance to those that may be measured in the field (ABEDI et al., 2012). Houck and Strauss (1985) pointed out, however, that laboratory functional response studies can be used to infer basic mechanisms underlying natural enemy-prey-host interactions. Such studies provide valuable information for biological control programs. For example, comparisons of the attributes of different parasitoid species can

be made, and baseline information can be established for quality control standards in mass-rearing projects (MONTOYA et al., 2000).

According Lenteren and Bakker (1976), only the response of type III shows a relationship dependent on the density, as determined by density (inflection point of the curve) the parasitism rate increases with increasing density of the host. Thus, even though only until a certain density, the relationship parasitoid host presents characteristics of stabilization.

Handling time is defined as time spent handling the host, parasitizing the host, and also the time spent cleaning and resting. The effect of handling time is to reduce the time available for search for other hosts (HASSEL, 1978). The handling time from *P. volucre* found in this study is difficult to compare to the ones reported by Farhad, Talebi and Fathipour (2011) and Stilmant (1996), because they used an exposition time of 24h period, and whereas we used a 2h period.

Host recognition may involve changes in the female's behaviour, and directed responses towards a host. Once a female has encountered a potential host, she examines its quality and suitability, by antenna and ovipositor probing, for offspring development (REHMAN; POWEL, 2010). Thereby in this study, the number and time of encounters (contacts) made by parasitoid's antennae and tarsus onto their hosts (encounters) increased gradually with higher host densities, with an abrupt increase in the highest density (64 hosts), indicating an increase in searching behaviour of the parasitoid *P.volucre*. Obviously the parasitoids discovered hosts more easily at higher host densities and thus they spent more of their time searching and ovipositing at these densities

(LENTEREN; BAKKER, 1978). According to Wickremasinghe (2007) frequent abdominal protractions by *A. rhopalosiphi* at host honeydew indicated that an oviposition stimulant may be present in the honeydew and this response would cause the parasitoid to increase its searching time. According this hypothesis, in the highest aphids' density, *A.solani* can produce more amounts of honeydew in the arena, consequently, stimulates the oviposition and attack of *P.volucre* females, as observed in this study.

After all observations on search behavior of *P. volucre* we have the impression that the parasitoid walks at random over the leaf disk and discovers a host mostly by touching it with the antennae and sometimes the female parasitoid may sting the host with her ovipositor and probe de host or and lay an egg. In this study, the oviposition took less than 3 seconds and when the female *P.volucre* just probes spent approximately 0.5 seconds. So, this difference between both time events makes less difficult to determine what actually happened. The probing test is the last mechanism used by Aphidiinae to evaluate the nutritional quality of the host and being evaluated sources gustative (MACKAUER; MICHAUD; VÖLKL, 1996; REHMAN; POWEL, 2010) and ours results showed that the number of touches short tends to increase with increasing density of the host as also recorded by Sampaio, Bueno and Pérez-Maluf (2001).

Normally, the female parasitoid *P. volucre* had a pre contact (probing or touch with antennas) on the host *A. solani* before to oviposite. Stilmant et al. (2008) stated that the pre-contact host preference was found for *A. ervi* but it did not correspond to the level of acceptance. However, the number of antennal contacts by *P. volucre* in this study

corresponded to the parasitization level of the aphid species. So, this observation is consistent with this current report.

The average of the probing and attack number in the host density 4 was higher than four aphids host *i.e.*, there was superparasitism during the encounter between host and female parasitoid. After the female attacked a parasitized aphid, it was possible to diagnose that this female walked slower and cleaned up excessively. The superparasitism behaviour is the deposition of an egg by a parasitoid into or on a host that has already been parasitized with one or more eggs, is commonly reported in field and laboratory studies (BUENO; SAMPAIO, 2009). The superparasitism may lead the female waste search time and eggs; this behaviour has often been considered maladaptive (TUNCA; KILINÇER, 2008). This behaviour could happen because the low number of aphids and long time exposure in contact to hosts. Consequently, this result proved that the *P.volucre* female wasp does not distinguish parasitized from unparasitized hosts in lower hosts' densities. Species with imperfect discrimination do not always avoid superparasitism and females of these species invest less time per patch, leaving them even if they still contain many unparasitized hosts (BAAREN et al., 2004).

After each female's attack, she spent a long time cleaning her ovipositor, legs, antenna and wings and one problem detected was when parasitoids stung the *A. solani*, the alarm pheromone lead individuals of the population to drop off from the plant or generally react by moving, leading to a possible spread in the arena. Various conditions may limit a parasitoid's oviposition success, and this condition includes the aphids' defensive behaviors as related to colony structure and parasitoid

searching behaviour. An aphid's defensive behaviors, such as kicking, can be important for the success or failure of parasitoid oviposition (MACKAUER; VOLKL, 1993). According to our observation in this study, the time that the female parasitoid spent in the top of Petri dish was due the reaction of *A.solani* against *P.volucre* attacks, *i.e.* they drop off from the leaf disk.

Nevertheless, females' *P. volucre* exposure to the host density 4 remained inactive in the leaf disk more time than when were in the 64 host density and this result shows how active the *P. volucre* female is in high density. However, Steenis and El-Khawass (1995) evaluated the behaviour of *A.colemani* searching for *Aphis gossypii* Glover and described that the duration of the first visit to a leaf disk increased with increasing aphid density. In the current work we did not detect difference among the host's densities. During on 12h period observing females' parasitoids in an arena containing 4, 16 and 64 host densities, we demonstrated that even in low density, the *P.volucre* female remained on the leaf disc. This information proves that even at low densities of aphids, the female of *P.volucre* not leave the plant host.

6 CONCLUSION

A significant positive linear coefficient of the logistic regression model indicates type III functional response to *P. volucre* at different densities *A.solani* as aphid host. This study provides information on host-parasitoid interactions, which are helpful in the management strategies of *A.solani.* The searching behaviour of *P.volucre* was affected by different aphids' densities, then can implicate on different effects on the pattern of patch exploration for biological control and field situations.

ABSTRACT

Behavioral responses are one of the most important tools in selecting natural enemies for biological control programs. The functional response and searching behaviour of *Praon volucre* Haliday (Hym.: Braconidae) parasitizing the foxglove aphid *Aulacorthum solani* (Kaltenbach) (Hem.: Aphididae), were investigated under different host densities at laboratory conditions at 22 ± 2°C, RH 70 ± 10% and 12h photophase. A significant positive linear coefficient (P1) of the logistic regression model indicates type III functional response to *P. volucre* at 4, 8, 16, and 64 densities of *A. solani.* The handling time (T_h) and searching efficiency (*a*) investigated using the Rogers equation, showed a handling time (*Th*) of 0.23 minutes and searching efficiency (*a*) of $0.05h^{-1}$. The maximum estimate of parasitism (*T/Th*) was 8.71 nymphs parasitized/female/2h. The time and number of encounters and attacks of female's parasitoid with the hosts increased with an increase in host density. The time of probes with the ovipositor by females was longer when the arena forage contained higher number of aphids (64 host densities = 8.8 seconds) than on 4 (2.2 seconds) and 16 (2.9 seconds) host densities. Female's exposure to the 4 host density remained inactive in the leaf disk more time (13.6 min.) than to females exposed to the 64 host density (5.0 min.). The results suggested that *P.volucre* has potential as biological control agent of *A.solani* and his searching behavior was affected by the different *A. solani* densities. That information's can implicate on different effects on the pattern of patch exploration for biological control and field situations by this parasitoid.

Key words: Aphid parasitoids. Aphids. Parasitism. Behavioral responses.

