

# Evaluation of *Artemia* sp. (Branchiopoda, Artemiidae) as an alternative prey for laboratory rearing of *Tupiocoris cucurbitaceus* (Hemiptera: Miridae), a predator of horticultural pests

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## SUMMARY

*Tupiocoris cucurbitaceus* (Spinola) (Hemiptera: Miridae) is a predator of whiteflies (Hemiptera: Aleyrodidae) present in several American countries. Rearing this bug requires the use of plants as ovipositional substrate and a prey to feed on to complete its development. *Artemia* sp. (Branchiopoda, Artemiidae) cyst, a crustacean tested for the laboratory rearing of other hemipteran species, could be used as food, being less expensive than the species commonly employed (*Sitotroga cerealella* [Olivier] [Lepidoptera: Gelechiidae]). The objective of the present study was to determine nymphal developmental time, nymphal mortality, fertility, and sex ratio of *T. cucurbitaceus* fed on *Artemia* sp. cysts. Three diets were used during the rearing of three generations: *S. cerealella* eggs, *Artemia* sp. cysts and a mix of both species. The nymphal developmental time (11 to 13 days), nymphal mortality (40 to 66%), and adult fertility (74 to 101 nymphs / female) were similar for all diets. In all cases, the adults showed a high female proportion (0.67-0.97). In conclusion, *Artemia* sp. cysts are suitable for *T. cucurbitaceus* laboratory rearing, at least for three generations.

**Keywords:** biological control, predatory bug, brine shrimp cysts, laboratory rearing, food resource.

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## RESUMEN

*Tupiocoris cucurbitaceus* (Spinola) (Hemiptera: Miridae) es un predador de moscas blancas (Hemiptera: Aleyrodidae) presente en varios países de

América. La cría de este insecto requiere el uso de plantas como sustrato de oviposición y una presa como alimento para completar su desarrollo. Cistos de *Artemia* sp. (Branchiopoda, Artemiidae), crustáceo evaluado para la cría en laboratorio de otras especies de hemípteros, podría utilizarse como alimento alternativo, siendo menos costoso que el empleado habitualmente (huevos de *Sitotroga cerealella* [Olivier] [Lepidoptera: Gelechiidae]). El objetivo del presente trabajo fue determinar el tiempo de desarrollo y la mortalidad ninfal, la fertilidad y la proporción de sexos de *T. cucurbitaceus* alimentados con cistos de *Artemia* sp. Se utilizaron tres dietas durante la cría de tres generaciones: huevos de *S. cerealella*, cistos de *Artemia* sp. y una mezcla de ambas especies. El tiempo de desarrollo ninfal (11 a 13 días), la mortalidad ninfal (40 a 66%) y la fertilidad (74 a 101 ninfas / hembra) fueron similares con todas las dietas. En todos los casos, los adultos mostraron una alta proporción de hembras (0,67-0,97). En conclusión, los cistos de *Artemia* sp. son adecuados para criar *T. cucurbitaceus* en laboratorio, al menos durante tres generaciones.

**Palabras clave:** control biológico, chinche predatora, cistos de *Artemia*, cría en laboratorio, recurso alimenticio.

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## INTRODUCTION

Whiteflies *Trialeurodes vaporariorum* (Westwood) and *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) are worldwide pests. They cause significant reductions in crop yield through two types of damage: by feeding directly on plant leaves and by producing honeydew, on which sooty mold develops reducing photosynthesis and respiration surface. In addition, whiteflies serve as vectors to a variety of plant viruses that severely affect crops (Byrne and Bellows, 1991; Tellez et al., 2017).

Biological control agents like coccinellid beetles, lacewings, parasitic wasps, predatory heteropterans, and predatory mites have become commercially available worldwide to reduce whiteflies damage (Pilkington et al., 2010; van Lenteren, 2012; Messelink et al., 2014). Some of the major natural enemies of whiteflies are predator bugs of the subfamily Dicyphinae, family Miridae (Peri et al., 2009; Perdikis et al., 2011; Moreno-Ripoll et al., 2012; Abd-Rabou and Simmons, 2014; Silva et al., 2016). At present the species used against these and other pests in conservative/augmentative

