



Host species suitability and instar preference of *Aphidius ervi* and *Aphelinus abdominalis*

M.C. Velasco-Hernández¹, N. Desneux², M.M. Ramírez-Martínez³,
L. Cicero⁴ and R. Ramírez-Romero^{1*}

¹ Biological Control Laboratory, Department of Agricultural Production, CUCBA, University of Guadalajara, Zapopan, Jalisco, México

² French National Institute for Agricultural Research (INRA), UMR1355, 400 Route des Chappes, 06903, Sophia-Antipolis, France

³ Department of Ecology and Natural Resources, CUCSUR, University of Guadalajara, Autlán de Navarro, Jalisco, México

⁴ Forest Health and Agriculture Program, C.E. Mocochoá – CIRSE, National Institute for Research in Forestry, Agriculture, and Livestock (INIFAP), Mocochoá 97454, Yucatán, México

* Corresponding author: rramirez@cucba.udg.mx

With 3 figures and 1 table

Abstract: Parasitism rates and parasitoid development can be influenced by the species and developmental stage of the host, both of these factors can influence parasitoid performance and fitness. In this study, parasitism rates and developmental parameters were assessed for two widely distributed and commercially available species of aphid parasitoid: *Aphidius ervi* (Hymenoptera: Braconidae) and *Aphelinus abdominalis* (Hymenoptera: Aphelinidae). In a first bioassay, parasitism rates and parasitoid development were investigated in different host species. The wasp *A. ervi* was tested on *Macrosiphum euphorbiae*, *Rhopalosiphum padi*, and *Myzus persicae* (Hemiptera: Aphididae) and *A. abdominalis* was tested on *M. persicae*, *R. padi*, and *Rhopalosiphum maidis* (Hemiptera: Aphididae). The results indicated that *A. ervi* had a greater percentage of emergence, higher percentage of parasitized aphids, longer developmental time, and higher proportion of females in *M. persicae* than in the other hosts. *A. abdominalis* had a greater percentage of emergence, larger progeny, and shorter developmental time in *R. padi* than in the other hosts. A second bioassay evaluated the preference of the parasitoids for different instars of their respective optimum host aphids in terms of parasitism and development, as determined in the first bioassay. The results showed that *A. ervi* produced a greater number of mummies in the fourth instar and in adults of *M. persicae*. In contrast, *A. abdominalis* preferred the first instar of *R. padi*. In conclusion, our results indicate that both parasitoid species exhibit different parasitism parameters depending upon the host species and the host stage. This suggests that these parasitoid species could be potentially complementary on multiple or combined releases of biological control programs.

Keywords: host preference, biological control, natural enemies, parasitoids, aphids

1 Introduction

Biological control is a pest control strategy that has been effective in several cases (Van Driesche & Belows 1996). Besides, it can be an alternative or complementary strategy to the traditional use of synthetic pesticides for pest control (Van Driesche et al. 2008). However, its use involves knowledge on the implication of several factors (e.g. interactions among organisms), which in some cases are not fully known or understood. For example, if the control of an aphid pest is intended using a parasitoid species, once released the parasitoid it could attack other aphid species, producing unexpected results on the efficiency of our pest control strategy. Then, previous knowledge on the host species suitability or instar preference of natural enemies on different hosts will be helpful to better predict or understand pest control results (Godfray 1994, Brodeur & Vet 1995, Desneux et al. 2009a, 2009b, 2009c, Desneux et al. 2012).

Aphids are an important pest group throughout the world. Because of the damage they cause to vegetable, fruit, and grain crops, a few species stand out as particularly important: *Macrosiphum euphorbiae* Thomas, *Myzus persicae* Sulzer, *Rhopalosiphum padi* Linnaeus and *Rhopalosiphum maidis* Fitch (Hemiptera: Aphididae) (Blackman & Eastop 2007, Gavkare et al. 2014). The main damage they cause is due to extraction of sap and fungal proliferation due to honeydew, but above all, they are vectors of a number of viral diseases (Boquel et al. 2014, Xiao et al. 2015). One of the methods for their control has been the use of natural enemies such as predators and parasitoids. For example, *Aphidius ervi* Haliday (Hymenoptera: Braconidae) and *Aphelinus abdominalis* Dalman (Hymenoptera: Aphelinidae) are two species of solitary parasitoids increasingly being used in America and Europe for biological control of aphids (Daza-Bustamante et al. 2003, Ceballos & Duarte 2012, Legarrea et al. 2014, Shrestha et al. 2015). They are commercially available and can share some hosts attacking common crops such as eggplant, tomato, pepper and beans. The wasp *A. ervi* can be used to control species of the tribe Macrosiphini such as *M. euphorbiae*, *M. persicae*, and *Acyrtosiphon pisum* Harris (Takada & Tada 2000, Acheampong et al. 2012, Daza-Bustamante et al. 2003). For its part, *A. abdominalis* is a wasp that attack species of aphids including *M. persicae*, *R. padi*, *R. maidis*, and *M. euphorbiae*, among others (Duarte et al. 2012, Acheampong et al. 2012, Ceballos & Duarte 2012, Japoshvili & Abrantes 2006, Botto 1981). The quality of the host, from the perspective of the parasitoids, can be related to its nutritional content (Mackauer et al. 1996). At the same time, the nutritional content of the host will be influenced by its species (Sequeira & Mackauer 1993). Thus, the host species can determine its quality and whether or not it would be chosen by a parasitoid. In an ecosystem, it is common for different host species to coexist (Van Alphen & Jervis 1996), and it is predicted that the host of the best quality for parasitoid progeny development will be chosen for reproduction (Godfray 1994, Heimpel et al. 1996, Cingolani et al. 2014). The mechanisms used by female parasitoids to locate and discriminate among hosts are widely described by Isidoro

et al. (2001) and van Baaren et al. (2007). In this process, antennae play a key role for the recognition of cues associated to hosts, both, volatile and contact semiochemicals (Romano et al. 2016). Thus, faced with a host of good quality, it is expected that a parasitoid will choose this host over others and will have high rates of parasitism and survival to the adult stage, as well as progeny with greater size and fertility, shorter development time, and a biased female sex-ratio (at least a 1:1 female:male proportion (i.e. 50% females) is expected) (Mackauer 1996, Van Driesche et al. 2008, Teppa-Yotto et al. 2013, Ghimire & Phillips 2014).

