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The sex ratio of the koinobiont parasitoid *Microcharops anticarsiae* Gupta remains female-biased on young larvae of velvetbean in the laboratory environment

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ABSTRACT. The natural parasitic behavior of parasitoids should be known by those in charge of planning strategies for the biological control of pests; therefore, the aim of the present study was to determine the larval instar of *Anticarsia gemmatalis* Hübner parasitized by *Microcharops anticarsiae* Gupta in the field and the implication of such parasitic behavior in the sex ratio in the laboratory environment. The length of each larval instar of *A. gemmatalis* parasitized by *M. anticarsiae* in the field was determined, and the egg-to-pupa period of the parasitoid and its larval instar lengths were plotted in Gantt charts. According to the chart, *A. gemmatalis* was parasitized at the first (15%) and second (85%) larval instars in the field, but the length of the first, second and third larval instars of this species was not affected by the parasitism by *M. anticarsiae* in the field; however, its fourth larval instar was extended and the fifth one was shortened in 2015 but not affected in 2016. The sex ratio of *A. gemmatalis* larvae parasitized by *M. anticarsiae* in the field was female-biased, and the sex ratio of early parasitized larvae (3-day old) in the laboratory environment was also female-biased for three cultivated generations. The 'generation' factor has affected the egg-to-pupa, pupal and egg-to-adult periods of *M. anticarsiae*, since females pupated earlier than males in the egg-to-pupa period. Based on the results, *M. anticarsiae* mostly parasitized the second larval instar of *A. gemmatalis* in the field, and parasitism in 3-day old larvae in the laboratory environment produced female-biased sex ratio in *M. anticarsiae*, regardless of the generation.

Keywords: behavior; preference; offspring; soybean; Gantt chart.

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Introduction

Successful parasitism in natural environment involves complex factors, such as choosing the right host (Ueno, 2015), number of ovipositions (Huang, Hua, Wang, Zhang, & Li, 2017), injecting venom to overcome the immune response of the host, and adapting or regulating the physiology of the parasitoid through larvae development (Kaeslin et al., 2010). According to Bernal, Prasifka, Sétamou, and Heinz (2004), parasitoids are the most documented examples of biological control worldwide, mainly species belonging to the order Hymenoptera. Studies on parasitoid wasps and their behavior are needed in order to help better understanding them and to use these species in agricultural biological control.

Microcharops anticarsiae Gupta (Hymenoptera: Ichneumonidae) is an efficient natural enemy of *Anticarsia gemmatalis* Hübner (Lepidoptera: Erebidae), but also parasitizes other lepidopteran species (Patel & Habib, 1998). Although *M. anticarsiae* is a koinobiont parasite, adult females belonging to this species lay eggs inside the host. The parasitic larva feed upon the host from inside of it; however, the host continues to feed, grow and defend itself at least during the initial parasitism phase (Harvey, Poelman, & Tanaka, 2013).

Studies have determined that *M. anticarsiae* has four larval instars when parasitize *A. gemmatalis* larvae feeding on conventional (non-transgenic) soybean leaves. *Microcharops anticarsiae* parasitize the host from the first to fourth larval instar, but laboratory results showed no preference for a particular instar under laboratory conditions (Patel & Habib, 1993). Other studies have shown that parasitism on different instars did not have any effect on the larval development of *M. anticarsiae*; however, despite its potential to be a

biological control agent in crops, the literature lacks studies focused on determining the parasitic behavior of parasitoids, such as *M. anticarsiae,* in the field and the implication of such parasitism on sex ratio in the laboratory environment.

The present study results from an investigation about the natural parasitic behavior of *M. anticarsiae*, variation in its sex ratio throughout three generations in the laboratory environment and its effect on the parasitoid development time.

Material and methods

Study Site

The experiments were conducted at the Teaching, Research and Extension Farm (21º15'04" S; 48º17'04" W) and at the Biological Control and Integrated Pest Management Laboratory of the Agricultural and Veterinarian Sciences School of *Universidade Estadual Paulista* "Júlio de Mesquita Filho", Jaboticabal Campus, State of São Paulo, Brazil (21º14'28" S; 48º17'23" W) during the 2015 and 2016 seasons. Samples were collected in January and February in both seasons. The Department of Exact Sciences of *Universidade Estadual Paulista* "Júlio de Mesquita Filho" provided the meteorological data used in the study.

