

Development and prey preference of *Macrolophus basicornis* (Hemiptera: Miridae) feeding on *Myzus persicae* and *Macrosiphum euphorbiae* (Hemiptera: Aphididae)

Desarrollo y preferencia de *Macrolophus basicornis* (Hemiptera: Miridae) frente a las presas *Myzus persicae* and *Macrosiphum euphorbiae* (Hemiptera: Aphididae)

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ABSTRACT: Biological characteristics, consumption, and prey preference of the predatory bug *Macrolophus basicornis* preying on *Myzus persicae* or *Macrosiphum euphorbiae* on tomato plants were studied. Incubation period of the predator's eggs was of 8 days and its nymphs presented five instars regardless of the prey consumed. All the nymphal stages showed a similar development time with the exception of the 2nd instar. There were no significant differences between the size and weight of *M. basicornis* nymphs feeding on *M. persicae* or on *M. euphorbiae*. The average rate of aphid consumption revealed significant differences between the 5th instar nymph and the females of the predator when they had no option to choose the prey. In addition, the fifth nymphal stage consumed significantly more aphids in 24 h than the other stages. The females had a high rate of predation comparable to the fifth nymphal stage. *M. basicornis* was shown to be capable of completing its cycle on preys like *Myzus persicae* or *Macrosiphum euphorbiae*. The biological characteristics and prey consumption by *M. basicornis* were strongly influenced by the prey species and size, the latter being able to influence the prey preference of the predator.

Key words: aphids, biological characteristics, consumption, *Macrolophus basicornis*, prey preference

RESUMEN: Se estudiaron las características biológicas, el consumo y la preferencia de presa del depredador *Macrolophus basicornis* usando como presas *Myzus persicae* y *Macrosiphum euphorbiae*, que se alimentan de plantas de tomate bajo condiciones controladas. El tiempo de incubación de los huevos del depredador fue de ocho días y las ninfas de *M. basicornis* presentaron cinco instares sin importar la presa consumida. Todos los estadios ninfales mostraron un tiempo de desarrollo similar, con excepción del segundo instar. No existen diferencias significativas entre las tallas y el peso de las ninfas de *M. basicornis* que se alimentaron con *M. persicae* o las que fueron alimentadas con *M. euphorbiae*. La tasa media de consumo de áfidos reveló que hay diferencias significativas para el quinto instar ninfal y las hembras del depredador cuando no tienen opción de escoger la presa. Además, el quinto estadio ninfal consumió significativamente más áfidos en 24 h que el resto. Los machos tuvieron una alta tasa de depredación en comparación con

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la hembra y baja en comparación con el quinto estadio ninfal. Los resultados muestran que el depredador *M. basicornis* es capaz de completar su ciclo con presas como *Myzus persicae* y *Macrosiphum euphorbiae*. Las características biológicas y el consumo de *M. basicornis* están fuertemente influenciadas por la especie presa, el tamaño y la presa; esta última pudiera influir en la preferencia de presa del depredador.

Palabras clave: áfidos, características biológicas, consumo, *Macrolophus basicornis*, preferencia de presa.

INTRODUCTION

Species of the genus *Macrolophus* Fieber (Hemiptera: Miridae) are polyphagous predators that are considered effective as biological control agents for many greenhouse pests, such as thrips, aphids, mites, and leaf miners, and on eggs of some lepidopteran species (1). They have been successfully used in temperate and Mediterranean Europe for augmentative biological control and many of them marketed for controlling arthropod pests in greenhouses (2).

Some investigations on the occurrence, abundance, and effect of various parasitoids and predators on tomato and other crops under greenhouse and field environments have shown the substantial importance of the species within the genus *Macrolophus* (3).