In order to effectively employ parasitoids in biological control programs, it is important to know about their host selection behavior and host suitability in different host species (Rakhshani et al. 2004). Indeed, during their life cycle, one of the important decisions facing female parasitoids is whether or not to parasitize certain host species and developmental stages (Barrete et al. 2009). These decisions will determine whether or not her progeny will survive and develop adequately.

Previous studies have approached this subject of host suitability and selection of parasitoids in different species and stages of aphids (Rakhshani et al. 2004, Tomanovick et al. 2009, Sidney et al. 2010, Silva et al. 2011, Monje et al. 1999). Among the principal biological parameters measuring host suitability are rates of parasitism, survival to the adult stage, sex-ratio, immature developmental times and offspring size (Sidney et al. 2010, Gavkare et al. 2014, Duarte et al. 2012, Gavkare et al. 2013, Stilmant et al. 2008). For the specific case of *A. ervi* and *A. abdominalis*, some of these parameters have been previously studied using some aphid hosts (Table 1). However, information taking into account other biological parameters or host species is lacking (see details in Table 1). For example, for *A. ervi*, Sidney et al. (2010) studied developmental parameters using the host *M. euphorbiae*. Percentage of parasitism has been assessed on *M. persicae* (Gavkare et al. 2014) and *R. padi* (Stilmant et al. 2008). However, important parameters such as mean number of parasitized hosts, sex-ratio and offspring size have not still been assessed for *M. persicae* and *R. padi* (Table 1). Information on *A. abdominalis* is less available. For example, Duarte et al. (2012) evaluated some biological parameters (e.g., percentage of emergence and developmental time) in *M. persicae*. However, the sex-ratio and offspring size of *A. abdominalis* developing in *M. persicae* was not reported. Furthermore, all these developmental parameters have not been assessed using *R. maidis* or *R. padi* as hosts (Table 1).

In another hand, it has been observed that some species of parasitoids are able to oviposit and develop in various developmental stages of a host (Rakhshani et al. 2004, López et al. 2009, Latham & Mills 2012). Nevertheless, some species of parasitoids show a preference for parasitism of certain stages of development of the host (Weisser 1994, Rakhshani et al. 2004, He et al. 2005, Talebi et al. 2006). In the case of *A. ervi*, its preference for specific developmental stages has been assessed in species such as *M. persicae*, *Acyrtosiphon kondoi*, and *Aulacorthum solani* (Hemiptera: Aphidoidea) (Colinet et al. 2005, He & Wang 2006, He et al. 2011). These studies found that *A. ervi* has a preference for intermediate stages of development. As a possible explanation, these authors proposed defensive behavior and nutritional value of the host. Instar preference of *A. abdominalis* has been studied in *Nasonovia ribisnigri*

and *Myzus ascalonicus* (Hemiptera: Aphidoidea) (Shrestha et al. 2015, Wahab 1985). These studies report that for *N. ribisnigri*, the preference is for first, second, and third stages, while in *M. ascalonicus*, the preference is for third and fourth stages, as well as for adults. The authors relate this preference to the presence of effective defensive mechanisms in the other stages.

Table 1. Detailed information on the contributions of the present study. For each parasitoid species, we formed 18 cells by crossing 6 biological parameters studied and 3 hosts species analyzed. For each cell it is indicated: 'Not reported previously' (when we found no previous published literature reporting that information) or 'Reported previously' (when previous literature reports information for that specific cell). In the last case, we provide also the reference that reports such information. 'Reported here' indicates that we generated data in this study for that specific cell.

<i>Aphidius ervi</i>			
	Hosts		
	<i>M. persicae</i>	<i>R. padi</i>	<i>M. euphorbiae</i>
Mean of parasitized aphids	Not reported previously (Reported here)	Not reported previously (Reported here)	Not reported previously (Reported here)
Percentage of parasitism	Reported previously: Gavkare et al. 2014 (Reported here)	Reported previously: Stilman et al. 2008 (Reported here)	Reported previously: Sidney et al. 2010 (Reported here)
Percentage of emergence	Reported previously: Hofsvang & Hagvar 1975 (Reported here)	Not reported previously (Reported here)	Reported previously: Sidney et al. 2010 (Reported here)
Sex-ratio	Not reported previously (Reported here)	Not reported previously (Reported here)	Reported previously: Sidney et al. 2010 (Reported here)
Development time	Reported previously: Hofsvang & Hagvar 1975 (Reported here)	Not reported previously (Reported here)	Reported previously: Sidney et al. 2010 (Reported here)
Offspring size	Not reported previously (Reported here)	Not reported previously (Reported here)	Reported previously: Sidney et al. 2010 (Reported here)

<i>Aphelinus abdominalis</i>			
	Hosts		
	<i>M. persicae</i>	<i>R. padi</i>	<i>R. maidis</i>
Mean of parasitized aphids	Not reported previously (Reported here)	Not reported previously (Reported here)	Not reported previously (Reported here)
Percentage of parasitism	Reported previously: Duarte et al. 2012 (Reported here)	Not reported previously (Reported here)	Not reported previously (Reported here)
Percentage of emergence	Reported previously: Duarte et al. 2012 (Reported here)	Not reported previously (Reported here)	Not reported previously (Reported here)
Sex-ratio	Not reported previously (Reported here)	Not reported previously (Reported here)	Not reported previously (Reported here)
Development time	Reported previously: Duarte et al. 2012 (Reported here)	Not reported previously (Reported here)	Not reported previously (Reported here)
Offspring size	Not reported previously (Reported here)	Not reported previously (Reported here)	Not reported previously (Reported here)

An understanding of the preference of parasitoids for certain developmental stages is important because it forms a part of the life history of these organisms (Trotta et al 2014), and can contribute to the understanding of foraging and host-selection strategies (Henry et al. 2009), as well as their population dynamics (Lin & Ives 2003, Sengonca et al. 2008). From an applied point of view, this information can be valuable during mass rearing optimization (He & Wang 2006), and release of these parasitoid species (Shrestha et al. 2015).