biological control strategies are: *Macrolophus pygmaeus* (Hemiptera: Miridae) (Rambur) (before *M. caliginosus*) (Martínez-Cascales et al., 2006; Sánchez et al., 2006; Franco Villamizar, 2010), *Dicyphus tamaninii* (Wagner) (Gessé Sole, 1992; Lucas and Alomar, 2002; Arnó et al., 2005; Agustí and Gabarra, 2009a, 2009b; Castañé et al., 2009) and *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) (Perdikis et al., 2008; Sylla et al., 2018). In especial, *M. pygmaeus* and *N. tenuis* are available as commercial products (De Puyssseleyr et al., 2013). In addition to the use of these species against whiteflies, they can also attack thrips, aphids, mites, and several lepidopteran species including *Tuta absoluta* (Meyrick) (Perdikis et al., 2008; Urbaneja et al., 2009; Castañé et al., 2011; Perdikis et al., 2011).

The mirid *Tupiocoris cucurbitaceus* (Spinola) is a predator collected in Argentina on tomato crops affected by *T. vaporariorum* (Del Pino et al., 2009). This bug was also found on several wild and cultivated plant species (Carpintero and Carvalho, 1993; Carpintero, 1998, 2004), and in other American countries (Carvalho, 1947; Carvalho and Ferreira, 1972; Carvalho and Afonso, 1977; Ferreira

et al., 2001; Ferreira and Henry, 2011). As a species of the Dicyphini tribe, *T. cucurbitaceus* is primarily a predaceous bug (Orozco et al., 2012), although its phytophagy has been described by Carpintero (1998) and Ohashi and Urdampilleta (2003). Previous studies have analyzed the main biological characteristics of *T. cucurbitaceus* feeding on whiteflies nymphs and *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) eggs (López et al., 2012; Orozco Muñoz et al., 2012). This predator is also capable of consuming other pests like aphids, mites and eggs and neonate larvae of *T. absoluta* (Cagnotti et al., 2016; López et al., 2019; Cagnotti et al., 2021; van Lenteren et al., 2021).

*Corcyra cephalonica* (Stainton), *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and *S. cerealella* eggs are the main food supply used worldwide to rear several species of natural enemies such as egg parasitoids, lacewings, coccinellid beetles and predatory heteropteran (Parra, 1997; Put et al., 2012; Pomari-Fernandes et al., 2016). Cysts of the crustacean brine shrimp *Artemia* sp. (Branchiopoda, Artemiidae) were tested in crop fields as food supply for *N. tenuis* (Owashi et al., 2020) and also in laboratory rearing for other predatory species like *Harmonia axyridis* (L.) (Coleoptera: Coccinellidae) (Hongo and Obayashi, 1997), *Iphiseius degenerans* (Berlese) (Acari: Phytoseiidae) (Vantornhout et al., 2004), *M. pygmaeus* (Castañé, Quero and Riudaverts, 2006) and *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) (Arijs and De Clercq, 2001). The use of *Artemia* sp. cysts as a suitable supplemental food for ladybirds (Seko et al., 2019), mirids (Messelink et al., 2015; Oveja et al., 2016) and predatory mites (Leman and Messelink, 2015; Vangansbeke et al., 2016) in crop fields has also been assessed. In addition, some studies suggested that these cysts used as a complementary food resource could be beneficial for the fertility and survival of predatory mirids and ladybugs released in crops (Messelink et al., 2015; Oveja et al., 2016; Seko et al., 2019).

*Tupiocoris cucurbitaceus* is a natural enemy that is currently used in inoculative releases to control whiteflies in greenhouses (Polack et al., 2017). Therefore, the rearing and availability of this bug is an important issue for successful biological control programs. Usually, *E. kuehniella* or *S. cerealella* eggs are used as food to rear this natural enemy although this substrate is very expensive and sometimes difficult to obtain (Arijs and De Clercq, 2001; Castañé et al., 2006; Vandekerkhove et al., 2008). Thus, *Artemia* sp. cysts could be an alternative and less expensive food supply to rear *T. cucurbitaceus*.

The objective of the present work was to evaluate

the main biological characteristics (nymphal development time and survival, fertility and sex ratio) of individuals of *T. cucurbitaceus* feeding on three different diets over three generations under laboratory conditions. This knowledge is useful to evaluate the viability of *Artemia* sp. cysts as an alternative diet to rear this predator.