Some species, such as *Macrolophus pygmaeus* (Rambur), can successfully develop on *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae), which are important pests of eggplant (*Solanum melongena* L.) and tomato (*Solanum lycopersicum* L.). (4)

In Cuba, two species of this genus, *Macrolophus basicornis* (Stal) (5) and *Macrolophus preclarus* (Distant), have been reported associated with lepidopteran species and aphids in tobacco (*Nicotiana tabacum* L.). *Nesidiocoris tenuis* (Dist) and *Engytatus varians* (Distant) (syn. *Cyrtopeltis varians*) have been other plant bug species also found (6).

At present, the use of natural enemies as biological control agents, especially the use of predators of this genus, have been booming (7,8,9) due to the increase of the occurrence of

resistance to pesticides and the interest in environmentally sustainable agriculture. However, before polyphagous predators can be used effectively as biological control agents, it is important to obtain a robust information on their biology, predation rate, and prey preference. The latter item is relevant to the amount of energy obtained concerning search costs and consumption of prey (10). All these limitations are closely associated with the prey size and behavior.

Considering the above information, we found important to determine the biological characteristics and the consumption and prey preference of *Macrolophus basicornis* feeding on *M. persicae* and *M. euphorbiae* on tomato plants.

MATERIAL AND METHODS

Collection of specimens

Adult insects were collected from an area with tomato in Lavras (21° 14. 45' S, 44° 59.59' W, 920 m altitude), and from another area with tobacco in Ribeirão Vermelho (21° 08,596' S and 045° 03,466' W, 808 m altitude) in June and August 2012. The adults were captured with the aid of an exhauster and placed in glass test tubes (2.5 x 8.0 cm), sealed with PVC plastic film, and carried to the Biological Control Laboratory, Department of Entomology, Federal University of Lavras, Minas Gerais.

Prey rearing

The preys were reared on a leaf of *Nicandra physalodes* (L.) Gaertn, for *M. persicae*, and of *Lectuca sativa*. var *Veronica*, for *M. euphorbiae*, in 15 cm Petri dishes with a layer

of 1% water agar (5 dishes/sp.). In both cases, 20 aphid adults of each species were placed on each leaf. The dishes were covered with a paper towel with a rubber band and placed into a controlled climatic chamber (BIOD) at 21 ± 1 °C, RH 70 ± 10 % and photoperiod of 12 hours; the plates were changed twice a week.

The leaves for the aphid rearing were disinfected with a solution of 1 % sodium hypochlorite for 5 min, washed with tap water, and finally rinsed with distilled water for 10 min. This guaranteed high quality lettuce and nicandra leaves.

Predator rearing. Once in the laboratory, the adult predators were released in acrylic cages (30x30x60 cm) containing *Nicotiana tabacum* L. plants with approximately 25 cm of height, which were used as the oviposition substrate. The predators were fed with eggs of *Anagasta kuehniella* (Zeller) *ad libitum*. The climatic conditions for the rearing were establish a temperature of 25 ± 2 °C, a relative humidity of 70 ± 10 %, and a photoperiod of 12 hours.

The plants with the adult predators remained in their cages for seven days. Afterward, plants with new eggs were placed in cages. Once the nymphs were born, eggs of *A. kuehniella* were offered twice a week until the emergence of new adults.

Egg embryonic development of the predator *Macrolophus basicornis*

To obtain *M. basicornis* eggs, ten couples of the predator were placed in glass containers (1,7 liters) containing a small tomato plant (*Solanum lycopersicum* L). cv. Santa Clara with two pairs of leaves, and a mixture of 1st to 3rd instar nymphs of *M. persicae* or *M. euphorbiae* was used as food. The glass containers were sealed with a voile-type fabric and kept in a room at 28 ± 2 °C, RH 70 ± 10 %, and photoperiod of 12 hours. After 24 hours and every day until the death of adults, the plants with eggs were replaced by new ones, and the number of eggs counted under a stereo microscope. Finally, the roots of these plants

were wrapped with cotton, placed in Petri dishes (15 cm), and sealed with a PVC film.

The embryonic period length was evaluated by observations made on egg development and survival; the number of eggs and percentage of fertile eggs were also taken into account. The female ovipositional period was estimated from the first to the last oviposition day.