For the above reasons, the aims of the present study were: 1) to assess the suitability of different aphid species for *A. ervi* and *A. abdominalis* and, 2) to determine which host developmental stage is preferred by the parasitoids *A. ervi* and *A. abdominalis*, when faced with different developmental stages of *M. persicae* and *R. padi*, respectively. We choose these host species for the second bioassay on the basis of the results of the first bioassay that indicated that these hosts were the best for parasitoid development.

2 Materials and Methods

2.1 Biological material

Plants

To maintain the different species of aphids, the plants used were chili (*Capsicum annuum* L. var. Serrano), oat (*Avena sativa* L. var. Avemex), potato (*Solanum tuberosum* L. var. Blanca), and maize (*Zea mays* L. var. Tuxpeño). The chili plants were grown from commercial seeds acquired at La Casa del Hortelano S.A. de C.V. (Guadalajara, Jalisco, México). Seeds for the oat plants were acquired at Grupo Industrial Vida S.A. de C.V. (Venta del Astillero, Jalisco, México). Potato plants were grown from tubers acquired from a local commercial market (Zapopan, Jalisco, México). The seeds for the maize, were provided by CIMMyT (Centro Internacional de Mejoramiento de Maíz y Trigo, México State, México).

All of the seeds and tubers were planted in square pots (8.5 cm × 8.5 cm × 9 cm) containing Nutrigarden® soil: 50% black soil and 50% compost (Sulfatos y Derivados, S.A. de C.V., México) and perlite (Agrolita de México, S.A. de C.V.) mixed in a 1:1 ratio. The plants were fertilized with “triple 18” fertilizer (SQM Comercial de México S.A. de C.V.) every two days. All of the plants were used at 1–2 months of age and were maintained free of herbivores until the experiment. The chili plants were maintained in an acclimatized room with 24 ± 3 °C, relative humidity (RH) of $50 \pm 10\%$ and a light:dark (L:D) photoperiod of 12:12 hours in the Biological Control Laboratory, CUCBA, Universidad de Guadalajara, Mexico (BCL-UdG). The maize plants and oat were maintained under greenhouse conditions (17 ± 6.5 °C, RH: $68 \pm 26.5\%$) in cages covered with anti-aphid mesh to preclude the entrance of herbivores. The maize and oat plants were grown in the greenhouse because, during previous growing attempts, we observed that development proceeded better under greenhouse conditions than under laboratory conditions. In contrast, the chili and potato plants developed well for our purposes in the laboratory.

The plants used to maintain the aphid colonies were: chili plants for *M. persicae*, oat plants for *R. padi*, potato plants for *M. euphorbiae*, and maize plants for *R. maidis*.

Insects

All insects were maintained under laboratory conditions, at 24 ± 3 °C, RH of $50 \pm 10\%$ and a photoperiod of 12:12 (L:D) hours.

Aphid colonies were established in the BCL-UdG. The colonies of *R. padi*, *M. euphorbiae*, and *R. maidis* were initiated with individuals collected in crops adjacent to the BCL-UdG campus (Latitude: North 20° 44' 47", Longitude: West 103° 30' 43") between August and November of 2013. *R. padi* was collected on oat plants, *R. maidis* on maize plants, and *M. euphorbiae* on tomatillo plants (*Physalis philadelphica* Lam.). To collect aphids, we carefully sampled about 20 individuals per aphid species using a fine paintbrush. These individuals were left on its corresponding host plant placed in an acrylic cage (45 cm high × 38 cm long × 30 cm wide) covered

with anti-aphid mesh. After sampling, cages with plants and aphids were transferred to a rearing room and provided with new host plants as required. These cages were supervised daily and newborn aphids were carefully transferred to a different cage containing new host plants. The newborn aphids were used to increase populations and perform experiments. The colony of *M. persicae* was established from individuals provided by Mary Carmen Torres Quintero (Laboratorio de control Biológico del Centro de Investigaciones en Biotecnología de la Universidad Autónoma del Estado de Morelos, México) in February of 2012. All the aphid species were taxonomically verified by the Aphidoidea specialist MSc. Rebeca Peña Martínez (Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, IPN, México) (Blackman & Eastop 2000, Blackman & Eastop 2007).

Parasitoids of both species, *A. ervi* and *A. abdominalis*, were provided as mummies by the company Koppert México S.A. de C.V. (Querétaro, México). After their arrival at the laboratory, they were maintained in acrylic cages (45 cm high × 38 cm long × 30 cm wide) and fed *ad libitum* with a honey solution (Carlota, McCormick de México S.A. de C.V., Morelos, México) diluted to a honey:water ratio of 7:3, v/v, and drinking water in folded absorbent paper (7 cm × 7 cm) placed in the base of a petri dish (8.5 cm in diameter) (Bao-Fundora et al. 2016). After adult emergence of these mummies, pairs of parasitoids were randomly selected and using an insect aspirator were placed together in petri dishes (8.5 cm in diameter) to increase the probability of mating. The pairs were fed with a honey solution (7:3 ml honey:water) and drinking water in 1 cm³ of cotton. For the experiments, 24- to 48-hour old females of both species (*A. ervi* and *A. abdominalis*) were used. From previous studies, it is known that both parasitoids are able to oviposit 1 day after their emergence (Wahab 1985, He et al. 2004).