## MATERIALS AND METHODS

### Insects rearing

Adults of *T. cucurbitaceus* used in the experiments were obtained from the colony reared in the Insectario de Investigaciones para Lucha Biológica (Insectarium for Biological Control Research), Instituto de Microbiología y Zoología Agrícola (IMYZA, Institute for Agricultural Microbiology and Zoology), Instituto Nacional de Tecnología Agropecuaria (INTA, National Institute of Agricultural Technology). They were originally collected in February 2009 on tomato crops in Buenos Aires and identified by Diego Carpintero (Museo Argentino de Ciencias Naturales, Buenos Aires). Since then, the colony was refreshed each spring-summer with insects from local greenhouses. The bugs were maintained on tomato (*Solanum lycopersicum* L.) and tobacco (*Nicotiana tabacum* L.) (Solanaceae) plants and fed on *S. cerealella* eggs that were reared according to Méndez et al. (2016).

### Development time and mortality of nymphs in the generation 0 (F0)

A piece of a tobacco leaf was placed in a Petri dish with an agar base (experimental units, e.u.). The agar was prepared with 2% Benomyl (methyl-1-butylcarbomyl-2-benzimidazole carbamate: Capxan F®) in a concentration of 10ml l<sup>-1</sup>. The tobacco leaf was cut into a circle maintaining the central rib and at least one edge. Then it was sprayed with Nystatin (C<sub>47</sub>H<sub>75</sub>NO<sub>17</sub>; Nystatin Denver Farma®) prepared in a concentration of 1ml/250ml. Capxan and Nystatin were used to prevent fungal development.

In order to obtain the first tested generation (F0), four adults (3 ♀ and 1 ♂) of *T. cucurbitaceus* from the insectary were placed in each e.u. and fed on *S. cerealella* sterile eggs *ad libitum* during 48 hours. After this, the dishes (n= 30) were kept in a climate-controlled chamber at T: 25.3 ± 0.6 °C, R.H.: 43 ± 4% and a 12L: 12D photoperiod until the emergence of the 1<sup>st</sup> instar nymphs of F0 (10-15 days).

Once nymphs emerged (F0), they were transferred all together to a new e.u. prepared as described above and randomly assigned to one of the following three diets (*ad libitum*): diet S: *S. cerealella* sterile eggs used as control (routine rearing); diet A: *Artemia* sp. cysts (acquired from a pet supplies trade), and diet M: a mixed diet consisting of *S. cerealella* eggs and *Artemia* sp. cysts (1:1). Ten Petri dishes (e.u.) per treatment (diet) were used. Number of nymphs (F0) obtained was  $25.50 \pm 3.23$  (diet S),  $24.10 \pm 4.48$  (diet A) and  $21.60 \pm 3.44$  (diet M) (mean  $\pm$  standard error). The diet was supplied *ad libitum* on pieces of cardboard and replaced every 48 hours. The number of nymphs 1 and the number of adults were monitored in each replicate daily. The percentage of nymphal mortality was estimated as  $(1 - (\text{number of adults obtained} / \text{initial number of nymphs 1} / \text{replicate} / \text{treatment})) * 100$ . For each replicate, the mean nymphal development time was estimated from the developmental time of all nymphs emerging on it.

### Fertility of generation 2 (F2), nymphal mortality and sex ratio of generation 3 (F3)

The total of the adults (F0) emerged in the 10 Petri dishes per treatment was, diet S: 81 adults (55 ♀ and 26 ♂, sex ratio 0.68); diet A: 72 adults (45 ♀ and 27 ♂, sex ratio 0.62), and diet M: 67 adults (41 ♀ and 26 ♂, sex ratio 0.61). They were transferred to tobacco plants placed in acrylic cages (50 cm x 30 cm x 30 cm) in order to allow the next two generations to develop. Two cages per treatment were used with two or three tobacco plants inside as oviposition substrate to avoid overcrowding. The adults of the F0 and F1 were supplied with the same food they received as nymphs (i.e., S, A, or M). The diets were offered *ad libitum* on pieces of cardboard and replaced every 48 hours. To avoid the proliferation of fungi, food remains were removed using a hand aspirator. To avoid any overlap between the adults of the successive generations, the F0 and F1 adults were maintained in the cages during two weeks after their emergence (period of highest fertility) and then removed. The assay was carried out at T:  $25 \pm 2$  °C, R.H.: 40-60% and a 12L: 12D photoperiod.