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ARTICLE 4 **Predation and biological parameters of *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) on several pest aphids as prey**

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Artigo normalizado de acordo com a norma do periódico Biological Control

ABSTRACT

The role of generalist predators in biological control and their interactions with prey, of which aphids constitute one part, has received considerable attention. The generalist predatory bug *Macrolophus pygmaeus* (Rambur) is commonly encountered on vegetable greenhouse crops, and also is sold commercially as biological control agent. The aim of this study was to investigate the consumption capacity of two instars and adults *M. pygmaeus* having as prey *Myzus persicae* (Sulzer), *Myzus persicae nicotianae* Blackman, *Aulacorthum solani* (Kaltenbach) and *Macrosiphum euphorbiae* (Thomas); also to evaluate the developmental time and reproductive parameters on *A. solani*, *M. persicae* and eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). To evaluate the aphid consumption, the 1st and 4th instars or adult females of *M. pygmaeus* were maintained individually in a Petri dish (9 cm diameter) containing leaf disc of each aphid host plant on a layer of water/ agar 1% and 30 1st, 3rd instars or wingless adult's aphids as prey. For the 1st instar of *M. pygmaeus* was offered the 1st instar of each aphid species. The number of aphids consumed was counted after 24h, and a total of 20 replicates were tested. Fifth nymphs and twenty-five pairs of newly-emerged-females and males of *M. pygmaeus* (less than 1-day old) were evaluated concerning to development and fecundity, respectively. The experiments were carried out in climatic chamber at 25° ± 2° C, 65% ± 10% RH and 16h photophase. The results showed that the 4th instar and adult's stages of *M. pygmaeus* would be effective on the aphid consumption, however, the 1st instar predator besides presented the lowest feeding rate, only feed on 1st instar aphids. Aphids no exposed to the predator have high survival rate on the 24h period, showing that no influence of aphid natural death on the daily predation of *M. pygameus*. The higher daily predation, in general, was on *M. persicae* and *M. persicae nicotianae* over *A. solani* and *M. euphorbiae* individuals spite a comparable size and the different reactions of these aphids when attacked by the predator. In addition, the female of *M. pygmaeus* fed on *E. kuehniella* eggs showed higher fecundity (47.0 eggs/15 days) compared to females feed on aphids *M. persicae* (32.4 eggs/15days) and *A.solani* (32.3/15 days eggs). *M. pygmaeus* might contribute to restrict aphid populations prior to the arrival of specialist natural enemies, and or in combination with other natural enemy and then, helping to the aphid management in greenhouse crops.

Keywords: Predatory capacity, reproductive parameter, prey, sweet pepper, tomato.

RESUMO

Macrolophus pygmaeus (Rambur) é um predador comumente encontrado em cultivos protegidos de hortaliças e sua capacidade de controlar populações de algumas pragas é considerada importante, além de ser vendido comercialmente como agente de controle biológico. O objetivo deste estudo foi investigar o desempenho biológico de *M. pygmaeus*, avaliando sua capacidade de consumo quando no 1° e 4° instars e adultos no 1°, 3° instars e adultos ápteros de *Myzus persicae* (Sulzer), *Myzus persicae nicotianae* Blackman, *Aulacorthum solani* (Kaltenbach) e *Macrosiphum euphorbiae* (Thomas); tambem avaliar o seu desenvolvimento e parâmetros reprodutivos quando alimentado com *A. solani*, *M. persicae* e ovos de *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). O consumo das ninfas de 1° e 4° instars e adultos de *M.pygmaeus* nos diferentes estadios de pulgões foram conduzidos em placas de Petri em câmara climática a 25 º ± 2 º C, 65% ± 10% UR e 16h fotofase. O numero de pulgoes consumidos foi avaliado após 24h em um total de 20 repetições. O desenvolvimento ninfal e fecundidade de *M.pygmaeus* foram determinados quando os indivíduos se alimentaram dos pulgões *M.persicae*, *A.solani* ou ovos de *E. kuehniella*. Os resultados mostram que o 4° instar e adultos de *M. pygmaeus* podem ser eficazes no consumo de pulgões, entretanto, o 1° instar do predador alem de apresentar a menor taxa de predação, somente predou o 1° ínstar dos pulgoes. Pulgões nao expostos ao predador apresentaram elevada taxa de sobrevivência no período de 24h, mostrando que não há influência de mortalidade natural de pulgões na predação diária de *M. pygameus*. A elevada predação, em geral, de *M. persicae* e *M. persicae nicotianae* sobre *A. solani* e *M. euphorbiae* pode ter relação com o tamanho dos pulgões e tambem com as diferentes reações destes pulgões quando atacados pelo predador. A fêmea de *M. pygmaeus* alimentada com ovos de *E. kuehniella* apresentaram maior fecundidade (47,0 ovos/15 dias) em comparação com as fêmeas que se alimentam dos pulgões *M. persicae* (32,4 ovos/15 dias) e *A.solani* (32,3 ovos/15 dias). O predador *M. pygmaeus* devera contribuir para restringir populações de pulgoes antes da chegada de inimigos naturais mais especialistas e ou em combinação com outro inimigo natural e entao, ajudar no manejo de pulgões em cultivos em casas de vegetação.

Palavras-Chave: Capacidade predatória, parâmetro reprodutivo, presa, pimentão, tomate.

1 INTRODUCTION

Sweet pepper crops under protected cultivation are attacked by several key pests, including the aphids *Myzus persicae* (Sulzer), *Myzus persicae nicotianae* Blackman and *Aulacorthum solani* (Kaltenbach) (MESSELINK et al., 2011). *Macrosiphum euphorbiae* (Thomas) is an important aphid pest on tomato crops. These aphids are polyphagous species, with a wide range of host plants (MALAIS; RAVENSBERG, 2003). Particularly, *A.solani* has become a very common pest in sweet pepper in greenhouses (SHELT; WACKERS, 2003) and growers and agricultural technicians have complained that the particular impact of this aphid have been increasing over the years and no effective control has been found yet.

Biological control is an important strategy for controlling these pests in greenhouse, as they are attacked by several natural enemies, as parasitoids, predators and pathogens (CRINITI et al., 2008; PERDIKIS; KAPAXIDI; PAPADOULIS, 2008). In general aphid parasitoids are the first choice to control aphids; however the natural occurrence of hyperparasitoids often disrupts this approach (BRODEUR, 2000), or the aphid parasitoid is not sufficiently efficient to control the target aphid. Then, in case of failure of aphid control, a choice for other strategies or natural enemy is important to permit the biological control program continuity in greenhouse crops.

The role of generalist predators in biological control and their interactions with prey, of which aphids constitute one part, has received considerable attention (SYMONDSON; SUNDERLAND; GREENSTONE, 2002) due to their importance in agroecosystems and the unsustainable nature of conventional methods of pest control (HARWOOD; OBRYCKI, 2005). In tomato crops, the mirid *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) actually is used to control whiteflies (*Bemisia s*pp.) and *Tuta absoluta* (Meyrick)

(CALVO; BOLCKMANS; BELDA, 2012), but also preys leafminer larvae, caterpillars, lepidopteran eggs and aphids (RASDI; FAUZIAH; MOHAMAD, 2009). This predatory bug has the ability to survive on plant juices, high numbers can be maintained in the crop even when prey is scarce (INGEGNO; PANSA; TAVELLA, 2011), is commonly encountered on vegetable greenhouse crops (EUBANKS; DENNO, 2000), have the ability to suppress pest populations (PERDIKIS; LYKOURESSIS, 2002) and is sold commercially as biological control agent (LENTEREN, 2012).