2.2 Bioassay 1: Host species suitability

All bioassays were performed at 24 ± 3 °C and a RH of $50 \pm 10\%$, an L12:D12 photoperiod and 1600 lux. We followed a randomized block design (with days as the blocking factor) in which the different aphid species were tested (no-choice tests) with the two species of parasitoids. For *A. ervi*, the aphid species *M. persicae*, *R. padi*, and *M. euphorbiae* were tested. For *A. abdominalis*, the aphid species *M. persicae*, *R. padi*, and *R. maidis* were tested. These aphid species were chosen taking into account that they can share some host plants and be present simultaneously in the field. For example, *R. padi* and *R. maidis* can be found simultaneously on maize (*Zea mays*) and grasses (SAGARPA 2013). *Myzus persicae* and *M. euphorbiae* can be simultaneously present on plants of the Solanacea family (Srinivasan & Alvarez 2011, Davis et al. 2006).

We tested host species suitability based on the methodology described by Desneux et al. (2009b). In this methodology, each host plant corresponding to a specific species of aphid was covered with a cylinder of transparent plastic (11 cm in diameter, 21 cm high). Each cylinder had 12 holes (3.5 cm in diameter) and an additional hole at the upper end, these were all covered with anti-aphid mesh for ventilation. Fifty aphids

of the corresponding species were placed on each host plant (i.e. *M. persicae* on chili, *R. padi* on oat, *R. maidis* on maize, and *M. euphorbiae* on potato). These 50 aphids were taken at random from the general colony of each species and included individuals from different developmental stages following Desneux et al. (2009b) and with the aim of simulating what can be found in the field (i.e. several aphid instars present concomitantly). After this time, a female parasitoid was introduced into the cylinder. For this, we used an insect aspirator to take the parasitoid from the petri dish and release it over the leaves of the plant. Afterwards, the plant was immediately covered with the plastic cylinder. Once released, the parasitoid was allowed to forage for 24 hours. After this period of time, the female parasitoid was withdrawn and the plants were checked daily for the presence of mummies (Capinera 2008). When mummies were detected, they were separated into individual gelatin capsules (Capsugel quality^{MR}, Envases Velasco, Guadalajara, Jalisco, México) and checked every 24 hours in order to register the day of emergence and the sex of the emerging wasps.

For this bioassay, the response variables were the mean number of aphids parasitized (i.e., number of mummies formed), the sex of the emergent wasps, and the development time in days (time after oviposition until emergence of the parasitoid adult), for each wasp. Using these variables, three values were calculated: the percentage of parasitism (number of parasitized aphids*100/total exposed aphids), the percentage of emergence (number of emerged wasps*100/number of parasitized aphids), and sex ratio (number of female emerged wasps/total number of emerged wasps). In addition, the posterior right tibia of emerged wasps was measured as an estimation of size. The tibias were measured with a stereoscope (Stemi 2000-C Carl Zeiss) connected to an AxioCam ICc1 camera, using AxioVision 4.8 software (Carl Zeiss, Guadalajara, Jalisco, México). Fifteen replicates were performed for *A. ervi* (in total 45 *A. ervi* wasps were tested) and 20 replicates for *A. abdominalis* (in total 60 *A. abdominalis* wasps were tested).

For *A. ervi*, the response variables (mean number of parasitized aphids, percentage of parasitism, percentage of emergence, sex ratio, and development time) were compared among aphid species using linear mixed models (LMM) with the host species as the fixed factor and the block (days) as the random factor (Crawley 2007). Size was analyzed using a linear model with the host species as the main factor and the block integrated into the model (Crawley 2007), but after $\sqrt{x + 0.5}$ transformation of data (Zar 1998) to fit normality and homoscedasticity requirements.

For *A. abdominalis*, the four response variables, mean number of parasitized aphids, percentage of parasitism, percentage of emergence, and development time, were compared among host species using an LMM with the host species as the fixed factor and the block as the random factor. The sex ratio was not compared because only females emerged in the three species of aphids evaluated. The size of the progeny was compared among aphid host species in the same way as for *A. ervi*. All analyses were performed using R, version 3.3.1 R Core Team (2013).

2.3 Bioassay 2: Instar preference

For this bioassay, we also followed a randomized block design, with time (days) as the blocking factor. Ten individuals from each nymphal developmental stage, first to fourth instars (Perdikis et al. 2004) as well as wingless adults (Tahriri et al. 2007), from each aphid species, were selected. With these 50 aphids in total, we sought to provide the wasps with a sufficient number of aphids to parasitize (see Results, Fig. 1). For this bioassay, for *A. ervi* we used *M. persicae* as host species and for *A. abdominalis* we used *R. padi*, since these were the species with the highest rates of parasitism in the first bioassay (see Results, Figs. 1 and 2). The aphids were placed on a leaf of their corresponding host plant that was inserted into a piece (1 cm²) of floral foam (OASIS®, Smithers-Oasis, Zapopan, Jalisco, México) to maintain hydration, afterwards they were transferred to a petri dish (8.5 cm in diameter). The leaves were changed every three days to ensure their turgidity. Damp filter paper (ISOLAB average porosity, 8.3 cm in diameter, Prolab, Guadalajara, Jalisco, México) was placed in the base of each petri dish and to provide food to wasps, a drop of undiluted honey was placed on the inside of the lid. One leaf of the host plant for each species of aphid was placed on the damp filter paper, and different stages of aphids were randomly placed on the leaf and allowed to establish themselves for one hour. After this period, one female parasitoid was taken using an insect aspirator and then released into each petri dish and allowed to forage there for 24 hours. At the end of this time period, the parasitoid was withdrawn and the aphids were separated according to their developmental stage. The different stages of aphids were placed on a leaf of their corresponding host plant in a petri dish (described earlier) in order to allow development of the aphids and parasitoids to the mummy phase (Capinera 2008). The leaf was changed every three days in order to maintain nourishment for the aphids. After the day of exposure to the parasitoid, the aphids were observed every 24 hours and the number of mummies produced for each stage was recorded. For each parasitoid species, this procedure was replicated fifteen times (i.e. the total number of tested individuals was: 15 *A. ervi*, 750 *M. persicae*, 15 *A. abdominalis* and 750 *R. padi*). For *A. ervi*, the mean number of mummies was compared among stages using a linear model with the host instar as the main factor and the block integrated into the model (Crawley 2007), after $\sqrt{x + 0.5}$ transformation of data (Zar 1998) to fit normality and homoscedasticity requirements.