Sample collection

Soybean cultivars SYN 1365 RR® and BRX Potência RR® (both are tolerant to glyphosate) were sown in 1 ha during the 2015 and 2016 seasons, respectively – crops were not treated with insecticide during the experiment. *Anticarsia gemmatalis* larvae were collected with a beat sheet (length: 1 m - width: 0.5 m) in a crop row (length: 2 m) according to Souza et al. (2018); in total, 20 points were randomly sampled on a weekly basis after plant emergence. The collected larvae were stored in plastic containers and sent to the laboratory.

Sample monitoring in laboratory environment

The collected larvae were stored in the Biological Control and Integrated Pest Management Laboratory under controlled temperature (25 ± 2°C) and relative humidity (60 ± 10%) conditions, in a 14:8-h photoperiod. Larvae were grouped based on their field instar; and individually placed in Petri dishes. Leaflets, which were daily replaced, covered the bottom of the Petri dishes for larvae feeding. Soybean leaves were previously cleaned with sodium hypochlorite (2.5%) for five minutes and washed three times in water. Petri dishes were sealed with polyvinyl chloride film to avoid leaflet dehydration. It was not possible differentiating the parasitized larvae from those non-parasitized; therefore, it was only possible to separate the parasitized group from the non-parasitized one when parasitoid larvae left the hosts to form the cocoon. A brief description of the experimental groups is presented in Table 1.

Table 1. Description of the groups.

Grouped by instar	Grouped by parasitism	Groups	Description
1st	Parasitized	G1p	Parasitized larvae collected at the first instar
	Non-parasitized	G1	Non-parasitized larvae collected at the first instar
2nd	Parasitized	G2p	Parasitized larvae collected at the second instar
	Non-parasitized	G2	Non-parasitized larvae collected at the second instar
3rd	Parasitized	G3p	Parasitized larvae collected at the third instar
	Non-parasitized	G3	Non-parasitized larvae collected at the third instar
4th	Parasitized	G4p	Parasitized larvae collected at the fourth instar
	Non-parasitized	G4	Non-parasitized larvae collected at the fourth instar
5th	Parasitized	G5p	Parasitized larvae collected at the fifth instar
	Non-parasitized	G5	Non-parasitized larvae collected at the fifth instar

The following parameters were measured after larval collection in the field in the 2015 and 2016 seasons: Length of each larval instar, larval period of *A. gemmatalis* and the sex ratio of *M. anticarsiae*. Larval instars of *A. gemmatalis* grown in the laboratory environment were observed on a daily basis and changes on them were recorded at the same frequency. Larvae molt was characterized by the presence of shed head capsules in each Petri dish. Sex ratio was determined based on the ratio of females to the total population. Gantt charts of larval instar length and the parasitoid egg-to-pupa period were plotted to determine the larval instar of *A. gemmatalis* parasitized by *M. anticarsiae* in the field.

Based on the Gantt charts, larval instar lengths were organized in ascending order (from first to fifth instar). The egg-to-pupa period of *M. anticarsiae* parasitizing *A. gemmatalis* larvae was characterized by the first instar of larvae parasitized in the field, and this variable was used to determine the larval instar of *A. gemmatalis* parasitized by *M. anticarsiae* in the field. Laboratory culture was based on measurements of the following parameters: Egg-to-pupa, pupal and egg-to-adult periods.

Microcharops anticarsiae culture in laboratory environment

Microcharops anticarsiae emerged from larvae collected in the field in the 2016 season were used to start the culture in the laboratory environment. Adult specimens represented the parental generation of the three cultivated generations. In total, 10 larvae (3-day old) of *A. gemmatalis* were conditioned in plastic container (5 L) with one drop of pure honey and a coffee cup (10 mL) of wet cotton; the container had two lateral openings (10 x 5 cm) covered with a mesh for airflow purposes. A glass pot (10 mL) was used to hold the soybean leaf. Larvae were placed on soybean leaves before parasitism began. *Microcharops anticarsiae* females were mated at the age of 8 days, prior to parasitism. One mated female was placed in the experimental container, and stayed there for 2 hours; this procedure was repeated 10 times, but with different females and larvae in each repetition. Larvae were placed in Petri dishes in separate, and fed soybean leaflet, similar to the procedure adopted for larvae collected in the field. All parasitoid females were released at Jaboticabal Campus after parasitism. *Microcharops anticarsiae* males and females fed on pure honey and wet cotton.