Once the F2 adults were obtained in the rearing cages described above, seven couples newly emerged per treatment were individually exposed to a piece of tobacco leaf in a Petri dish as previously described. The piece of leaf was replaced three times at four days intervals or until the female died, whatever happened first. These pieces of tobacco leaves were kept in a climate controlled chamber

at T:  $25.3 \pm 0.6$  °C, R.H.:  $43 \pm 4$ % and a 12L: 12D photoperiod until the emergence of the F3 nymphs. Fertility of F2 was estimated as the number of F3 first instar nymphs / female / treatment, adding up the fertility of each period of female lifespan.

Since the number of nymphs obtained in the second leaf exposed to F2 parents (between days five and eight of their lifespan) was the highest for all the pairs and all the diets, the nymphs corresponding to this leaf per each pair were transferred to new tobacco plants inside an acrylic cage and fed on the corresponding diet to obtain the F3 adults. Then, the nymphs completed their development until reaching the adult stage in five individual tobacco plants per diet.

The percentage of nymphal mortality of the F3 generation was estimated as  $(1 - (\text{number of adults obtained} / \text{initial number of nymphs 1} / \text{replicate} / \text{treatment})) * 100$ .

F3 adults were sexed and sex ratio was estimated as number of females / total number of individuals / plant.

### Statistical analysis

The mortality of the F0 and F3 nymphs, the fertility of F2 and the sex ratio of the F3 (these data were previously transformed using square root arcsine function in order to fit ANOVA assumptions) were analyzed by one-way ANOVA with three levels (S, A and M).

Means were compared using the Tukey test at the 0.05 level. Since the nymphal developmental time of the F0 data did not meet the ANOVA assumptions, a non-parametric (Kruskal-Wallis test) analysis was performed. Software Statistix 7.0 was used for the statistical analysis.

## RESULTS AND DISCUSSION

Predatory nymphs of the first generation developed successfully in all the diets tested. There were no significant differences in the developmental time of F0 nymphs among diets (K-W= 3.22, df= 28,  $P= 0.20$ ) (Table 1), reaching the adulthood after 11 to 13 days. This value was slightly lower than those recorded for this predator feeding on *S. cerealella* eggs (López et al., 2012), *B. tabaci* nymphs (Orozco Muñoz et al., 2012) and *E. kuehniella* eggs (Burla et al., 2014). Neither did other authors find differences in the developmental time of other mirid species, such as *M. pygmaeus* (Castañé et al., 2006) and *Dicyphus hesperus* (Knight) (Miridae) (Labbé et al., 2018) when they used *Artemia* sp. cysts as

**Table 1.** Biological attributes of *Tupiocoris cucurbitaceus* feeding on different diets (mean  $\pm$  standard error)

BIOLOGICAL ATTRIBUTE	DIET		
	<i>Sitotroga cerealella</i> eggs	<i>Artemia</i> sp. cysts	Mix ( <i>Artemia</i> sp. cysts + <i>Sitotroga cerealella</i> eggs)
Nymphal developmental time F0 (days)	11.79 $\pm$ 0.73 <b>a</b>	12.80 $\pm$ 0.22 <b>a</b>	13.03 $\pm$ 0.28 <b>a</b>
Nymphal mortality F0 (%)	66.88 $\pm$ 7.15 <b>a</b>	61.82 $\pm$ 8.54 <b>a</b>	64.66 $\pm$ 7.15 <b>a</b>
Fertility F2 (nymphs / female)	74.60 $\pm$ 22.07 <b>a</b>	85.28 $\pm$ 19.86 <b>a</b>	101 $\pm$ 15.04 <b>a</b>
Nymphal mortality F3 (%)	44.82 $\pm$ 9.75 <b>a</b>	43.22 $\pm$ 9.28 <b>a</b>	44.66 $\pm$ 8.98 <b>a</b>
Sex ratio F3 (females/[females+males])	0.97 $\pm$ 0.93 <b>a</b>	0.88 $\pm$ 0.07 <b>ab</b>	0.67 $\pm$ 0.10 <b>b</b>

Means followed by the same letter within rows are not significantly different ( $p > 0.05$ ).

an alternative food supply to lepidopteran eggs. However, Vandekerkhove et al. (2008) registered a higher nymphal development time for *M. pygmaeus* using non-hydrated and non-decapsulated *Artemia* sp. cysts instead of *E. kuehniella* eggs, suggesting that the young nymphs had problems to penetrate the *Artemia* capsules. Moreover Owashi et al. (2020) found nymphs of *N. tenuis* feeding on *E. kuehniella* eggs developed more quickly than those feeding on dry or hydrated cysts of *Artemia* sp., while nymphs feeding on dry cysts developed significantly faster than those feeding on hydrated cysts. In our study, even though the cysts were not hydrated or decapsulated, *T. cucurbitaceus* did not seem to have had problems to penetrate the *Artemia* sp. capsules.