Although the capacity of attacking diverse prey, the generalist predators could be considered an important tool in biological control, also its omnivorous behavior could influence their biological characteristics depending on the consumed prey species (EUBANKS; DENNO, 2000; MENDES et al., 2002), and this could interfere on its ability as biological control agent (LENTEREN, 2012). Thus, several biological parameters as predation rate, development, survival and fecundity should be measured to evaluate the potential of a biological control agent on the prey.

The objective of this work was to investigate the biological performance of *M. pygmaeus* evaluating (1) the daily predation of the two instars (1^{st} and 4^{th}) and adults on 1^{st}, 3^{rd} instars and wingless adults of *M. persicae, M. persicae nicotianae, A. solani and M. euphorbiae*; (2) nymphal development and survival on the aphids *A. solani*, *M. persicae* or on eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), and (3) to assess the effects of aphids *A. solani* and *M. persicae* or eggs of *A. kuehniella* on the reproductive parameters of *M. pygmaeus*.

2 MATERIAL AND METHODS

2.1 Rearing *Macrolophus pygmaeus*

The predatory insect *M. pygmaeus* is mass reared by Koppert Biological Systems and used as commercial biological product. This predator is reared having tomato and tobacco plants as oviposition substrate and frozen eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) as food. The individuals used in the tests were from this commercial stock colony.

2.2 Rearing *A. solani, M. persicae, M. persicae nicotianae* and *M. euphorbiae*

The aphids *A. solani, M.persicae* and *M. persicae nicotianae* are kept on sweet pepper plant (*Capsicum annuum* L.) variety 'Spider', and *M. euphorbiae* are reared on tomato plants (*Lycopersicon esculentum* L.) in Koppert Biological Systems greenhouses. Individuals from these rearing were used to start a stock colony of each aphid.

Stock colonies of *A. solani*, *M. persicae* and *M. persicae nicotianae* took place on sweet pepper leaf discs (9 cm diameter, 1.5 cm heights) in Petri dish (9 cm diameter, 1.5 cm height), and colonies of *M. euphorbiae* were reared on tomato leaf discs. The leaf discs were placed upside down during the aphid rearing. Twice per week, the old leaf discs were replaced by new ones to provide continuous fresh tissue to the aphids feeding. The aphid colonies were maintained in climatic chamber at $22^{o} \pm 2^{o}$ C, 65% ± 10% RH and 16h photophase. The individual of each aphid used in the experiments were from this stock colony. This methodology was adapted according to Sidney et al. (2010a, 2010b).

2.3 Daily predation of *Macrolophus pygmaeus*

The daily predation of the two instars (1st and 4th) and adults of *M. pygmaeus* on 1st, 3rd instars and wingless adults of *M. persicae, M. persicae nicotianae, A. solani and M. euphorbiae* were evaluated to access the aphid's suitability as prey to the predatory bug.

To obtain the 1st and 4th instars and adults of *M. pygmaeus*, the eggs or the earlier instar from the commercial stock colonies were used. Until they reached to the instars 1st or 4th or adult of *M. pygmaeus*, they were kept individually in Petri dishes (9 cm diameter) containing leaf disc of sweet pepper or tomato according to each aphid host plant on a layer water / agar 1% and *E.kuehniella* as food. Those Petri dishes were maintained in a climatic chamber at 25° ± 2° C, 65% ± 10% RH and 16h photophase.

As soon they reached to instars 1st or 4th or adult they were removed from the Petri dish with a help of a suction mouth, and transferred to a new Petri dish. The 4th instar and adult's predator were kept isolated in the Petri dish without food for a 24h period. The 1st instar *M. pygmaeus* was not conditioned to 24h of starvation as we observed in a pre-test that this newly - instar did not survive without food for 24h, thereby, as soon they hatching from the eggs, they were evaluated on their aphid consumption.

To evaluate the aphid consumption, the 1st and 4th instars or adult females of *M. pygmaeus* were maintained individually in a Petri dish (9 cm diameter) containing leaf disc of each aphid host plant on a layer of water/ agar 1% and 30 1st, 3rd instars or wingless adult's aphids as prey. For the 1st instar of *M. pygmaeus* was offered only the 1st instar of each aphid species. We observed in the pre-tests that the 1st instar predator was not able to consume the others instars or adults of these aphids. The predator individuals were removed from the Petri dish after 24h and the number of aphids consumed were counted and

recorded.

Twenty replicates (n=20) for each instar (1^{st}, 4^{th}) or adult of *M.pygmaeus* on each aphid's species (*M. euphorbiae, M. persicae, M. nicotianae* and *A.solani*) at 1^{st}, 3^{rd} instars and wingless adults were tested. Simultaneously, a control treatment without the predator *M. pygmaeus* under the same conditions as described before was carried out. However, in this case, 5 replicates for each aphid (1^{st}, 3^{rd} instars and wingless adults) and each one consisting of 30 individuals were evaluated. The mortality of these aphids in the absence of the predator was recorded after 24h. This test was developed in order to determine whether or not there would be spontaneous aphid death without the presence of the predator in 24h.

2.4 Nymphal development and survival of *Macrolophus pygmaeus*

Both the nymphal development and survival of *M. pygmaeus* fed on the aphids *M.persicae* and *A.solani* or eggs of *E. kuehniella* were determined.

The 1^{st} instar less than 24 h old of *M. pygmaeus* were placed individually on Petri dish (9 cm diameter, 1.5 cm height) contained leaf discs of sweet pepper (*Capsicum annuum* L. cv Spider) on a layer of 1% agar / water and mixed-instars plus adults aphids *M. persicae* or *A. solani* or *E. kuehniella* eggs as food *ad libitum*. The Petri dishes were covered with muslin tissue to permit exchange ventilation and kept in a climatic chamber at $25^{o} \pm 2^{o}$ C, 65% ± 10% RH, and 16 h photophase. New aphids or E. *kuehniella* eggs were added regularly to ensure sufficient food for nymphal development. These Petri dishes were checked daily by inspecting the predator nymphal cast-skins. The nymphal developmental time and survival of *M. pygmaeus* fed on those different preys was recorded.

The emerged-adults were sexed by observation of the external genitalia on the ventral abdomen and the sex ratio was recorded. These adults were paired and used on reproduction tests. A total of 50 nymphs (replicates) of *M. pygmaeus* per treatment were used whereas each treatment was represented by three different prey species, *M. persicae*, *A. solani* and *E. kuehniella* eggs (control treatment).

2.5 Fecundity of *Macrolophus pygmaeus*

Twenty-five pairs of newly-emerged-females and males of *M. pygmaeus* (less than 1-day old) were maintained individually in Petri dishes (9 cm diameter, 1.5 cm height), contained leaf discs of sweet pepper (*Capsicum annuum* L. cv Spider), as oviposition substrate, with midrib in the middle on a layer of 1% agar / water and the mixed-instars plus adults of the aphids *M. persicae* or *A. solani* and or *E. kuehneilla* eggs as prey *ad libitum*. A drop of water was placed on the walls of each Petri dish and the preys were offered daily to maintain enough food to the female oviposition. The Petri dishes were replaced twice a week, and were covered with muslin tissue to allow adequate ventilation. The oviposition period was evaluated for a 15 days period, and each 3 days, the couples were transferred to a new Petri dish with the preys on a new leaf disc substrate.