Statistical analysis

Statistical analyses were carried out in the R software for Windows (R Core Team, 2016). Normality was checked by the Shapiro-Wilk test and the Levene test was used to evaluate homogeneity of variances ($\alpha \leqslant 0.05$). Data were tested by one-way ANOVA, followed by Duncan's test for comparison between factors. Differences were significant at $\alpha \leqslant 0.05$. Data were transformed into y^{λ} by Box-Cox transformation, whenever necessary. Data of the laboratory culture were analyzed by repeated measures ANOVA.

Ethical note

Parasitism condition and the sex of larvae collected in the field were unknown at sampling. Parasitoids emerged from larvae collected in the field were fed on water and honey (10%), and were used in laboratory studies. Sample collection in the field was carried out based on the Brazilian legislation for scientific activities. The first author was granted with Authorization 49516-1 to carry out the research between 2014 and 2016. Adult parasitoids were sent to the American Entomological Institute for identification and those identified were deposited in the Museum of Entomology of *Universidade Estadual Paulista* "Júlio de Mesquita Filho" at the Agricultural and Veterinarian Sciences School, Jaboticabal Campus, State of São Paulo, Brazil.

Results

Larval development of *A. gemmatalis* parasitized with *M. anticarsiae* in the field

Based on results of the 2015 season, there were no differences in length for the first, second and third larval instars of *A. gemmatalis* parasitized by *M. anticarsiae* in the field (Table 2). However, the length of the fourth larval instar was extended by parasitism, in turn, the length of the fifth larval instar was shortened; consequently, the larval period was in general shorter (Tables 2 and 3). The egg-to-pupa period (11.07 ± 0.46 days) of *M. anticarsiae* parasitizing *A. gemmatalis* was determined based on *A. gemmatalis* larvae collected in the field at the first instar (G1p).

The natural infestation of *A. gemmatalis* was low in 2016; therefore, there were only few larvae to be sampled in the field and to form the experimental groups (Table 4 and 5). Based on the results, there were no differences in the length of the third larval instar of *A. gemmatalis* parasitized by *M. anticarsiae* in the field (G3 vs G3p), but the length of the fourth larval instar of this species was extended due to parasitism (G3 vs G3p and G4 vs G4p), and the length of the fifth larval instar was not affected in the field (G4 vs G4p). The total larval period of *A. gemmatalis* was shorter.

Some relevant factors about parasitism by *M. anticarsiae* were observed: its larvae ate *A. gemmatalis* larvae from inside of it and at the end of the development period, only the integument and the head of the

hosts remained intact. *Microcharops anticarsiae* larvae wove a cocoon and pupated on the sides of their host. *M. anticarsiae* males were ready to copulate right after their emergence, but females were not.

Table 2. Length of the first to fourth instar development (mean ± SD) of non-parasitized and parasitized *Anticarsia gemmatalis* larvae, 2015.

Groups	N[1]	First instar (days)		*N*	Second instar (days)		*N*	Third instar (days)		*N*	Fourth instar (days)	
G1	17	2.29±1.31	a[2]	17	2.47±1.07	a	17	2.53±0.80	a	17	2.47±0.80	b
G1p	14	2.21±1.25	a	14	2.29±0.61	a	14	2.21±0.80	a	14	3.79±1.37	a
df		1, 29			1, 29			1, 29			1, 29	
p		0.86			0.62			0.22			0.01	
CV		16.97			9.63			9.70			12.10	
G2				20	2.10±1.25	a	20	2.20±0.70	a	20	2.60±0.60	b
G2p				17	2.24±1.03	a	17	2.18±0.64	a	17	3.65±1.46	a
df					1, 35			1, 35			1, 35	
p					0.60			0.97			0.02	
CV					15.27			8.83			11.25	
G3							17	1.94±0.75	a	17	2.88±0.78	a
G3p							28	2.21±1.07	a	26	3.10±1.38	a
df								1, 43			1, 42	
p								0.47			0.99	
CV								13.24			14.14	
G4										36	2.03±1.00	b
G4p										34	3.29±1.00	a
df											1, 68	
p											0.00	
CV											13.10	

[1]N: Total number of insects sampled in the field. [2]Means with the same letters in columns indicate no significant differences (Duncan, $p \leq 0.05$).

