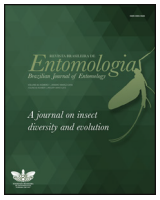
Revista Brasileira de Entomologia 66(3):e20220016, 2022

SOCIEDADE BRASILEIRA DE ENTOMOLOGIA
FUNDADA EM 1937

REVISTA BRASILEIRA DE
Entomologia
A Journal on Insect Diversity and Evolution



Morphology of immature stages, biological parameters and life table of *Microtechnites bractatus* (Hemiptera: Miridae) on different host plants

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ARTICLE INFO

Article history:
Received 16 March 2022
Accepted 24 August 2022
Available online 23 September 2022
Associate Editor: Adeney Bueno

Keywords:
Garden fleahopper
Insect plant interaction
Plant bug

ABSTRACT

The garden fleahopper, *Microtechnites bractatus* (Say) (Hemiptera: Miridae), is associated with several cultivated plant species and, despite its economic importance, little is known about its development and performance in such hosts. We described here, the morphology of immature stages, and evaluated the biology of *M. bractatus* in beans, potatoes, white clover, alfalfa, and wheat. The bioassays were carried out in the laboratory under controlled temperature (25±2ºC), humidity (UR70±15%), and photoperiod (12L:12D). The eggs of *M. bractatus* are elongated and slightly curved, without respiratory projections and light yellow in color, becoming dark at the end of the incubation period. Nymphs present an oval-shaped body, a reddish color that intensifies along with the development and dimorphic wing pads in the fifth instar. In the bioassays, the host plants influenced the biological aspects of *M. bractatus*, both in the immature and adult stages. Nymph survival was higher in clover and alfalfa, while in wheat, it was lower. Clover-fed insects had the longest longevity. The fecundity parameters and egg viability were favored in insects that fed on clover and alfalfa. The fertility life table showed that feeding *M. bractatus* with clover provides a higher net reproduction rate (R_0) and a higher finite ratio of population increase (λ). This study contributes to bioecological and behavioral studies on *M. bractatus* and provides data for the recognition and characterization of individuals in the immature stage.

Introduction

Microtechnites bractatus (Say) (Hemiptera: Miridae) is a polyphagous insect that feeds on several plants of economic importance, occurring in 17 families, such as Fabacceae, Solanaceae, Poaceae, Brassicaceae, Curcubitaceae, among others (Henry, 1983; Capinera, 2001; Wheeler, 2001; Ferreira et al., 2015; Nogueira et al., 2019). The species was discovered by Say (1832) in Indiana, US, and named *Capsus (Cylapus) bractatus*. It was later included in the genus *Halticus* Hahn, 1832 as *Halticus bractatus* (Say, 1832). Well known in the literature since 1890 (Atkinson, 1890) for its economic importance, from 2012, the species moved to the *Microtechnites* genus (new combination) by Tatarnic and Cassis, 2012 (Tatarnic and Cassis, 2012) and is currently known as *Microtechnites bractatus* (Say, 1832). Nymphs and adults suck the plant sap, causing whitish spots on the leaves and reducing the photosynthetic rate (Beyer, 1921). More serious occurrences can result in growth delay and death of plants at an early stage of development (Capinera, 2001). There are even reports that *M. bractatus* (syn. n. *Halticus bractatus*) acts as a transmitter of Sowbane mosaic virus and Tobacco velvet mottle virus (Sobemovirus) (Bennett and Costa, 1961; Butter, 2018).

Studies have addressed the insect-plant associations, geographic distribution (Silva et al., 1968; Carvalho, 1989; Ferreira et al., 2001; Schuh, 2013; Nogueira et al., 2019; Ferreira et al., 2021) and even possible damage to crops, such as the severe infestation in Nicaragua, United States, Canada, and Mexico, with 60% losses in crops of alfalfa (Beyer, 1921; Gbif, 2021). However, despite this large number of records and the economic losses already attributed to the insect, the ontogeny of *M. bractatus* is minimally known and studied, which limits bioecological, behavioral, and applied studies on the species.

Here, we evaluated the biology of *M. bractatus* maintained in different host plant species, making a life table and characterizing the effect of plants on insect ontogeny. The plants used in our bioassays,

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https://doi.org/10.1590/1806-9665-RBENT-2022-0016

were previously described as hosts of *M. bractatus* (Henry, 1983; Capinera, 2001; Wheeler, 2001; Ferreira et al., 2015; Nogueira et al., 2019; Ribeiro et al., 2020) and represent a remarkable agronomic importance as cultivated crops around the world. Three species of Fabaceae were chosen, since this family is the preferred as host of this insects (Nogueira et al., 2019). Additionally, we added one Poaceae and one Solanaceae species as contrasting treatment, aiming to understand the biology of *M. bractatus* maintained on non-fabaceae plants. Moreover, in order to improve knowledge about this species, we also performed a morphometric study describing the main characters of the immature stages (eggs and nymphs) throughout their development.