Prior to counting the number of eggs on the substrate, the pair of predator was previously removed, and the leaf discs were examined under a stereomicroscope. The eggs were recorded on the 3^{rd} / 7^{th} / 11^{th} and 15^{th} days of the female lifespan according to methodology proposed by Margaritopoulos, Tsitsipis and Perdikis (2003) and Perdikis and Lykouressis (2002, 2003). Twenty-five couples of *M. pygmaeus* representing 25 replicates/ treatment were

used, whereas each treatment was composed by the three different preys *M. persicae*, *A. solani* and *E. kuehniella* eggs (control treatment).

2.6 Measure tibias length from *Macrolophus pygmaeus* adults

Fifth individuals, 25 females and 25 males of *M. pygmaeus* developed on *A.solani* or *M.persicae* or *E. kuehniella* eggs to measure the adult size were used. From each predator individuals, the right hind tibia was removed and placed in a drop of 70% alcohol under a coverslip slide and measured under an optical microscope (5 x increases) with ocular micrometer, according to Sidney et al. (2010b).

2.7 Data Analyses

The daily predation data of *M. pygmaeus* were analyzed using either 1 or 2-way ANOVA with factors the equal vs. unequal numbers, the aphid instars and wingless adults or the aphid species. Means were separate using the Tukey test ($p < 0.05$) using the statistical package JMP IN (STATISTICAL ANALYSIS SYSTEM INSTITUTE - SAS INSTITUTE, 2007).

The nymphal development of *M. pygmaeus* were analyzed using one-way analysis of variance (ANOVA) and the means were compared using the Tukey test ($p < 0.05$). The sex ratio was subjected to chi-square test ($p < 0.05$). These statistical analyzes were performed using SigmaPlot 11.0, program (SIGMAPLOT…, 2008).

The oviposition patterns of *M.pygmaeus* fed on each prey (eggs of *E. kuehniella*, *A.solani* or *M.persicae*) were obtained by the average daily oviposition, considering the accumulated percentage of the number of eggs / female / day, according to methodology proposed by Mendes et al. (2005) and

Tomanović et al. (2003) and was analyzed using one-way analysis of variance (ANOVA). The means were compared using the Tukey test ($p < 0.05$).

3 RESULTS

3.1 Daily predation of *Macrolophus pygmaeus*

3.1.1 Daily predation of the 1st instar of *M. pygmaeus*

The 1st instar *M. pygmaeus* fed on all aphids' species offered as prey. However, this instar was able to prey only on the 1st instar of these aphids (Fig 1). There was a significant effect of the prey species on the 1st instar *M. pygmaeus* predation rate (*df* = 3 F = 7.9632, p (>F) = 0.0001101). The predation/24h of 1st instar *M. pygmaeus* was higher on *M. euphorbiae* and *M. persicae nicotianae* compared to those fed on *A. solani* and *M. persicae* (Fig 1).

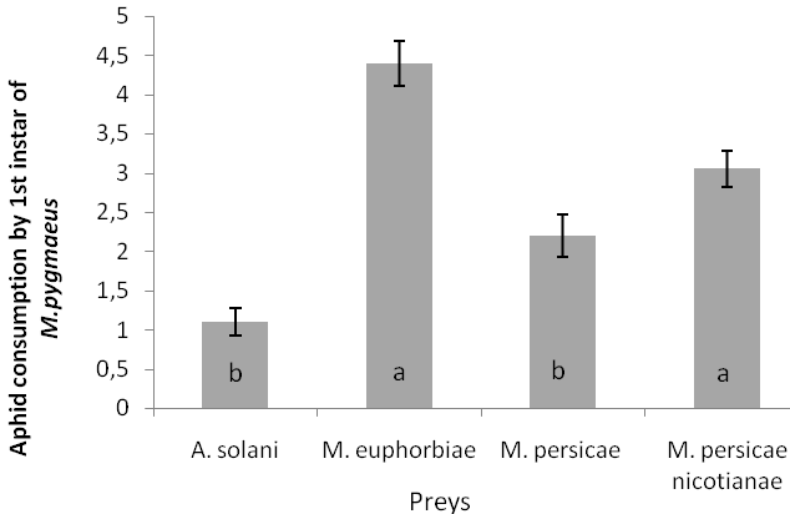

Figure 1. Daily predation of 1st instar *Macrolophus pygmaeus* on 1st instar of *Aulacorthum solani, Macrosiphum euphorbiae, Myzus persicae* and *Myzus persicae nicotianae,* 25±2°C, 65±10% RH and 16 h photophase.
Note: Means followed by different small letters within a column are significantly different (Tukey test, *P* □ 0.05).

3.1.2 Daily predation of 4th instar *M. pygmaeus*

The daily predation of 4th instar *M. pygmaeus* was influenced by prey aphid species and there was difference among the instars and wingless adult aphids as prey. There were significant differences on the predation of the 1st instar aphid (10.2 ± 1.54), 3rd instar (2.6 ± 0.38) and wingless adults of *M. euphorbiae* (0.5 ± 0.14) (*df* = 2, *F* = 47.183, *Pr* (□*F*) = 1.16*10 ^ (-13) (Table 1). However, the daily predation of 4th instar *M. pygmaeus* on the 1st instar of all aphids evaluated was higher compared to the predation on 3rd instar and wingless adults of *A. solani* (1st instar 8.1 ± 1.4; 3rd instar 1.0 ± 0.25 and wingless adult 0.8 ± 0.17 (*df* = 2, *F* = 35.096, *Pr* (□*F*) = 1.163*10^(-10)); *M. persicae* (1st instar 18.25 ± 1.24; 3rd instar 2.6 ± 0.40 and wingless adult 1.8 ± 0.53 (*df* = 2, *F* = 100.88, *Pr* (□*F*) = 2.2e-16)) and *M. persicae nicotianae* (1st instar 20.3 ± 1.26; 3rd instar 5.7 ± 1.00 and wingless adult 4.0 ± 0.61(*df* = 2, *F* = 63.633, *Pr* (□*F*) = 3.001*10^(-15) (Figure 2).

The daily predation by *M. pygmaeus* on the aphids *M. persicae* and *M. persicae nicotianae* was higher compared to predation on 1st instar *M. euphorbiae* and *A. solani* (*df* = 3, *F* = 16.522, *Pr* (□*F*) = 2.327*10 ^ (-08)) (Figure 2). The consumption of 4th instar *M. pygmaeus* on *M. persicae nicotianae* (3rd instar 5.7 ± 1.00) was consistently higher than on *M. euphorbiae* (3rd instar 2.6 ± 0.38), *M. persicae* (3rd instar 2.6 ± 0.40) and *A.solani* (3rd instar 1.0 ± 0.25) when these species were offered in their 3rd instar (*df* = 3, *F* = 13.57, *Pr* (□*F*) = 3.538*10 ^ (-07)). However, the daily predation on wingless adults of *M. euphorbiae* (0.6 ± 0.14) and *A.solani* (0.8 ± 0.17) was lower than that on *M. persicae* (1.8 ± 0.53) and *M. persicae nicotianae* (4.0 ± 0.61). In this case, the predation/24h of the 4th instar predator was higher on *M. persicae nicotianae* (4.0 ± 0.61) than on the others aphids offered as prey (*df* = 3, *F* = 15.891, *Pr* (□*F*) = 4.098*10^(-08)) (Figure 2).