For *A. abdominalis*, the mean number of mummies produced was compared among the different stages using a linear mixed model (LMM) with the stage as the fixed factor and the block as the random factor.

3 Results

3.1 Bioassay 1: Host species

For *A. ervi*, we found significant differences in the mean number of parasitized aphids ($F_{2,28} = 75.554$, $P < 0.0001$) (Fig. 1A), percentage of parasitism ($F_{2,28} = 61.987$, $P < 0.001$) (Fig. 1B), percentage of emergence ($F_{2,28} = 26.256$, $P < 0.001$) (Fig. 1C),

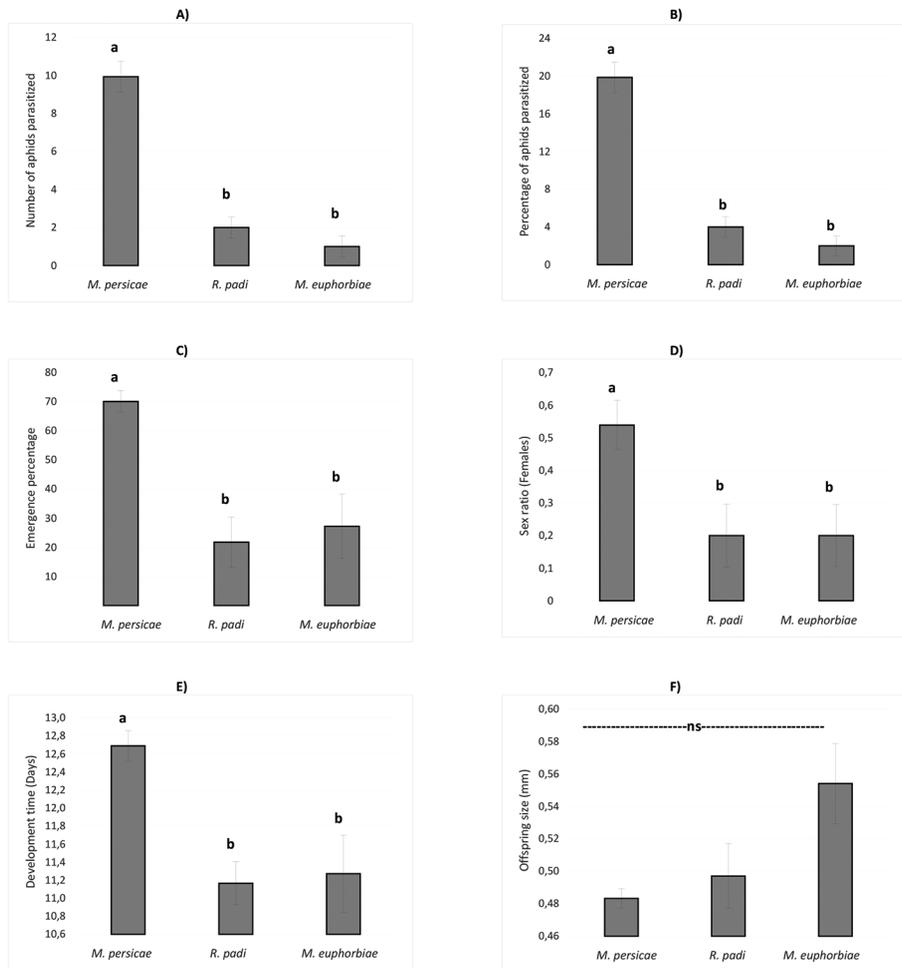


Fig. 1. Variables analyzed for the species *A. ervi*. **A)** Mean (\pm SE) number of parasitized aphids, **B)** Mean (\pm SE) percentage of parasitism, **C)** Mean (\pm SE) percentage of emergence, **D)** Mean (\pm SE) proportion of females, **E)** Mean (\pm SE) development time (in days), **F)** Mean (\pm SE) size of the progeny tibia (in millimeters). Different letters indicate significant differences among the host species ($P < 0.05$).