Materials and methods

Insect rearing

Adult *M. bractatus* were collected in the experimental area of the Midwestern State University, Cedeteg Campus, Guarapuava, Paraná, Brazil (25°23'03.33"S, 51°29'39.24"W and an average altitude of 1100 meters). The adults were placed in a plastic cage (40 cm x 30 cm x 35 cm) with lateral openings lined with voile tissue. Inside the cage, glass jars containing water and bean leaves (*Phaseolus vulgaris* L.) were added for feeding and laying. Three times a week, the plants were replaced by new ones, while the old ones were inspected to remove eggs. The eggs were transferred to Petri dishes (9 cm x 1 cm) lined with filter paper moistened with sodium hypochlorite solution (10%) to prevent fungi growth. Newly hatched nymphs were kept in gerbox® boxes lined with moistened filter paper and fed with bean leaves until emergence. The insects were kept under controlled conditions of temperature (25±2 ºC), humidity (65%±15), and photoperiod (12L:12D).

For species confirmation, insects were identified by PhD. Paulo Sérgio Fiuza Ferreira, professor (retired) and taxonomy specialist of Miridae at Federal University of Viçosa, in Viçosa, Minas Gerais, Brazil. The insects were deposited in the reference collection of the Agriculture Entomology of UNICENTRO, Guarapuava-PR.

Plant material

The bioassays were carried out using white clover (*Trifolium repens* L.), alfalfa (*Medicago sativa* L.), beans (*Phaseolus vulgaris* L.), wheat (*Triticum aestivum* L.), and potatoes (*Solanum tuberosum* L. cv. Agata). These plants are registered as hosts of this insect (Nogueira et al., 2019) and were selected because they have agricultural importance as grain crops, pastures, and vegetables. Plants were grown in pots (5dm^3) containing commercial substrate and shallow soil (1:1) and kept in a greenhouse. White clover, alfalfa, beans, and wheat plants were sown at one centimeter deep, with approximately 100 seeds per pot for alfalfa, white clover, and wheat and 50 seeds for beans. In turn, the potato plants were obtained from minitubers, totaling two minitubers seeds per pot. During plant development, NPK fertilization (4-14-8) was performed at planting and daily irrigation. The plants used in the bioassays had leaves that were always formed in the vegetative stage.

Description of immature stages

To proceed with the description of the immature stages of *M. bractatus*, rearing was maintained with total control of oviposition, hatching, ecdysis, and emergence, based on daily observations throughout the development period. From this rearing, 20 newly laid eggs and 20 specimens of each instar were removed so that they could be measured. The number of instars was determined based on observations of exuvian, and the insects of each instar were collected after ecdysis to morphometric analyses. The individuals were fixed in 70% ethyl alcohol to study their morphology and morphometric parameters. The description of the color was based on live specimens. Morphometric data were obtained from a stereoscopic microscope (Leica M205 C) coupled to a microcomputer. For eggs, height and diameter measurements were obtained; for the nymphs, the body length (BL) measurements were obtained from the apex of the head to the extremity of the abdomen; head length (HL); head width at eye level (HW); interocular distance at mean eye height (ID); pronotum length (PL), pronotum width (PW), wing pads length (WPL), posterior tibia length (PTL); and antennal length (AL) segments I, II, III and IV. Mean, minimum, and maximum data were obtained from 20 repetitions.

Biology and life table

Newly hatched nymphs of *M. bractatus* (up to 12 hours) were individualized in Petri dishes (9 cm diameter) containing moistened filter paper (1.5 mL of water). Leaves of (i) white clover; (ii) alfalfa; (iii) beans; (iv) potatoes, or (v) wheat were inserted into these containers. Each repetition consisted of groups of two nymphs, making up 30 repetitions and 60 individuals (N=30). At two-day intervals, food was exchanged, and evaluations were carried out regarding the period of nymphal development, duration of each instar, and survival.