Table 3. Length of the fifth, sixth instar and total larval development (mean ± SD) of non-parasitized and parasitized *Anticarsia gemmatalis* larvae and the sex ratio of *Microcharops anticarsiae*, 2015.

Groups	N[3]	Fifth instar (days)		*N*	Sixth instar (days)	*N*	Larval period (days)		Sex ratio (%)
G1	17	5.47±1.50	a[4]	3	5.00±1.00	17	16.12±2.29	a	
G1p	3	2.67±0.58	b		-[2]	14	11.07±1.73	b	0.57
df		1, 18			-		1, 29		
p		0.01			-		0.00		
CV		12.47			-		5.96		
G2	20	5.95±2.33	a	3	6.33 ±1.53	20	13.80±2.14	a	
G2p	6	3.50±1.38	b		-	17	9.29±1.86	b	0.73
df		1, 24			-		1, 35		
p		0.02			-		0.00		
CV		16.02			-		7.20		
G3	17	5.35±1.54	a		4.50 ± 0.71	17	10.71±1.10	a	
G3p	6	3.33±1.37	b		-	28	5.89±1.97	b	0.49
df		1, 21			-		1, 43		
p		0.01			-		0.00		
CV		13.10			-		26.27		
G4	35	5.86±1.65	a	2	4.00±1.41	36	7.94±2.14	a	
G4p	6	3.33±1.03	b		-	34	3.88±1.32	b	0.70
df		1, 39			-		1, 68		
p		0.00			-		0.00		
CV		11.49			-		12.04		

[3]N: Total number of insects sampled in the field. [4]Means with the same letters in columns indicate no significant differences (Duncan, $p \leq 0.05$).

Determination of *A. gemmatalis* larval instar parasitized by *M. anticarsiae* in the field

The egg-to-pupa period (11.07 ± 0.46 days) was assessed through Gantt chart plots and results were used to determine the larval instar parasitized by *M. anticarsiae* in the field in the 2015 season (Table 2). Considering Figure 1, *A. gemmatalis* larvae in G1p were parasitized at their first larval instar and

larvae at G2p, G3p and G4p were parasitized at their second instar, as shown in the Gantt chart. In total, 79 larvae from groups G2p (*N* = 17), G3p (*N* = 28) and G4p (*N* = 34) were parasitized by *M. anticarsiae* at their second larval instar in the field (Table 2). In addition, 14 larvae of *A. gemmatalis* from G1p were parasitized at their first larval instar; therefore, the host larvae were parasitized at the first (15%) and second larval instars (85%) in the 2015 season. Unfortunately, it was not possible to arrange groups with individuals collected in the 2016 season, given the low natural infestation with *A. gemmatalis* in the field (Tables 4 and 5).

Table 4. Length of the first to fourth instar (mean ± SD) of non-parasitized and parasitized *Anticarsia gemmatalis* larvae, 2016.

Groups	N[5]	First instar (days)	N	Second instar (days)	N	Third instar (days)		N	Fourth instar (days)	
G1	2	1.50±0.71	2	2.00±0.00	2	2.00±0.00		2	2.00±0.00	
G1p	-	-	-	-	-	-		-	-	
G2			3	2.00	3	2.00±0.00		3	2.00±0.00	
G2p			4	1.00±0.00	4	2.00±0.00		4	6.50±5.07	
G3					6	1.83±0.75	a[6]	6	2.17±0.41	b
G3p					6	1.67±0.82	a	6	4.33±0.52	a
df						1, 10			1, 10	
p						0.721			0.00	
CV						78.53			46.58	
G4								7	1.71±0.49	b
G4p								14	3.07±1.38	a
df									1, 19	
p									0.01	
CV									2.62	

[5]N: Total number of insects sampled in the field. [6]Means with the same letters in columns indicate no significant differences (Duncan, $p \leq 0.05$).

Table 5. Length of the fifth, sixth instar and total larval development (mean ± SD) of non-parasitized and parasitized *Anticarsia gemmatalis* larvae and the sex ratio of *Microcharops anticarsiae*, 2016.