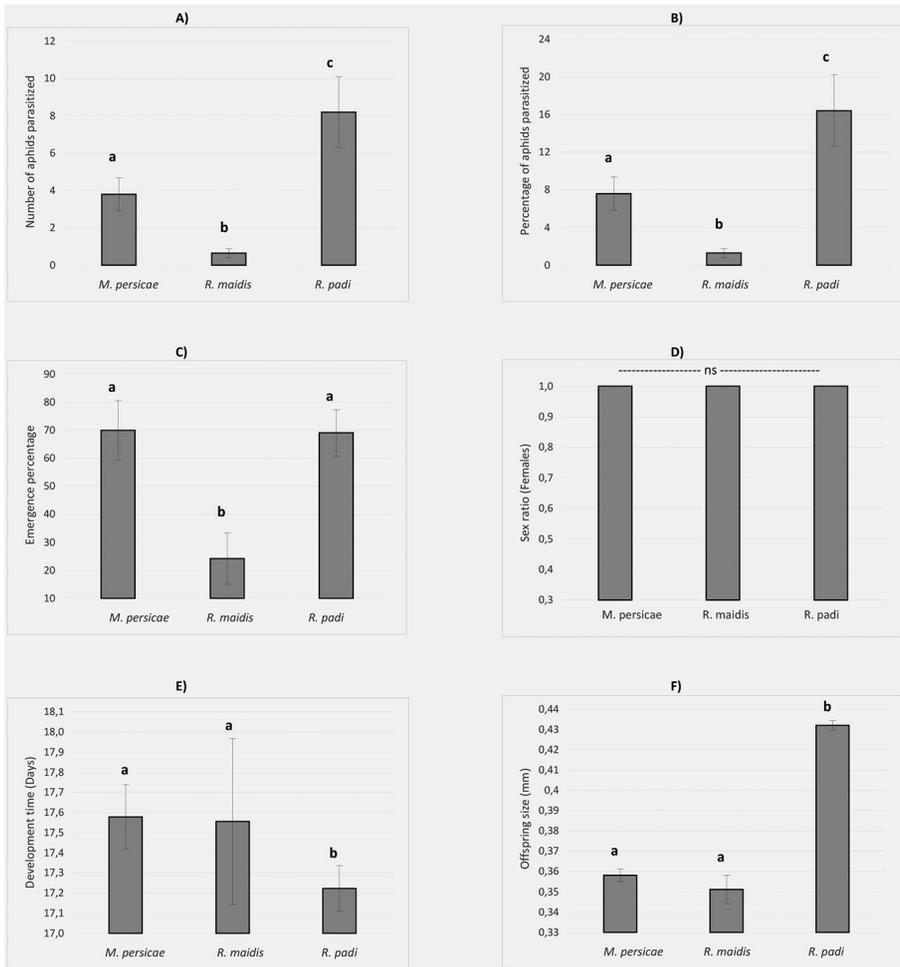


Fig. 2. Variables analyzed for the species *A. abdominalis*. **A)** Mean (\pm SE) number of parasitized aphids, **B)** Mean (\pm SE) percentage of parasitism, **C)** Mean (\pm SE) percentage of emergence, **D)** Mean (\pm SE) proportion of females, **E)** Mean (\pm SE) development time (in days), **F)** Mean (\pm SE) size of the progeny tibia (in millimeters). Different letters indicate significant differences between the host species ($p < 0.05$).

sex ratio ($F_{2,28} = 9.822$, $P < 0.001$) (Fig. 1D), and development time ($F_{2,108} = 13.098$, $P < 0.001$) (Fig. 1E). However, no significant differences were found in the size of the emerged wasps from different hosts ($F_{2,108} = 2.665$, $P = 0.074$) (Fig. 1F). In terms of mean number of parasitized aphids, percentage of parasitism, percentage of emergence, and female:male sex ratio, the parasitoid *A. ervi* had significantly higher levels in *M. persicae* than in *R. padi* and *M. euphorbiae* (Figs. 1A, B, C and D). Furthermore, development time of *A. ervi* was significantly longer in *M. persicae* than in the other

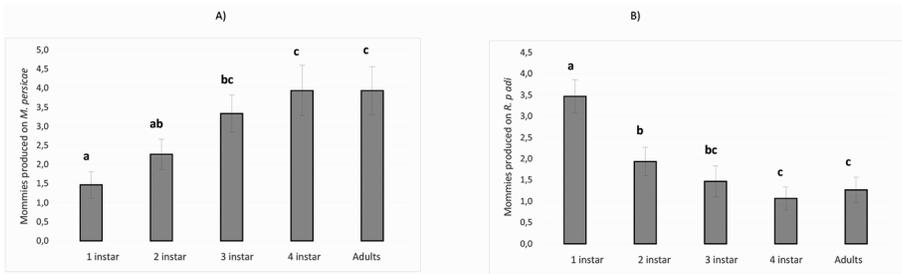


Fig. 3. Instar preference bioassay. **A)** Mean (\pm SE) number of mummies produced by *A. ervi* in each nymphal stage of the host aphid species *M. persicae*, **B)** Mean (\pm SE) number of mummies produced by *A. abdominalis* in each nymphal stage of the host aphid species *R. padi*. Different letters indicate significant differences between the host species ($p < 0.05$).

two aphid species (Fig. 1E). In contrast, the size of the *A. ervi* offspring emerging from the three aphid host species was not significantly different (Fig. 1F).

For the parasitoid *A. abdominalis*, significant differences were found for the mean number of parasitized aphids ($F_{2,38} = 16.550$, $P < 0.0001$) (Fig. 2A), percentages of parasitism ($F_{2,38} = 18.765$, $P < 0.0001$) (Fig. 2B), percentages of emergence ($F_{2,38} = 6.439$, $P = 0.003$) (Fig. 2C), development time ($F_{2,204} = 10.610$, $P < 0.001$) (Fig. 2E), and size of the progeny ($F_{2,202} = 121.270$, $P < 0.001$) (Fig. 2F). The highest percentage of parasitism was obtained in *R. padi*, and the lowest was obtained in *R. maidis* (Fig. 2B). We did not find significant differences in sex ratio because in all three aphid species, only female wasps were obtained (Fig. 2D). Regarding the percentage of emergence, more *A. abdominalis* individuals were obtained from *M. persicae* and *R. padi* compared with *R. maidis* (Fig. 2C). Development time for *A. abdominalis* was shorter in *R. padi* than in *M. persicae* and *R. maidis* (Fig. 2E). When the size of the offspring was analyzed, *A. abdominalis* was found to produce significantly larger daughters in *R. padi* compared with those obtained from *M. persicae* and *R. maidis* (Fig. 3D).

3.2 Bioassay 2: Instar preference

Each species of parasitoid showed a preference for parasitism of some host instars over others (*A. ervi*: $F_{4,56} = 5.813$, $P < 0.001$, *A. abdominalis*: $F_{4,56} = 15.598$, $P < 0.001$). *A. ervi* produced a significant larger number of mummies in the fourth nymphal stage and in adult aphids (Fig. 3A). In the third instar, the number of produced mummies was marginally larger relative to those produced in the second instar (Fig. 3A). This indicates that *A. ervi* can produce more offspring in more advanced stages of development in the aphid *M. persicae*.