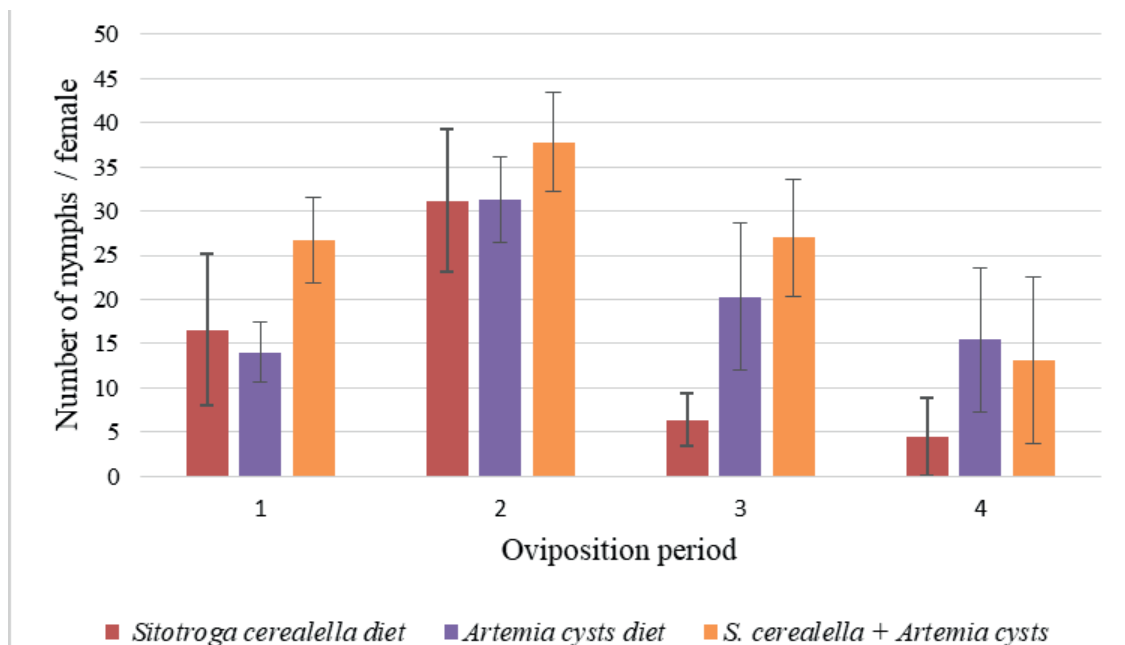
Mortality of the F0 nymphs was not significantly different among diets ( $F_{2,27} = 0.13$ ,  $P = 0.88$ ) (Table 1). More than 60% died regardless the prey on which they had fed. This value was higher than the reported by Burla et al. (2014) for the same mirid on tobacco plants after feeding on *E. kuehniella* eggs. Nevertheless, the mortality values here registered were similar (Grenier et al., 1989; Vandekerkhove et al., 2008; Labbé et al., 2018) or higher than the recorded for other mirids fed on *Artemia* sp. cysts (Castañé et al., 2006; Owashi et al., 2020). In the present study, although no treatment was applied to *Artemia* cysts and vegetable material was provided, the mortality was high but similar for all diets, including *S. cerealella* eggs. Experimental conditions (e.g. manipulation effect, humidity accumulation inside the Petri dishes) are likely to have affected the nymphal survival, particularly during the early stages.

The fertility of the F2 females was not significantly different among diets ( $F_{2,15} = 0.44$ ,  $P = 0.65$ ) (Table 1). The fertility values registered were higher than those recorded for *T. cucurbitaceus* fed with *S. cerealella* eggs, *T. vaporariorum* and *B. tabaci* nymphs (López et al., 2012; Orozco Muñoz et al., 2012). However, these authors estimated this variable with

adult offspring instead of the nymphal one, which resulted in lower values since it includes nymphal mortality. Similar to *T. cucurbitaceus*, Castañé et al. (2006) did not find significant differences for *M. pygmaeus* fertility (n° eggs / female) during the study of generations fed with *Artemia* sp. cysts (dried or hydrated) as an alternative prey when compared to *E. kuehniella* eggs. Other authors reported no significant differences in the number of eggs laid by *N. tenuis* (Owashi et al., 2020).