Development and reproduction of *M. basicornis* preying on *M. persicae* and *M. euphorbiae* on tomato plants

Plants of tomato cv. Santa Clara with approximately 25 cm of height were placed into a rearing cage of *M. basicornis*. After 24 hours, they were moved to a rearing cage and placed into a controlled climatic chamber (BIOD) at 28 ± 1 °C, RH 70 ± 10 % and a 12 hour photoperiod. They were examined daily until appearance of nymphs. Then, 100 newly hatched nymphs were individually placed on 5 cm discs of *S. lycopersicum* leaves located on a 1 % water agar layer in 5 cm dishes. They were changed twice a week. The nymphs were daily fed with five 1st instar nymphs of *M. persicae* or *M. euphorbiae* under the same previously described conditions. They were daily examined for instar changes indicated by the presence of exuvias. Duration time of each instar, nymph mortality, and sex ratio were recorded.

Once nymphs turned into adults, 20 couples of 24 hour- old *M. basicornis* were kept in glass jars (1.7 L) containing a small tomato plant with three leaves as ovipositional substrate under the same conditions in which the nymphs were born. Each predator couple were fed daily with 10 nymphs of *M. persicae* or *M. euphorbiae*. The oviposition substrate was changed daily, and the plants placed in 15 cm Petri dishes with the roots covered with moistened cotton until nymph emergence.

The number of eggs layed, mortality of adults, and number of nymphs emerged were recorded daily. From the data, pre-oviposition and oviposition periods, fecundity (daily and

total eggs / female) and longevity were attained.

Measuring and weighing *M. basicornis*

The size of 20 *M. basicornis* nymphs from the 1st to the 5th instar were determined. They were measured with a reticle coupled to the micrometrical stereomicroscope (Olympus SZ40). The width of the head (head capsule) (distance between the outer edges of the compound eyes) was also measured.

Twenty 4th and 5th instar nymphs were weighed individually using an analytical balance (Shimadzu, AW 220). They were weighed one day after each instar change.

Assessment of the consumption capacity of *M. basicornis* predation feeding on *M. persicae* or *M. euphorbiae* on tomato plants

The foraging arena consisted of a leaf of *S. lycopersicum* cv. Santa Clara with mixed nymphs from the 1st to the 4th instar of *M. persicae* and *M. euphorbiae* placed on a 1 % water agar layer in a 5 cm Petri dish

Without chance to choose. One predator female up to one day old and one 5th instar predator nymph were collected from the laboratory maintenance predator culture and kept individually in a glass tube with moistened cotton and without access to food for 24 hours prior to the experiments. Then, they were released into 9 cm Petri dishes each containing 10 mixed instar nymphs of *M. persicae* or *M. euphorbiae*. After 24 hours, the consumed preys were determined.

With chance to choose. In this case the methodology above described was followed, but 40 mixed instar nymphs were placed on this occasion, that is 20 of each prey, *M. persicae* and *M. euphorbiae*. After 24 hours the number of consumed preys were determined.

The trials were conducted in a controlled climatic chamber (BIOD) at 28± 2 °C, 70 ±10 % RH and 12h photophase.

The number of aphids consumed per 24h were used to evaluate the predator's preference index determined by Manly's index, which

considers the depletion of prey due to predation (12). Manly's index is given by:

$$\beta_j = \frac{\ln\left(\frac{r_j}{A_j}\right)}{\sum_{j=1}^4 \ln\left(\frac{r_j}{A_j}\right)}, j = 1,2,3,4$$

where:

β_j - Manly's Beta for aphid instar j

r_j - the number of individuals in aphid instar j not select by the predator

A_j - the number of individuals in aphid instar j available to the predator

Statistical Analysis. All the data were analyzed using Simple ANOVA. The means were compared using the post hoc Tukey test ($P \leq 0.01$). The statistical analyses were conducted using the statistical software package InfosStat version 2.0 (11).