In contrast to *A. ervi*, the parasitoid *A. abdominalis* produced more mummies in early stage nymphs ($F_{4,56} = 15.598$, $P < 0.001$) than in more advanced developmental stages (Fig. 3B). In particular, the number of mummies produced in first

instar nymphs was significantly higher compared with other stages of development (Fig. 3B). Mummy production followed in descending order from the second to the third nymphal stage, with the fourth nymphal stage and the adults producing the smallest number of mummies (Fig. 3B).

4 Discussion

4.1 Host species suitability

Parasitoids can attack, reproduce, and develop in different host species. However, they must choose the most optimal species for their development (Ghimire & Phillips 2014). Because of this, it is expected that female parasitoids would prefer to select hosts with high nutritional quality to ensure development of their offspring (Wang & Liu 2002). In the present study, the suitability of three aphid host species for two species of widely employed aphid parasitoids was tested. In addition, it was assessed whether some developmental stages of some host species were preferred over others. We found that each species of parasitoid developed better in different host species, as well as in distinct developmental stages. That is to say, they can exploit distinct niches, which could prove useful in aphid control programs.

Studies on host preference and quality have been conducted for the parasitoid *A. ervi* (Sidney et al. 2010, Bueno et al. 1993). Our work brings new information on the development of this parasitoid species in different host species and the way that the host species can affect some developmental traits of the parasitoid. Previous studies have reported parasitism rates for *A. ervi* as 74% in *M. euphorbiae* (Sidney et al. 2010), 16.9% or 4.1% in *M. persicae* (Kavallieratos et al. 2004, Gavkare et al. 2014), 14.3% or no parasitism in *R. padi* (Rakhshani et al. 2008, Stilmant et al. 2008, Tomanović et al. 2008). These results differ from our findings in the present study, as we found parasitism rates of 2%, 19.8%, and 4% in *M. euphorbiae*, *M. persicae*, and *R. padi*, respectively. For other species of aphids such as *A. pisum* and *A. kondoi*, reported percentages of parasitism are of 34% and 32%, respectively (Bueno et al. 1993). Considering a related measurement, we found that the greatest percentage of emergence of *A. ervi* (70%) was from the host aphid *M. persicae*, a higher rate than the 50% reported by Hofsvang & Hagvar (1975). The percentage of emergence we found in the potato aphid, *M. euphorbiae*, was 25%, in contrast to the 92% found by Sidney et al. (2010).

Regarding sex ratio of *A. ervi* progeny emerged from *M. euphorbiae*, we found a lower percentage of females (20%), compared with that reported by Sidney et al. (2010) (68%). Further, despite the assertion in the literature that sex ratio will be biased toward females in large hosts (Spitzen & van Huis 2005), we found a lower quantity of females in the largest host (*M. euphorbiae*: 1.7–3.6 mm > *M. persicae*: 1.2 a 2.5 mm and *R. padi*: 1.2–2.4 mm (Enciclop'Aphid 2016)).

The mean development time of *A. ervi* in *M. persicae* in our study was 12.6 days on average, in contrast to the 19.9 days reported by Hofsvang & Hagvar (1975).

In our analysis of the size of the emerged wasps of *A. ervi*, our results coincided with those of Sidney et al. (2010) in that *M. euphorbiae* produced wasps of the greatest size, although the size we observed (0.55 mm) was lower than that reported by them (0.73 mm). For the most part, parasitoids evaluate the size of the host as an indication of quality when choosing hosts of greater size (Sequeira & Mackauer 1992). Nevertheless, our results contradict this trend since, although *M. euphorbiae* is the largest host (1.7–3.6 mm), it produces fewer parasitoids, and, within these, few females, whereas *M. persicae* produces more parasitoids and, within these, the most females. In other words, if the production of adults and especially females is important for the fitness of the parasitoid, *M. persicae* represents a good quality host for *A. ervi*, in fact, the best among the studied species. Likewise, *M. persicae* has been reported as the most optimal host for the development of other species of parasitoids, such as *Aphidius colemani* Viereck, *Diaeretiella rapae* Stary and *Praon volucre* (Haliday) (Hymenoptera: Braconidae) (Kavallieratos et al. 2004, Silva et al. 2011).

A preference for parasitizing some species over others has also been found in the genus *Aphelinus* (Kang et al. 2012). For example, Prinsloo (2000) found that *Aphelinus* sp. nr. *varipes* (Hymenoptera: Aphelinidae) oviposited in *Diuraphis noxia* Kurdjumov, *Schizaphis graminum* Rondani, *Sitobion avenae* Fabricius, *R. padi*, and *Metopolophium dirhodum* Walker, but development was not achieved in *R. padi* and *S. avenae*. Moreover, a greater number of eggs were laid in *D. noxia*. Their results suggest that some hosts are more adequate for the parasitoid.

The percentage of parasitism of *A. abdominalis* in the hosts evaluated in the present study differed from the results found in other studies (Duarte et al. 2012), supporting the hypothesis of specialization of populations of parasitoids in accordance with their origins.

In this way, differences among populations could explain the variations of host acceptance and parasitism rates. For example, differences among mechanoreceptor sensilla (i.e. number and distribution) of female antennae could perhaps result on different rates of host location and acceptance (Isidoro et al. 2001, van Baaren, et al. 2007, Romano et al. 2016). In general, the percentage of emergence of *A. abdominalis* in *M. persicae* was high (70%), coinciding with that reported by Duarte et al. (2012) on the same species.