Groups	N[7]	Fifth instar (days)	N	Sixth instar (days)	N	Larval period (days)		Sex ratio (%)
G1	2	6.50±0.71	-	-	2	14.00±1.41		
G1p	-	-	-	-	-	-		-
G2	3	4.33±2.08	1	6.00	3	12.33±2.65		
G2p	-	-	-	-	4	9.50±5.07		0.75
G3	6	5.33±2.50	1	6.00	6	10.33±1.63		
G3p	-	-	-	-	6	6.00±1.10		0.50
df						1, 10		
p						0.00		
CV						14.83		
G4	7	5.43±0.98	a[8] -	-	7	7.14±1.07	a	
G4p	2	4.00±1.41	a		14	3.64±1.78	b	0.50
df		1, 7				1, 19		
p		0.133				0.00		
CV		73.81				16.55		

[7]N: Total number of insects sampled in the field. [8]Means with the same letters in columns indicate no significant differences (Duncan, $p \leq 0.05$).

Sex ratio and development time of *M. anticarsiae* parasitizing *A. gemmatalis*

The sex ratio of *M. anticarsiae* was female-biased in the 2015 season, except for G3p, which recorded sex ratio of 0.49 (Table 2). Based on the observed sex ratio in the 2016 season, the population of *M. anticarsiae* was composed of 50% females, except for G2p, which was female-biased (Tables 4 and 5). Adult *M. anticarsiae* emerged in the 2016 season were used to start the laboratory culture, based on the preliminary results of the 2015 season.

The sex ratios of the first, second and third cultured generations of *M. anticarsiae* parasitizing 3-day old *A. gemmatalis* larvae were 0.56, 0.48 and 0.58, respectively; therefore, parasitism rate in these generations reached 92%, 81% and 90%, respectively (Table 6). The sex ratio of *M. anticarsiae* was female-biased when *A. gemmatalis* larvae were parasitized at the age of 3 days.

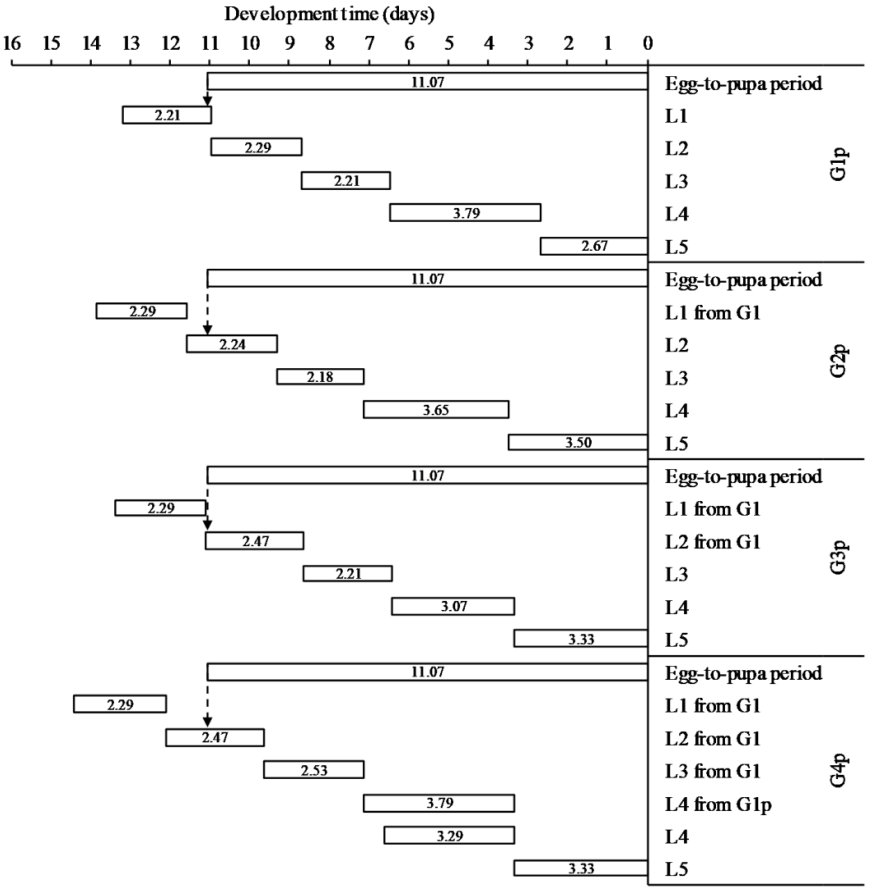

Figure 1. Gantt chart illustrating the larval instar lengths of *Anticarsia gemmatalis* parasitized by *Microcharops anticarsiae* in the field, during the 2015 season. L1, L2, L3, L4, and L5 represent the first, second, third, fourth, and fifth larval instar of *A. gemmatalis*. G1p, G2p, G3p, and G4p represent the parasitized larvae sampled at the first, second, third, and fourth instar in the field. G1 represents the non-parasitized larvae sampled at the first instar in the field.