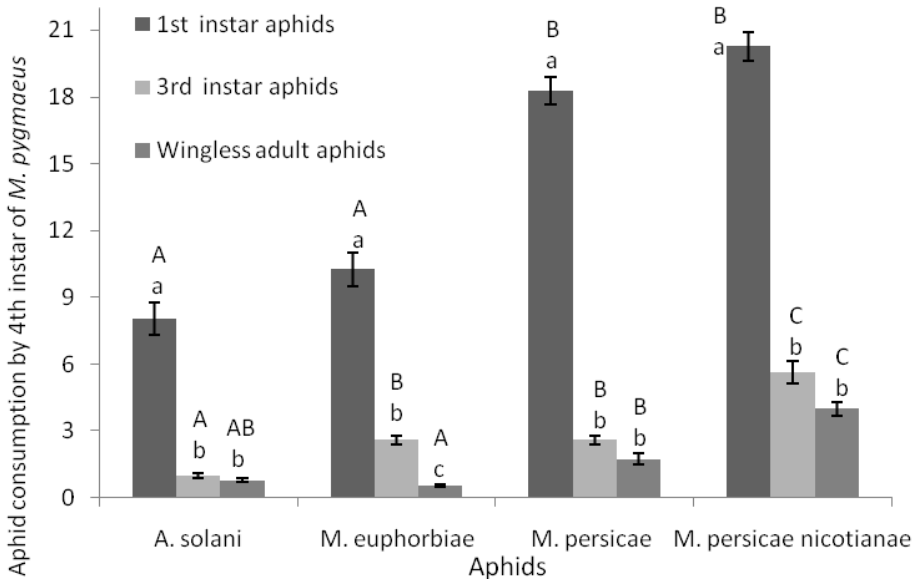

Figure 2. Daily predation of 4th instar *Macrolophus pygmaeus* on instars 1st and 3rd and wingless adults of *Aulacorthum solani, Macrosiphum euphorbiae, Myzus persicae and Myzus persicae nicotianae,* 25±2°C, 65±10% RH and 16 h photophase.
Note: Means followed by different capital letters (instars, wingless adult vs. aphid prey species) and small letters (different instars and wingless adult vs. each aphid prey species) within bars are significantly different (Tukey test, P □ 0.05).

3.1.3 Daily predation by adults of *M. pygmaeus*

The daily predation of predator's adults showed significant difference among the two instars and wingless adult of each aphid species. The predation rate was higher on 1st instar aphid compared to the 3rd instar and wingless adults of *A. solani*, *M. euphorbiae* and *M. persicae* as prey (Figure 3). The daily predation on *M. persicae nicotianae* at the 3rd instar and wingless adults did not show difference (*df* = 2 F = 11.16 p (>F) = 8.131*10^(-05)). Also, there was no difference on the daily predation on the 1st instar of the different aphids as prey (*df* = 3 F = 3.0031 p (>F) = 0.0356). A high daily predation by the adult *M. pygmaeus* on 1st instar *M. persicae* (25.7 ± 0.95), *M. euphorbiae* (24.9 ±

0.98), *M. persicae nicotianae* (22.1 ± 1.25) and *A. solani* (22.2 ± 1.12) were found.

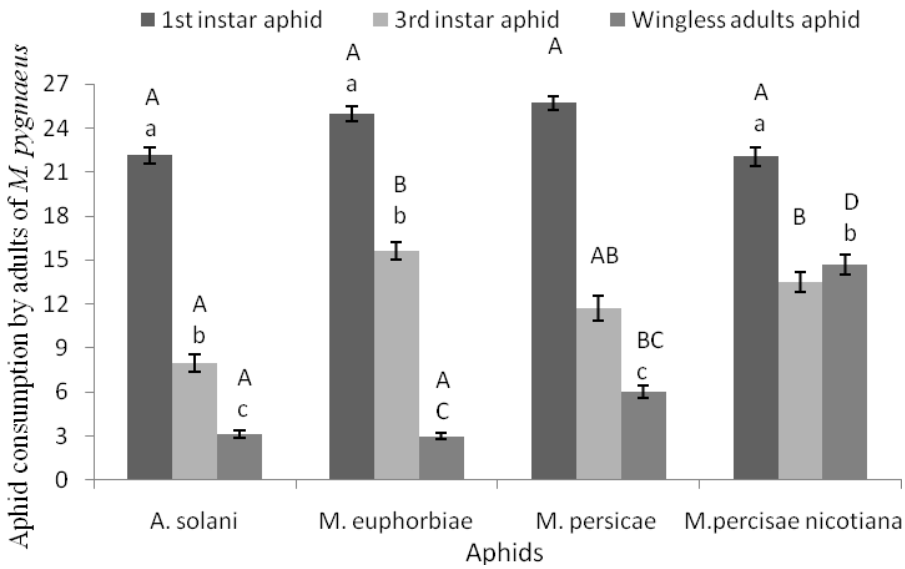

Figure 3. Daily predation of adults *Macrolophus pygmaeus* on 1st, 3rd instars and wingless adults of *Aulacorthum solani, Macrosiphum euphorbiae, Myzus persicae* and *Myzus persicae nicotianae,* 25±2°C, 65±10% RH and 16 h photophase.
Note: Means followed by different capital letters (compare the same instars and aphid prey species) and small letters (compare different instars in each aphid prey) within bars are significantly different (Tukey Test, P □ 0.05).

The daily predation of the adult *M. pygameus* on all aphids was highest when were offered the aphids 1st instar. The adult daily predation among the 1st instar of *M. persicae, M. persicae nicotianae*, *A. solani* and *M. euphorbiae* was not significantly different, and was 22.1 ± 1.25 *M. persicae nicotianae*, 22.2 ± 1.12 *A. solani*, 24.9 ± 0.98 *M. euphorbiae* and 25.7 ± 0.95 *M. persicae* (Table 1).

There was a significant difference on the consumption of the adult predator *M. pygmaeus* when feeding on the 3rd instar of the prey aphids. The lower daily predation of adult predators was on the 3rd instar of *A. solani* (8.0 ± 1.21) and *M. persicae* (11.7 ± 1.69) compared to the 3rd instar of *M. persicae*

nicotianae (13.5 ± 1.40) and *M. euphorbiae* (15.7 ± 1.20) (*df* = 3 *F* = 5.3642 *p* (>F) = 0.002101).

There was a difference in predation rate on the aphid species when the adult predator fed on wingless adults of aphids. The consumption by the adult predator on wingless adults of *M. persicae nicotianae* (14.7 ± 1.40) was higher compared to wingless adults of *M. persicae* (6.0 ± 0.88), *M. euphorbiae* (3.0 ± 0.49) and *A. solani* (3.2 ± 0.50) (Table 3). Moreover, the adults of *M. pygmaeus* feeding on *A. solani* and *M. euphorbiae* showed lower daily predation when compared to the predation on wingless adults of *M. persicae* and *M. persicae nicotianae* (*df* = 3 *F* = 35.207 *p* (>F) = 2.267*10^(-14)).

3.1.4 Absence of the predator

In the absence of the predator (control treatment) there was a high survival of these aphids. The survival rate of the instars 1st, 3rd and wingless adults *of M. persicae nicotianae* was 100%, and ranged from 100 to 95%, 100 to 95.85% and 100 to 98.33%, respectively, on *A. solani* and *M. euphorbiae.*

3.2 Nymphal development, survival and fecundity of *Macrolophus pygmaeus*

3.2.1 Nymphal development and survival of *M. pygmaeus*

M.pygmaeus was capable to complete its nymphal stage by feeding *E. kuehniella* eggs, or mixed-nymphs plus adults of *M. persicae* and *A.solani.* However, there was a significant effect on the nymphal developmental time according to the prey offered as food (*df* = 2; *F*= 12.8718; $p < 0.0001$) (Figure 4). The nymphal development time was significantly faster when the predator

feed on *E. kuehniella* (13.6 ± 0.10 days) eggs compared to the aphids *M.persicae* (14.1 ± 0.14 days) (F = 3.969; P = 0.001) and *A.solani* (14.7 ± 0.14 days) (F = 6.033; P = 0.0001).