It should be noted that in this study, *A. abdominalis* only produced female progeny in all three host species, in contrast to the findings of Honek et al. (1998), who observed the emergence of both sexes. If the models of sex ratio presuming that female parasitoids utilize hosts of the best quality (Godfray 1994) are correct, our results would indicate that the hosts utilized were perceived by the females as being of high quality. Nonetheless, it is important to consider that *A. abdominalis* has been reported as a species that exhibits deuterotoky, and that for the most part, its progeny are females (Wahab 1985).

In general, it was observed that the host conferring substantial adaptive advantages on *A. abdominalis* was *R. padi*, indicating that it can be considered to be of higher quality than the other two species evaluated.

Overall, we found that the results obtained in this study, together with those of the cited previous studies, differed in developmental and experimental conditions, and

therefore differences in results are not surprising. However, some of these differences may be due to the parasitoids belonging to different biotypes and/or populations. With respect to this, Takada & Tada (2000) reported that they found differences in variables such as percentage of parasitism when comparing different “lines” (strains) of *A. ervi*. Likewise, it has been previously reported that *A. ervi* is a species with differences between populations, due principally to geographic isolation and their adaptation to specific hosts (Atanassova et al. 1998, Kavallieratos et al. 2004).

4.2 Instar preference

Our work confirms that both species of parasitoids are capable of parasitizing all of the stages of their aphid hosts, but have a preference for some of these stages over others (Lopez et al. 2009, Latham & Mills 2012). In general, it can be said that this study confirms previous findings of instar preference in these parasitoids, which is an important point for their optimization for practical use.

We found that *A. ervi* more frequently parasitized the last two nymphal stages and adults of *M. persicae*, a similar finding to that reported by Colinet et al. (2005), who observed a majority of parasitism in intermediate stages (corresponding to instars 3 and 4) of *M. persicae*. Likewise, this behavior of choosing intermediate stages has been observed in other hosts, such as *Acyrtosiphon kondoi* Shinji (He & Wang 2006, He et al. 2011). The preference of *A. ervi* for more advanced instars in *M. persicae* is related to improved quality. This has also been observed by He et al. (2011), who reported that *A. ervi* prefers to parasitize more advanced instars of *Acyrtosiphon pisum* (Harris). This is similar to the behavior of the parasitoid *Lysiphlebus testaceipes* Cresson (Hymenoptera: Braconidae), which prefers larger stages of *Aphis gossypii* Glover (Hopkinson et al. 2013). In addition, the females of *A. ervi* have the advantage of an absence of defensive, aggressive behavior in more advanced instars, in contrast to what has been observed in other species of aphids like *Aphis fabae* Scopoli, *Aphis glycines* Matsumura, and *A. pisum* (Ameri et al. 2014, Wyckhuys et al. 2008).

Similarly to findings in other species of parasitoids, such as *Aphelinus spiraeocolae* Evans & Schauff and *Monoctonus paulensis* (Ashmead) (Tang & Yokomi 1996, Chau & Mackauer 2000), *A. abdominalis* prefers to oviposit in the smaller developmental stages (instars 1 and 2, principally). Although in general, hosts of larger size (in this case, more advanced instars) are preferred by female parasitoids, the preference for small instars could be related to the capacity of larger aphids to escape or damage the parasitoid as a part of a defensive strategy while the parasitoids are ovipositing (Wyckhuys et al. 2008). Indeed, it has been reported that in various species of aphids, defensive behavior is more pronounced in larger stages in comparison with smaller ones (Kouamé & Mackauer 1991). In addition, and in concordance with our results, Shrestha et al. (2015) observed that *A. abdominalis* displays greater parasitism in the first stages (instars 1, 2, and 3) in *Nasonovia ribisnigri* Mosley (Hemiptera: Aphididae), in comparison with fourth instar nymphs and adults. Although it was found that *A. abdominalis* is capable of developing in all stages of *N. ribisnigri*, significantly more successful parasitism was reported in the second instar than in the fourth. In addition, it has been reported that in other species of

Aphelinus, the preference for parasitizing the first stages is frequently seen (Gerling et al. 1990, Sengonca et al. 2008, Rohne 2002). In this sense, an advantage of parasitizing younger instars stems from the fact that the immune system of the host is less developed and tends to be more susceptible to wasp venom, which is convenient for endoparasitoids like *A. abdominalis* (Stoepler et al. 2013, Brodeur & Vet 1995, Chau & Mackauer 2000). In our observations, we could see that in the first instar, the parasitoid approaches the host, becoming sufficiently close for insertion of the ovipositor while remaining out of range of the defensive movements of the aphid. Nevertheless, in the case of the third and fourth instars, the parasitoid is forced to get as close as possible while still staying out of reach of the defense systems of the aphids, this has also been seen in other species (Gerling et al. 1990). Thus, there may exist certain tendencies of small parasitoids like *A. abdominalis*, which can more easily parasitize early stages because the defenses of the aphids do not affect the parasitoid. On the other hand, Wahab (1985) reported that *A. abdominalis* oviposited more in the third stage in *Myzus ascalonicus* van der Goot (Hemiptera: Aphididae) in comparison with the other stages (first, second, fourth, and adult). However, they did not account for the quantity of mummies produced, that is to say, of effective ovipositions. In addition, the parasitoids stayed for three days in the presence of their host, so that possibly, and in the absence of more individuals of other instars, the females may have opted to oviposit in the next most numerous host stage.

5 Conclusion

In general, we can conclude that *A. ervi* developed better on the host species *M. persicae*, over the hosts *R. padi* and *M. euphorbiae*. As for *A. abdominalis*, we found that *R. padi* is a better host for its development than the hosts *M. persicae* and *R. maidis*. Regarding instar preference, we observed that *A. ervi* prefers to parasitize larger/older instars over smaller/younger ones. In contrast, *A. abdominalis* prefers to parasitize smaller/younger stages. Considering the potential of these parasitoids in biological control programs, we estimate that they could be complementary to each other, since with their differences in preferred host species and instar, they will not be competing for the same targets.

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