Table 6. Sex ratio and parasitism percentage of *Microcharops anticarsiae* in the laboratory environment.

Generation	Sex	Parasitism (%)	Sex ratio (%)
F1	Female	51.00	
	Male	41.00	
	Total	92.00	0.56
F2	Female	39.00	
	Male	42.00	
	Total	81.00	0.48
F3	Female	52.00	
	Male	38.00	
	Total	90.00	0.58
Mean		87.67	0.54

F1, F2, and F3: First, second and third generation of *M. anticarsiae.*

The 'generation' factor affected the egg-to-pupa, pupal and egg-to-adult periods, and the factor 'sex' affected the egg-to-pupa period of *M. anticarsiae* parasitizing *A. gemmatalis* (Table 7).

Table 7. Repeated measures ANOVA for the development time of *Microcharops anticarsiae* in the laboratory environment.

Source	Models	DF	F value	Prob > F
Egg-to-pupa period	Generation (G)	2	51.0649	0.00
	Sex (S)	1	15.0796	0.00
	G x S	2	0.5463	0.58
Pupal period	Generation (G)	2	33.1626	0.00
	Sex (S)	1	1.5316	0.22
	G x S	2	0.4001	0.67
Egg-to-adult period	Generation (G)	2	94.9523	0.00
	Sex (S)	1	3.9737	0.05
	G x S	2	1.054	0.36

The egg-to-pupa, pupal and egg-to-adult periods were longer in the third generation of *M. anticarsiae*, and the egg-to-pupa period was shorter in females than in males (Figure 2).