The nymphal survival rates of *M. pygmaeus* were higher than 90% when feeding on E. *kuehniella* eggs (100%), *M. persicae* (96%) and *A. solani* (90%) (Figure 5).

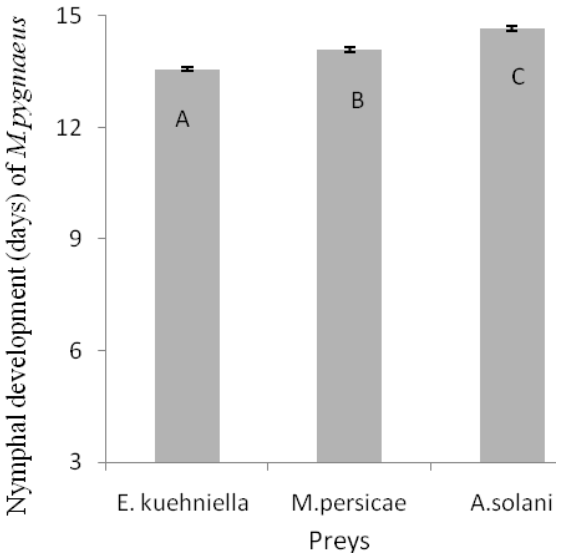

Figure 4. Nymphal development (days) of *Macrolophus pygmaeus* feed on *Ephestia kuehniella* eggs and mixed-instars plus adults of *Myzus persicae* and *Aulacorthum solani* at 25±2°C, 65±10% RH and 16 h photophase.
Note: Means followed by different capital letters (compare nymphal development time and different prey as food of *M.pygmaeus*) within bars are significantly different (Tukey test, P □ 0.05).

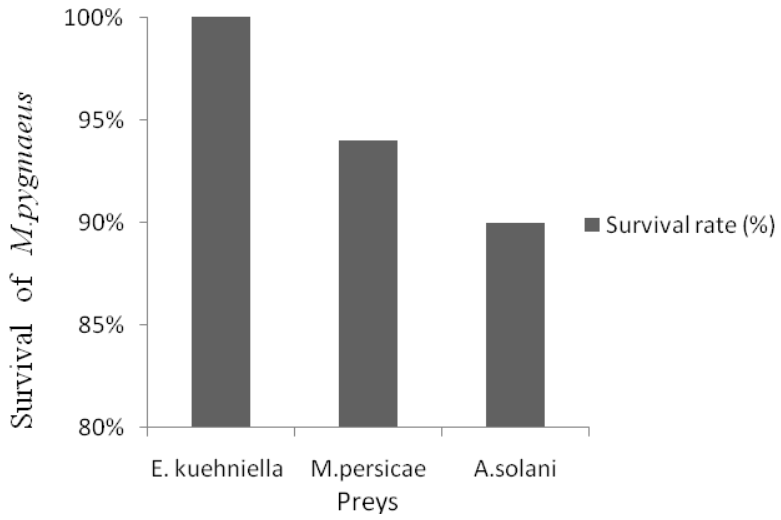

Figure 5. Nymphal survival (%) of *Macrolophus pygmaeus* when fed on mixed-instars plus adults of *Myzus persicae*, *Aulacorthum solani* and *Ephestia kuehniella* eggs. 25±2°C, 65±10% RH and 16 h photophase.

3.3 Fecundity of *Macrolophus pygmaeus*

The females of *M. pygmaeus* laid eggs dependent to the consumed prey species. There was significant difference on daily numbers of eggs regarding to the three preys consumed by the female predator ($P = 0.006765$) (Figure 6). The number of eggs/female/day of *M. pygmaeus* females fed on *E.kuehniella* eggs, *M.persicae* and *A.solani* were 3.1 ± 0.41, 2.2 ± 0.17 and 2.2 ± 0.26 eggs, respectively. The total fecundity of the female feeding on *E. kuehniella*, *M. persicae* and *A.solani* were 47.0 ± 6.09 eggs; 32.4 ± 2.55 eggs and 32.3 ± 3.84 eggs, respectively, in the 15 days period and having sweet pepper as oviposition substrate.

The female of *M. pygmaeus* fed on *E. kuehniella* eggs showed higher fecundity compared to females feed on aphids *A.solani* and *M. persicae* (P (>F) = 0.0141) (Figure 6).

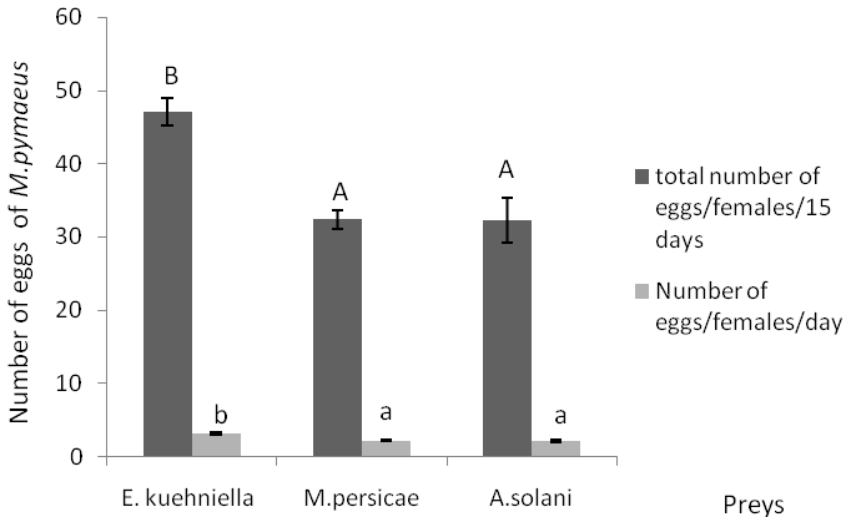

Figure 6. Total and daily fecundity of *Macrolophus pygmaeus* fed on mixed nymphs plus adults of *Myzus persicae*, *Aulacorthum solani* and *Ephestia kuehniella* eggs at 25±2°C, 65±10% RH and 16 h photophase.
Note: Means followed by different capital letters (compare total number of eggs / females/ 15 days) and small letters (compare number of eggs / females/ day) within a bar are significantly different by Tukey test (P □ 0.05).

The fecundity of *M. pygmaeus* was directed affected by the quality of the food, and they laid eggs on the sweet pepper leaves, independent of the food and started three days after the emergence of the female.

In the 3rd day, *M.pygmaeus* females fed on eggs of *E. kuehniella*, laid twice times eggs (16.5 eggs) than those females fed on *M. persicae* (7.5 eggs) and almost six times more eggs than females fed on *A. solani* (2.8 eggs) (Table 7). However, the number of eggs declined in the 7th day when the female fed on *E. kuehniella* compared to the number laid in the 3rd day of its lifespan (Figure 7). No difference on the number of eggs on the 3rd day (16.5 eggs) compared to the number on the 7th day (12.8 eggs), and in the 11th day (17.8 eggs). The female laid the lowest number of eggs on the 15th day (8.0 eggs) compared to the other days evaluated when having *E. kuehniella* eggs as food.

The female of *M.pygmaeus* laid fewer eggs (2.8 eggs) in the 3rd day of its lifespan when fed on *A.solani.* Moreover in the 7th (9.7 eggs) and 11th (13.6 eggs) days, the numbers of the eggs increased and were the highest values compared to the other days evaluated. The number of eggs decreased again on the 15th day (6.2 eggs) when having *A. solani* as prey (Figure 9).