RESULTS AND DISCUSION

Nymphal development

Like all predators of the genus *Macrolophus*, *Macrolophus basicornis* has a zoophytophagous feeding behavior. This means that he can feed on both, plant and prey. This behavior could be observed during the experimental work due to the appearance of necrotic rings in the feeding areas similar to those reported by Urbaneja *et al.* (2).

Regardless of the prey supplied, the predator egg incubation period on tomato plants was eight days. Similarly, the predator post-embryonic development included five nymphal stages feeding on each prey, each stage with a maximum duration of eight days. However, when *M. persicae* was supplied as a prey, an extra day was added to predator's fifth nymphal stage. Nevertheless, when *M. euphorbiae* was supplied as a prey, the predator's first nymphal stage reached eight days, consequently resulting in a longer nymphal development. With both preys, the stadiums of interest (fourth and fifth) were achieved in three days. The development and life cycles of the predator showed significant differences with both preys, being longer when

M. euphorbiae was the prey. In both cases, the life cycle duration was longer than one month. Therefore, considering this time and depending on the prey consumed, several annual predator generations per year can be possible, ranging from ten (on *M. persicae*) to seven (on *M. euphorbiae*). (Table 1)

Regarding the sexual coefficient, it was always favourable to females regardless of the prey supplied. This is a very convenient fact because of their role for preserving the species. They also had a higher rate of consumption. Females and males reached adulthood at the same time, so there were no difference in the period of development between the sexes. The mean longevity of the adult predator feeding on *M. persicae* was 8 days, however longevity of those adults kept feeding on *M. euphorbiae* was longer (25 days). This differences could be caused by the quality and size of the prey offered, maybe along with the predators prey preference.

The sex ratio always proved favorable to females regardless of the prey supplied, although, as Table 1 shows, it was slightly higher when the predator fed on *M. euphorbiae*. Females and males reached adulthood at the same time, so there was no difference in the period of development between the sexes.

The prey species also influenced on the reproductive parameters of *M. basicornis*. A higher oviposition period and daily fecundity of *M. basicornis* were observed when the prey was *M. euphorbiae* than when the prey was *M. persicae*. The predator's total fecundity showed the same behavior with an increase of ten eggs when it fed on *M. euphorbiae* (Table 2). These results could corroborate the above proposed influence of prey quality, size and predator's preference on its development and reproduction.

Not much information on development and reproduction of this species preying aphids is available, but there is a lot on other species in the same genus. For example, Hatherly *et al.* (13) observed a complete development of *M.*

caliginosus on *M. persicae* on different host plants. The predator sex ratio varied from 0.44 on Chinese cabbage to 0.47 on oil seed rape, and mortality lower than 50 % in both case. However, on each crop, the oviposition period was 5 and 10 days, the progenies per female were 45 and 20 nymphs/female with a survival of 60 and 65 %.

In tobacco, the development period of the predator *M. caliginosus* feeding on *M. persicae* was 17 days with 94 % of nymphal survival (14). On the other hand, Alvarado *et al.* (15) evaluated *A. gossypii* on cucumber and *M. euphorbiae* on tomato for their suitability as prey items for the development of *M. caliginosus* nymphs. Development of nymphs was shorter with *M. euphorbiae* on tomato than with *A. gossypii* on cucumber.

The results obtained with both preys studied in the present work differed from those achieved by other authors. The development period was longer than in other species such as *M. caliginosus* and *M. pygmaeus*. The differences between the nymphal development, sex ratio, fecundity and the rest of the biological characteristics could be influenced by the host plant and the prey. For instance, the incubation period could be influenced by the type and quality of the prey offered. Prey quality could affect the number of eggs laid (16). According to Urbaneja *et al.*, (17), the host plant is another factor influencing development period and survival.

In species such as *M. caliginosus*, any testing of host range with the proposal of informing a risk assessment of its establishment potential is further complicated by the zoophytophagous nature of the predator; that is, the capability of the insect to feed on both the plant and arthropod prey (18). In such cases, it is necessary to assess the survival, development, and reproduction of the species in the absence of any prey.