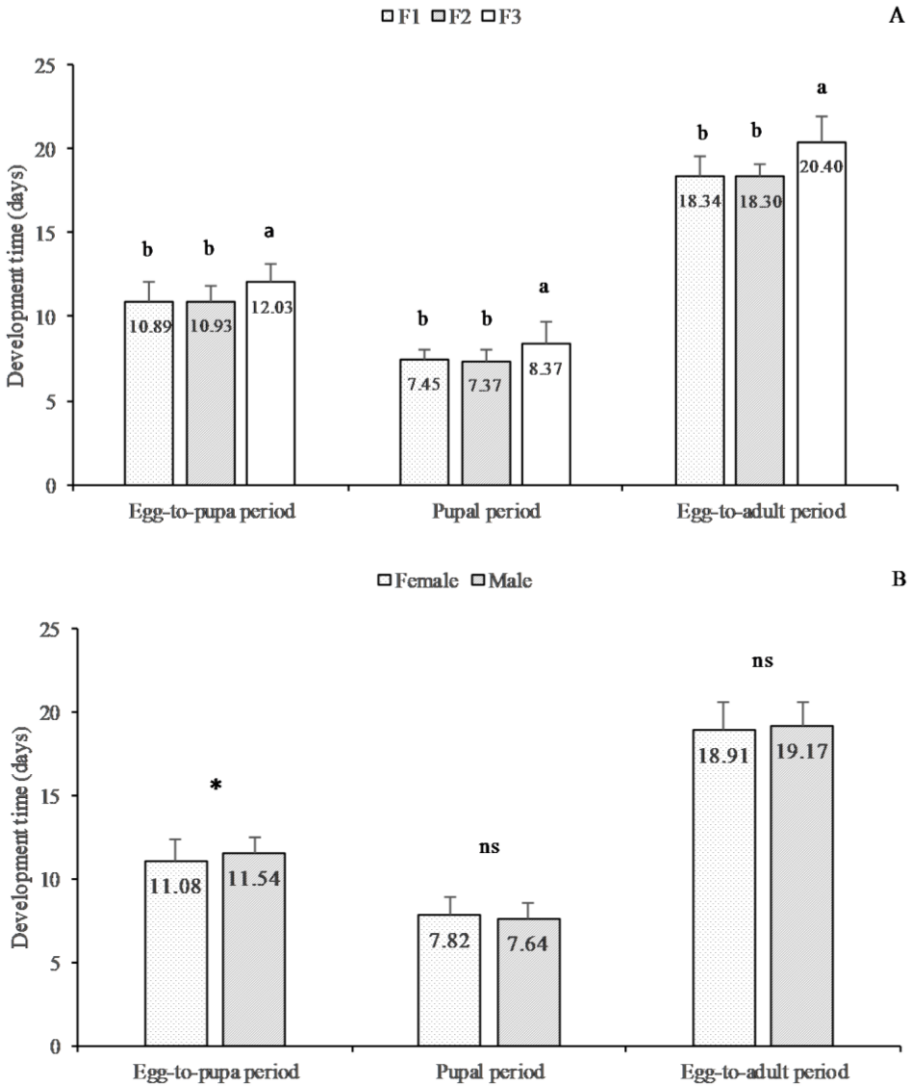

Figure 2. Development time of *Microcharops anticarsiae* parasitizing *Anticarsiae gemmatalis* larvae based on generation (A) and sex (B). Similar letters and ns on the columns indicate no significant differences and * on the columns indicates significant differences (Duncan, $p \leq 0.05$). F1, F2, and F3: First, second and third generation of *M. anticarsiae*. Female and Male: Female and male adults of *M. anticarsiae*.

Discussion

Larval development of *A. gemmatalis* parasitized by *M. anticarsiae* in the field

Parasitism by *M. anticarsiae* shortened the length of the fourth larval instar of *A. gemmatalis* and extended the fifth instar, but the overall larval period was extended. The effects of *M. anticarsiae* parasitism on the length of larval instars of parasitized *A. gemmatalis* larvae is explained by changes in growth hormone regulation, which resulted from the development of immature parasitoids (Kaeslin et al., 2010).

Larval period shortening was a positive outcome, because it shortened the time that parasitized *A. gemmatalis* larvae require to feed on soybean leaves. Therefore, parasitized *A. gemmatalis* larvae had shorter time to cause damage to soybean plants in the field.

Parasitoid larvae left the host and pupated on the sides of it. Assumingly, immature parasitoids allow their host larvae to develop to a certain size (mostly, to the fourth instar, when these host larvae meet their nutritional requirements) before they finally start eating the host from inside of it. A few parasitoid larvae left the host at the fifth larval instar; this finding can be likely explained by the higher cost associated with overcoming its immune defenses, with development control, and with the growth arrest of larger hosts (Kaeslin et al., 2010; Zhou, Meng, & Li, 2017).

Determination of *A. gemmatalis* larval instars parasitized by *M. anticarsiae* in the field

The egg-to-pupa period of *M. anticarsiae* in group G1p was used to determine the larval instar of parasitized *A. gemmatalis* larvae. This development time did not change when *A. gemmatalis* was parasitized at its first, second, third or fourth larval instars. This phenomenon occurs in other endoparasitoids; for instance, the development time of *Microplitis mediator* (Haliday) (Hymenoptera: Braconidae) is not affected by parasitizing host larvae of *Mamestra brassicae* (Linnaeus) (Lepidoptera: Noctuidae) at the first, second or third instar (Malcicka & Harvey, 2014), which indicates that endoparasitoids strategically maintain the development time changing other parameters like weight and sex ratio. Therefore, we considered the egg-to-pupa period needed by *M. anticarsiae* at the first instar (G1p) of *A. gemmatalis* was not different from that needed by *M. anticarsiae* to parasitize the second, third, and fourth larval instars of the assessed wasp species.

Based on Figure 1, *M. anticarsiae* parasitized the first and second larval instars of *A. gemmatalis* in the field; therefore, it is possible to state that *M. anticarsiae* more often parasitizes the early instars of *A. gemmatalis* in order to minimize mortality risks inherent in parasitism in older instars. Older larvae of *A. gemmatalis* are more efficient in attacking and harming the parasitoid during the parasitism process (Ameri, Rasekh, & Michaud, 2014; Firlej, Lucas, Coderre, & Boivin, 2010; Zhou et al., 2017); furthermore, parasitoid larvae have more time to develop better physiological conditions inside young hosts, since these hosts have less effective immune responses and/or produce fewer immune defenses than larger and more developed hosts (Kaeslin et al., 2010).