The female predator having *M.persicae* as food laid the highest number of eggs on the 7th (8.7 eggs) and 11th (16.1eggs) days and the lowest number on the 3rd (7.5 eggs) and 15th (6.6 eggs) days (Table 1). So, on the 11th day, the females of *M.pygmaeus* fed on *E. kuehniella, M. persicae* and *A. solani* increased their number egg-laying, which were 17.8; 16.1 and 13.6 eggs, respectively (Figure 7, 8 and 9). However on the 15th day the number of eggs declined. Females of *M.pygmaeus* fed on *M. persicae* laid 7.5 eggs /3rd day and 16.1 eggs/11th day and *A. solani* 2.8 eggs/3rd day and 13.6 eggs /11th day) (Figure 8).

In the 15th day, the number of eggs of the females of *M.pygmaeus* fed on *E. kuehniella, M. persicae* and *A. solani* declined by more than half of the number laid on the 11th day, and were 8.0 ± 1.89 eggs ; 6.6 ± 0.96 eggs and 6.2 ± 1.00 eggs, respectively (Table 1). The fecundity was significantly reduced in the 15th day (8.0 ± 1.89 eggs / 15th) when the females of *M.pygmaeus* fed on *E. kuehniella* comparing to the 3rd day (16.5 ± 2.19 eggs/3rd).

Table 1. Fecundity of *Macrolophus pygmaeus* fed on mixed-instars plus adults of *Myzus persicae, Aulacorthum solani* and *Ephestia kuehniella* eggs at 25±2°C, 65±10% RH and 16 h photophase.

	Fecundity of *Macrolophus pygmaeus*					
Prey	**3th Day**	**7th day**	**11th Day**	**15th Day**	**Eggs/female/day**	**Eggs/female/15 days**
E.kuehniella	16.5±2.19ab	12.8±1.84bc	17.8±2.98ab	8.0±1.89c	3.1±0.41A	47.0±6.09A
M.persicae	7.5±1.03ab	8.7±0.92bc	16.1±1.41cd	6.6±0.96[a]	2.2±0.17B	32.4±2.55B
A.solani	2.8±0.81a	9.7±1.41ab	13.6±1.70b	6.2±1.00ac	2.1±0.26B	32.3±3.84B

*Means followed by different small letters in a row (compare the day evaluated vs. each prey) and capital letters within a column (compare numbers of eggs among different preys) are significantly different (Tukey test (P □ 0.05). n=25

3.4 Sex ratio and tibia length of *Macrolophus pygmaeus* adults

The sex ratio (express by percentage of female offspring) of the emerged adults of *M. pygmaeus* was 66.0%; 60.9 % and 58.3% feeding on *E. kuehniella, A.solani* and *M.persicae*, respectively, (Table 2).

The females and males hind tibia lengths of *M. pygmaeus* fed on those preys didn't show significant differences (x^2= 0,635; P = 0,728). However, females and males feeding on *E. kuehniella* eggs showed larger size (1.9 ± 0.01 ♀; 2.0 ± 0.03 mm ♂) than those female and male fed on *M. persicae* (1.9 ± 0.01 ♀;1.9 ± 0.01 mm ♂) and *A.solani* (1.9 ± 0.01 ♀; 1.9 ± 0.02 mm♂). There was a significant influence of the prey species aphids as food on the female and male tibia length of *M. pygmaeus* (Female P (>F) = 3.3 * 10^{-13*} Male P (>F) = 0.0141) (Table 2), and consequently on the predator adult size.

Table 2. Sex ratio (♀/(♀ + ♂)) and adults tibias length (mm) (± S.E.) of *Macrolophus pygmaeus* fed on *E. kuehniella*, *A.solani* and *M.persicae.*

Macrolophus pygmaeus			
Prey species	Sex ratio (%)[1] (n=50)	Size of the adult (mm)[2] (n=15)	
		Female ♀	Male ♂
A. solani	60.87a	1.9± 0.01aA	1.9 ± 0.02 aA
E. kuehniella	66.00a	1.9 ± 0.01 bA	2.0 ± 0.03 bA
M. persicae	58.33a	1.9 ± 0.01 aA	1.9 ± 0.01 aA

[1]Means followed by the same small letter in the column, do not differ by chi-square test (P<0.05).
[2]Means followed by the same small letter in the column, same capital letter in the row, do not differ by Tukey test (P<0.05).

4 DISCUSSION

The availability and suitability of the food, preference (SADEGHI; GILBERT, 2000) and size are factors that could determine whether any particular insect feed on any particular food, and also most insects are rather specific when choosing their food, including predators. In this study the 4th instar and adult's stages of *M.pygmaeus* fed on 1st, 3rd instars and wingless adults of *A. solani*, *M. euphorbiae*, *M. persicae* and *M. persicae nicotianae*. Also they were able to develop and reproduce feeding on mixed-instars plus adults of *M. persicae* and *A. solani*. However, the 1st instar of this predatory bug was able to prey only on the 1st instar of each aphid offered as prey.

In this study, according to predation rate we can stated that the 4th instar and adult's stages of *M. pygmaeus* might be effective on the aphid consumption and contributing to the aphid control, as the 1st instar presented the lowest feeding rate on aphids and only preyed the 1st instar aphid. Also, aphids without the presence of the predator presented high survival rate on the 24h period, showing that no influence of aphid natural death on the daily predation of *M. pygameus*.

The reason for the consumption of *M. pygmaeus* on smaller aphids could be attributed to the higher mobility of the biggest *M. euphorbiae* and *A. solani*, which can escape from the predators more easily than the aphids *M. persicae* and *M. persicae nicotianae*.

As the biological control of *A. solani* in pepper using parasitoids is still very problematic and with failure, this information about predation rate of *M. pygmaeus* on *A. solani* are very important tool to the aphid biological control programs. In this study *M. pygmaeus* feed on 1st instar *A. solani* and this suggests that the predator could has impact on A. *solani* initial population since the release timing will be done as a preventive control or by the use of banker

plants. Occasionally, when pest densities reach high levels and infestations become out of control, growers tend to introduce higher-order predators in their crops. These predators constitute a second-line of defence (BRODEUR; CLOUTIER; GILLESPIE, 2002) and they are often release in combination with usual cocktail of more specialized natural enemies from the first line of defence.

The higher daily predation, in general on *M. persicae* and *M. persicae nicotianae* over *A. solani* and *M. euphorbiae* individuals spite a comparable size could be attributed also to the different reactions of these aphids when attacked by a predator. Aphids are well-known for their anti-predator responses, as they could kick at natural enemies, or walk way or drop off from the plants when perceiving parasitoid or predator (MESSELINK; SABELIS; JANSSEN, 2012). Also the aphids release alarm pheromones that alert conspecifics, and this could play an important role to avoid natural enemies. Large prey might be less profitable since it often results in increased handling time and mortality risk (PASTOROK, 1981; SABELIS, 1992). However the daily predation of 1st instar of *M. pygmaeus* on 1st instar aphids was higher on *M. euphorbiae* than on *M. persicae*, and the lower daily predation was found on *A. solani*. Sidney et al. (2010a) found significant differences in the hind tibial lengths between the 2nd instar of *M. euphorbiae* and *A.solani*, and in this case, the aphid *M. euphorbiae* was larger than that *A.solani,* indicating that the size of the aphids can influence the quality of host / prey for the natural enemy. One explanation for the higher consumption of 1st instar of *M. pygmaeus* on 1st instar *M. euphorbiae* could be attributed to the small difference in size between these 1st instar aphids and also, maybe there is no different reaction of these aphids when attacked by a1st instar predator.