Predator females showed their highest fertility values between the 5th and 8th day of the 16 days analyzed, regardless of the diet (Figure 1). This fertility peak was previously found for *T. cucurbitaceus* preying on *T. absoluta* and *S. cerealella* eggs (Cagnotti et al., 2021). However, this result does not agree with that of Burla et al. (2014) who registered the highest nymph production during the first, second and third weeks of female lifespan of *T. cucurbitaceus* fed on *E. kuehniella* eggs. We also observed that *T. cucurbitaceus* females laid off spring in the first 4 days of their lifespan, regardless of the diet evaluated. On the contrary, Vandekerkhove et al. (2006) reported a preovipositional period for *M. pygmaeus*. According to previous studies (Cagnotti et al., 2021) a preovipositional period is not present for *T. cucurbitaceus*.

*Artemia* sp. cysts did not affect the mortality recorded in the F3 generation which was similar to the other diets evaluated, 44.14% on average ( $F_{2,15} = 0.01$ ,  $P = 0.99$ ) (Table 1). This value was lower than the observed for the first generation tested, probably due to the use of plants and cages instead of Petri dishes as experimental units. Similarly, Castañé et al. (2006) recorded the same nymphal mortality for *M. pygmaeus* in the F6 and F8 generations fed on different diets. Besides, Vandekerkhove et al. (2008) registered a similar mortality for *M. pygmaeus* during four generations fed on *E. kuehniella* eggs or *Artemia* sp. cysts.



**Figure 1.** Fertility of *Tupiocoris cucurbitaceus* during different periods of oviposition (mean  $\pm$  standard error). Categories: **1:** days 1-4 of the adult female lifespan; **2:** days 5-8 of the adult female lifespan; **3:** days 9-12 of the adult female lifespan; **4:** days 13-16 of the adult female lifespan.

Significant differences among diets were found for the sex ratio of the F3 ( $F_{2,15} = 3.96$ ,  $P = 0.05$ ). The female proportion was significantly higher when *T. cucurbitaceus* fed on *S. cerealella* eggs compared to the mix diet. The sex ratio of individuals fed on *Artemia* sp. cysts was not different from the other diets (Table 1). The female proportion recorded in the present work was higher than those recorded by other authors in *M. pygmaeus* using *Artemia* sp. cysts and lepidopteran eggs as food, ca 1:1 (Castañé et al., 2006). Our results also differed from those reported by Orozco Muñoz et al. (2012), where the authors observed a  $0.5 \pm 0.1$  sex ratio for *T. cucurbitaceus* fed on *B. tabaci* nymphs. Recent studies performed on the same *T. cucurbitaceus* colony showed the presence of *Wolbachia* sp. in the predator individuals (Conte, personal communication). The same bacterium was detected on *S. cerealella* specimens in our laboratory rearing. The scarcity of males in the populations studied could be explained by the occurrence of parthenogenesis, a reproduction mechanism found in some mirids (Goula, 1986). In particular, parthenogenesis has been identified because of the infection with the endosymbionts bacteria, where *Wolbachia* is responsible for more than 50% of the affected arthropods (Weinert et al., 2015). A possible explanatory hypothesis is that the *Wolbachia* infection was transmitted from the prey (*S. cerealella* eggs) to the predator during the

laboratory rearing. *Wolbachia* can be transmitted by both horizontal and vertical ways and there is evidence that it can tolerate the passage through the digestive system (Rodríguez, 2013; Ferdj et al., 2016). This hypothesis could explain the high proportion of females in *T. cucurbitaceus* after several generations of laboratory rearing. However, as mentioned above, the F3 sex ratio was similar between diets, showing no treatment effect in this variable. In the present study, rearing *T. cucurbitaceus* on *Artemia* sp. cysts produce similar development time, survival, fertility and sex ratio at least until third generation.

## CONCLUSIONS

According to the results presented in this work, we can conclude that *Artemia* sp. cysts allow the suitable development of *T. cucurbitaceus* for at least three generations, without affecting some of its main biological characteristics. Therefore, the use of *Artemia* sp. cysts as an alternative prey (either alone or with *S. cerealella* eggs) for laboratory rearing of *T. cucurbitaceus* is an acceptable and valuable option.

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