Other studies investigated different host-parasitoid systems and showed that ichneumonid parasitoids rather parasitize young larvae; for example, second instar larvae of *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) (3-5 days old) represent the most favorable stage for the development of *Campoletis sonorensis* (Cameron) (Hymenoptera: Ichneumonidae) (Murillo, Hunt, & VanLaerhoven, 2012). In addition, the second larval instar of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) has proven to be suitable for the culture of *Hyposoter didymator* (Thunberg) (Hatem, Shawer, & Vargas-Osuna, 2016).

The present study sheds light on unanswered questions about the parasitic behavior of *M. anticarsiae* in the field, since the recorded findings provide important information about large-scale culture production methodologies focused on growing biological control agents to be applied to pests in crops.

Sex ratio and development time of *M. anticarsiae* parasitizing *A. gemmatalis*

Microcharops anticarsiae offspring was female-biased in most groups. The sex ratio is a critical factor to the mitigation of costs with the culture of this parasitoid, as well as an important attribute of biological control agents (Pandey, Kumar, & Tripathi, 2004). According to the literature, the sex ratio of Hymenopteran is influenced by factors such as diet (Ongaratto et al., 2019), instar (Poncio et al., 2018; Chu et al., 2014), superparasitism (Alvarenga, Dias, Stuhl, & Sivinski, 2015) and size/age of the host (Van Nieuwenhove & Ovruski, 2011; Ueno, 2015). Also, chemical cues are associated with the host characteristics, such as feces (Faraone, Svensson, & Anderbrant, 2017) and its ability to feed on different host plants (Morawo & Fadamiro, 2019). According to the present results, female *M. anticarsiae* individuals parasitize *A. gemmatalis* larvae at the end of the first and at the beginning of the second instar; these are the most favorable periods for parasitism for two reasons: larvae attach to the substrate at the end of the first instar and stop feeding, and the larvae stops moving at this point, *M. anticarsiae* has the advantage in parasitizing immobilized larvae; second reason, larvae remain weak at the beginning of the second instar and are easily exposed to parasitism - parasitoids can take advantage of this weak condition. Assumingly, *M. anticarsiae* can detect the volatiles emitted by early *A. gemmatalis* larvae (first and second instars) at molting; therefore, the end of the first and the beginning of the second instar may be the most favorable periods to parasitism, because they enable *M. anticarsiae* to produce female-biased sex ratio.

The effect of the factor 'generation' on the development time of *M. anticarsiae* indicates that the laboratory culture procedure needs improvements in order to avoid negative effects on the parasitoid development as reported by Bueno, Romero, Osorio, and Zaviezo (2017) for *Mastrus ridens* Horstmann (Hymenoptera: Ichneumonidae), because there were no introduced new parasitoids to the laboratory culture due to the lack of larvae in soybean crops in the field and laboratory. Using wind tunnels to help parasitoids finding the hosts, as well as olfactometers to determine the age of females to mate, could be instruments used to achieve such improvements. Otherwise, consanguinity had negative influence on the development time of third-generation parasitoids, because there were no new parasitoids in the laboratory culture due to the lack of larvae in soybean crops in the field. The difference between the egg-to-pupa period of males and

females helps separating parasitoids by sex, even before emergence. The rejection of recently emerged *M. anticarsiae* females indicates that they rather feed or develop before mating. Our results provide important information to those coordinating the production of *M. anticarsiae* females in large-scale culture production programs focused on biological control.

The current study is expected to help and motivate new researchers to conduct other investigations on this topic, since there are more questions to be answered about parasitic behavior and host-parasitoid interactions in the field. This research broadens the knowledge about the development of biological control programs focused on agricultural applications.

Conclusion

Parasitism of *M. anticarsiae* is female-biased in 3-day old *A. gemmatalis*, in the laboratory environment. The egg-to-pupa, pupal, and egg-to-adult periods of *M. anticarsiae* parasitizing 3-day old *A. gemmatalis* were affected only in the third generation when there is no introduction of new individuals in the culture. The egg-to-pupa period is shorter in females than in males of *M. anticarsiae*.

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