Predation increased from 1st instar to adult's stage of *M. pygmaeus*. The predation rate on *M.persicae* increased gradually as the nymphal stage raised in this study, and this was also observed by Perdikis, Lykouressis and Economou

(1999) on adult's females and fifth instar nymphs of *M. pygmaeus* feeding on *M. persicae* on pepper and eggplant at 20°C and 25 °C and showing a higher predation rate than the others instars and adults males. Also, in this study there was a higher daily predation on *M. persicae nicotianae* than on the others aphids offered as prey. The result suggests that *M. persicae nicotianae* is more susceptible on its different instars when compared to other species of aphids evaluated. Probably *M. persicae nicotianae* might be recognized easier by the predator *M. pygmaeus*, due to its red colour compared to the others "green aphids". In addition, wingless adults stage of *M. persicae nicotianae* (1.2 to 2.1 mm in length) and *A.solani* and *M. euphorbiae* have sizes (1.8 to 3.0mm and 1.7 to 3.6mm, respectively) (MALAIS; RAVENSBERG, 2003). Also, partial prey consumption or killing without consumption is known to occur in several predators and this is related to prey size, handling time, predation risk and prey defensive mechanisms (FANTINOU et al., 2008, 2009; MEYLING; ENKEGAARD; BRODSGAARD, 2003).

The host plant of the prey could also be a determining factor. The plant nutrient might affect the taste of the preys making them either more tasteful or less desirable to the predators. Thus, the host plants of aphids' species *M. euphorbiae* (tomato), *A.solani*, *M. persicae nicotianae* and *persicae* (sweet pepper), might have significant influence on the palate attractive for predatory bug *M. pygmaeus*.

Macrolophus sp. usually attacked the preys nearest to them (RASDI; FAUZIAH; MOHAMAD, 2009), and the ability of aphid natural enemies to control the target pest is influenced by plant features, like trichomes on tomatoes, or sticky or toxic exudates, that adversely affect the searching efficiency of predators' larvae (BLUMEL, 2004). However, aaccording to Bueno et al. (2012) and Malais and Ravensberg (2003), the mirids predators appear to have no trouble with the glandular hair of tomato plants, related to

walking on predation capacity. So, in our study, the predator there was no problem to consume *M.euphorbiae* on tomatoes plants.

In addition, the predator *M. pygmaeus* was capable of completing its nymphal stage by feeding on all the different aphid prey whether on eggs of *E.kuehniella* or nymphs of *M. persicae* and *A.solani*. Margaritopoulos, Tsitsipis and Perdikis (2003) demonstrated that *M. pygmaeus* and *M. caliginosus* preying *M. persicae* on tobacco leaves were also able to complete their development. In our study *M. pygmaeus* showed short development time, even when the predator fed on aphids or *E.kuehniella* eggs. The feeding habits of these predators are ambiguous, because even in the early attempts to evaluate their potential as biological control agents, their ability to use plant sap was suspected, due to their prolonged survival on crops during periods of prey scarcity (MALAUSA; DRESCHER; FRANCO, 1987). Furthermore, in biological control programs, this characteristic could play a key role in release strategies, because populations surviving on the crop during a period of prey scarcity could suppress a pest population as soon as it occurs and before it increases to high numbers (SYMONDSON; SUNDERLAND; GREENSTONE, 2002).

The nymphal development time was faster for *M.pygmaeus* fed on *E.kuehniella* eggs compared to the aphids *M. persicae* and *A. solani*. However, it's known that eggs of *E. kuehniella* are traditionally used in the professional rearing of this predator, providing optimization of all biological parameters. Therefore, even the results favored the eggs of *E. kuehniella*, aphids, as prey, also showed satisfactory results for the development of *M. pygmaeus*. Margaritopoulos, Tsitsipis and Perdikis (2003) demonstrated that the nymphal development time were 20.2 days when *M.pygmaeus* fed on *M.persicae* on tobacco plants. Our studies indicate that the sweet pepper as host plant seems that optimized the development time of the predator. Although plant saps stimulate better development of *M.pygmaeus* populations, the sap is insufficient

for complete development of individuals. When females are feeding only on plant sap they lay few eggs and show low survival (MALAIS; RAVENSBERG 2003).

The survival rates of *M.pygmaeus* was high for the three prey tested. In contrast, Perdikis and Lykouressis (2003) found that young nymphs of *M. pygmaeus* when fed on *A. gossypii* suffered higher mortality than the older ones, which could be attributed to accumulation of honeydew on their tarsi. This adverse effect on the host plant-prey system possibly results from the particular aphid *A. gossypii* on cucumber, leading to high nymphal mortality. In our results the host - plant, sweet pepper, did not showed adverse effect in the relationship between predators - preys.

The fecundity of females of *M. pygmaeus* was therefore directly affected by the quality of the consumed prey. When the female of *M. pygmaeus* fed on *E. kuehniella* showed higher eggs production during the 15 days period compared to females fed on *A.solani* and *M.persicae* as prey. These results show the nutritional superiority of Lepidoptera eggs as prey for the better development and reproduction of *M. pygmaeus*. This is probably due to the high nitrogen content present in these eggs, which guarantees a more adequate diet for the development of this predator in laboratory (EUBANKS; DENNO, 2000), and Lepidoptera eggs are prey of high quality for many generalist predators, while aphids are relatively of low nutritional quality. Riudavets and Castane (1998) found that *M. caliginosus* could lay 83 eggs per female and van Schelt et al. (1995) observed an oviposition rate of two to seven eggs per day for first 15 days when the female predator fed on *T. vaporariorum*. Therefore, although some variation in fecundity is evident between studies, *M. caliginosus* is highly fecund when fed on *T. vaporariorum*. The daily oviposition rate of *M. pygmaeus* females fed on *M. persicae* and *A.solani* was 2.16 and 2.15 eggs/day respectively. Margaritopoulos, Tsitsipis and Perdikis (2003) reported similar

values, for *M. pygmaeus* females fed on *M. persicae* (2 eggs / day). Eggs of *E. kuehniella* or aphids as prey is favorable to the oviposition, but aphids also could contribute for the oviposition of *M.pygmaeus* and also for the establishment and at least the maintenance for the next generation.

The difference in the number of progenies produced by a female can be determined by the host plant characteristics (GERLING; ALOMAR; ARNÓ, 2001) such as hardiness of the leaf petioles and midribs. Although many factors affect development and fecundity of predatory bugs, the most important one is food. Survival of populations of these natural enemies in habitats with a shortage or lack of prey depends on their capacity to allocate energetic resources to specific activities: at low prey densities, the energy reserved for reproduction may be limited and reproduction will reduce the survival capacity of these predators (BUENO; LENTEREN, 2011).

5 CONCLUSION

M. pygmaeus present a potential as aphid biological control agent and could contribute to the integrated management of these pests. The knowledge about predation rate, development, survival and fecundity of *M.pygmaeus* can be useful for the most appropriate implementation of aphid's biological control strategies. However, *M. pygmaeus* alone maybe is insufficient to restrain aphid populations, and then, good farming practices are needed to complement the effects of *M.pygmaeus* for controlling aphids. Future work should be carried out on sweet peppers crops releasing a combination between the predators *M.pygmaeus* and specialized natural enemies such as aphid's parasitoids, *P.volucre* and *A.ervi*.

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