In the case of *M. basicornis*, the offered preys were low quality foods. There was not marked influence on weight in none of the

TABLE 1. Biological parameters of *M. basicornis* feeding on *M. persicae* and *M. basicornis* on *Solanum lycopersicum* L. / Parámetros biológicos de *M. basicornis* alimentados con *M. persicae* y *M. basicornis* sobre *Solanum lycopersicum* L.

Prey	Nymphal development (days)					Incubation period (days)	Development period (days)	Life cycle (days)	Sex coef,
	X±Es(X)								
	1 st instar	2 nd instar	3 th instar	4 th instar	5 th instar	X±Es(X)	X±Es(X)		
<i>Myzus persicae</i>	3.86 ±1.14 a	8±0.00a	20.1±0.45 a	3.82± 1.55a	3.15± 2.50a	8±0,00a	20.1±0,45 a	36.45 a	0.427
<i>Macrosiphum euphorbiae</i>	8±1.44 b	8±0.00a	31±7.71 b	3.53±2.98a	3.9±2.85a	8±0.00a	31±7.71 b	47.05 b	0.466

*Means in the same column with letters in common are not significantly different.

TABLE 2. Reproductive parameters of *M. basicornis* feeding on *M. persicae* and *M. euphorbiae* on tomato plants. / Parámetros reproductivos de *M. basicornis* alimentados con *M. persicae* y *M. euphorbiae* sobre plantas de tomate.

Prey	Ovoposition period (days)	Daily fecundity	Total fecundity
	X±Es(X)	X±Es(X)	X±Es(X)
<i>Myzus persicae</i>	4.03±2,03 a	0.48±0.58 a	2.2±2.88 a
<i>Macrosiphum euphorbiae</i>	8.2±4.24 b	1.63±1.05b	12.15±8.22 b

* Means in the same column with letters in common are not significantly different.

instars, even when the size varied slightly in the 5th instar with *M. euphorbiae* as the prey (Table 3). Despite no significant differences were observed in the nymph size of *M. basicornis* feeding on *M. persicae* or *M. euphorbiae*, there were significant differences in the weight of the 5th instar nymphs, which were heavier when *M. euphorbiae* was the prey.

Aphid consumption

Usually, prey suitability and the innate prey preference of a predator are correlated because natural selection acts to optimize fitness (19). Additionally, if the optimal prey diet maximizes the

predator's net energy gain, the prey will be either accepted or rejected based on whether it fits within this optimal diet (20). Consequently, predators tend to be more selective in environments with high prey availability and less selective in environments where prey is scarce (21).

Observations on the mean aphid consumption rate revealed significant differences in both prey species for 5th instar nymphs and females when they were offered separately; however, no significant differences were observed when both species were offered together.

In addition, the 5th instar nymphal stage significantly consumed more aphids in 24 h than the other stages. Females had a higher predation rate compared with the 5th instar nymphal stage. Comparing the two prey species, the 5th instar nymph of *M. basicornis* consumed more nymphs of *M. persicae* than of *M. euphorbiae* (18.96 and 15.08 nymphs in 24 hours); however, the *M. basicornis* female consumed more nymphs of the aphid *M. euphorbiae*. (Table 4)

When the two prey species were offered together, no significant differences were found between the 5th instar nymph and the female of the predator, neither between Manly's preference index for each prey species in any stage evaluated or between stages.

Eubanks and Denno (22) suggest that not always a prey may be preferred. Prey size is another possible criteria involved in predator selection, and it can play a role in the predator preference under the experimental conditions in which is evaluated. According to Lins (23), the encounter rate can be higher in larger preys.

In similar studies, Perdikis *et al.*, (39) showed that the voracity of females and 5th instar nymphs of the predator *M. pygmaeus* on nymphs of *M. persicae* is higher than the voracity of other stages of the predator. The predation rate of *M. pygmaeus* was higher on *M. persicae* than on *Macrosiphum euphorbiae*, whereas its preference depended on the aphid species and the ratio of the available instars.

The predation rate per hour of *M. pygmaeus* in the light and dark phases was examined by Perdikis *et al.*, (24) in different plants. It was significantly higher in the dark than in the light phase. On the other hand, *M. pygmaeus* showed a strong preference for small preys. According to this author, the preference showed by *M. pygmaeus* for particular prey instars might also be valuable in the effort to explore its functional response under more realistic, mixed prey experiments.

Therefore, observed modifications of the

predatory behavior in mixed prey assemblages may have considerable consequences for population dynamics and regulatory mechanisms acting in predator-prey natural systems.

For predators, prey preference usually refers to active selection or else to the prey selectivity that is exhibited when they are foraging in mixed prey patches. Prey preference is determined not only by prey characteristics such as size, vulnerability, mobility, and caloric value, but also by predator-prey encounter rates (25).

According to the foraging theory, a predator makes the decision whether to attack an available prey type of low quality or shift to a patch of a more preferable prey type. Correspondingly, when a high quality prey is available, a low quality prey is most likely to be ignored (26). This implies that a predator is able to rank prey types according to their suitability, and that prey selection by preference is based on a mechanism of prey discrimination (11).

The ability to locate and utilize a wild prey is another essential requirement for the long term establishment of such non-native species. However, while the 'host range' is regarded as a critical component for rearing nonnative biocontrol agents, there is not a commonly agreed methodology as yet, and it is recognized that such methods, as proposed (27), require further refinement.

Our results are very similar to those obtained by other authors. *M. basicornis*, as the other *Macrolophus* spp., showed a strong preference for *M. persicae*. Prey preference can be influenced by the host plant on which the prey was feeding or their volatile emissions. The biological characteristics and the consumption of *M. basicornis* feeding on different preys are strongly influenced by the prey species and size and the prey host plant, and the latter can influence prey preference of *M. basicornis*.

TABLE 3. Standard parameters for *M. basicornis* feeding on *M. persicae* and *M. euphorbiae*. / Parámetros estándares para *M. basicornis* alimentado con *M. persicae* y *M. euphorbiae*

Prey	Size (mm)					Weight(mg)	
	X±Es(X)					X±Es(X)	
	Nymph1	Nymph2	Nymph3	Nymph4	Nymph5	Nymph4	Nymph5
<i>Myzus persicae</i>	0.02±0a	0.24±0,05a	0.31± 0a	0.35± 0a	0.40±001a	0.63±0.08a	1.01±0.155 a
<i>Macrosiphum euphorbiae</i>	0.02±0a	0.24±0.05a	0.30±0a	0.35±0a	0.40±0.01a	0.67±0.13a	1.16±0.13 b

* Means in the same column with letters in common are not significantly different.

TABLE 4. Predation rate of *Macrolophus basicornis* feeding on *Myzus persicae* and *Macrosiphum euphorbiae*. / Tasa de depredación de *M. basicornis* alimentado con *M. persicae* y *M. euphorbiae*.

Stage	Predation rate (nymphs/24h)			
	Separately		Together	
	<i>M. persicae</i>	<i>M. euphorbiae</i>	<i>M. persicae</i>	<i>M. euphorbiae</i>
5th instar nymph	18.96±1.0 b	15.08±1.00a	4.10±0.90a	4.43±0.90a
Female	11.92±0.83 a	16.28±0.83b	11.71±0,65a	11.29±0.65a

* Means in the same column letters in common are not significantly different.

TABLE 5. Manly's preference index for *M. basicornis* feeding on *M. persicae* and *M. euphorbiae*. / Índice de preferencia de Manly para *M. basicornis* alimentado con *M. persicae* y *M. euphorbiae*.

Stage	Manly's preference index	
	<i>M. persicae</i>	<i>M. euphorbiae</i>
5th instar nymph	0.17 ±0.93 a	0.17±0.17 a
Female	0.16±0.11 a	0.17±0,21a

* Means in the same column with letters in commons are not significantly different.

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