Newly emerged adults (up to 12 hours) were individualized in Petri dishes (9 cm diameter) containing moistened filter paper (1.5 mL of water). The maintenance of adults was similar to that performed for nymphs, feeding the insects on leaves from the plants referring to the treatments. Each replicate consisted of a couple (one male and one female), totaling 30 replicates, and therefore 60 adults were evaluated (N=30). The number of eggs deposited per female was evaluated by inspecting the leaves from each treatment, carefully removing the eggs from the leaf blade or petiole using a needle. At two-day intervals, observations were carried out to record and evaluate the longevity of males and females (sex ratio 1:1), number of eggs per female/day, and total eggs per female. To assess the incubation time and viability, eggs (N=50) from the third layer from ten couples per treatment were transferred to Petri dishes (9 cm diameter) containing moistened filter paper (1.5 mL of water). They were observed daily to characterize the hatching of nymphs.

The eggs, nymphs, and adult *M. bractatus* were kept in climatized chambers at 25±2°C, and there was controlled photoperiod (12L: 12D) throughout the bioassay.

Data analysis

Nymphal instar duration data were analyzed by the Kaplan-Meier estimator survival curve (Therneau, 2015), followed by a paired comparison of survival distributions by the Mantel-Cox test (Log Rank) using the SPSS software. The comparison of longevity between males and females in the same treatment was performed using the Mantel-Cox test (Log Rank), using SPSS software. The comparison between feeding substrates based on the biological parameters of fecundity, number of eggs per day, viability and incubation period of eggs were analyzed by the Kruskal-Wallis test (p<0.05). Each female's survival and oviposition data were analyzed using the statistical software "R", version 3.4.0 (R Core Team, 2017), from which a life table was constructed of fertility. The parameters of net reproduction rate (R_o), intrinsic population growth rate (rm), finite population increase ratio (λ), and mean generation time (T) were determined and compared by using 95% bootstrap confidence intervals from 10,000 simulations of

life-table parameters for each treatment. Morphometric data were submitted to analysis of variance (ANOVA, $p<0.05$), and the means of different instars were compared by the Tukey test ($p<0.05$).

Results

Description of immature stages

Egg stage: (Figs. 1A-D)

Flat, elongated and slightly curved shape, with an average length of 0.66 cm and an average width of 0.19 cm. Pearly white surface becoming reddish at the end of the incubation period (average duration of 10 days). Slightly reticulated chorion with few evident punctuations. Absent respiratory projections. Globular basal region and tapered apical region culminating in an oval operculum with an opercular rim with a protruding surface and intense white color in newly deposited eggs, which becomes opaque at the end of embryonic development (Fig. 1A). At the end of the incubation period, the nymph becomes perceptible by the reddish coloration in the central region of the egg (Fig. 1B). Eggs of *M. bractatus* are deposited individually below the epidermis of the petioles, leaves, and adjacent to the leaf veins, with only the opercular region exposed (Figs. 1C and D). Elongated region of the egg arranged longitudinally to the leaf veins and visible through the plant tissue.

Instar 1 (Table 1, Fig. 2A): oval, pinkish-yellow body with little pronounced segmentation. Sparse, erect, and yellowish bristles, simple in appearance and not branched. Total body length on average of 0.71 mm, smaller than in the other instars (ANOVA, Tukey, gl=5, $p<0.001$). Head in triangular-shape in dorsal view and rounded between antennae. Wider than long, with an average HW/HL ratio of 1.72. Light red eyes and an interocular distance of 0.19 mm, on average, are smaller than in the other instars (ANOVA, Tukey, gl=5, $p<0.001$). ID/HW ratio of 0.75, significantly higher than in the different instars (ANOVA, Tukey, gl=5, $p<0.001$). Antennae with 0.83mm, on average, formed by four pale yellow-colored antennae throughout. Longer than the body and AL/BL ratio of 1.17. Antenomers IV, III, II, and I measuring, on average, 0.29, 0.17, 0.13, and 0.09 mm, respectively. Short pronotum and significantly shorter length than in the other instars (ANOVA, Tukey, gl=5, $p<0.001$). PW/PL ratio of 2.71. Light yellow legs. Hind tibiae with an average length of 0.31 mm, smaller than in the other instars (ANOVA, Tukey, gl=5, $p<0.001$). Abdomen with sparse, erect, pale yellow bristles. Dorsal abdominal opening of scent glands inconspicuous.

Instar 2 (Table 1, Fig. 2B): oval body, reddish-yellow, with little pronounced segmentation. Sparse, erect, and yellowish bristles, simple in appearance and not branched. Total body length, on average, 1.03 mm and more significant than that of first-instar nymphs (ANOVA, Tukey, gl=5, $p<0.001$). Triangular head in dorsal view and rounded between antennas. HW/HL ratio of 1.91, on average. Light red eyes and an average interocular distance of 0.21 mm. ID/HW ratio of 0.67, different from other instars (ANOVA, Tukey, gl=5, $p<0.001$). Light yellow antennae with terminal regions of yellow-brown antennae. As long as the body, with an

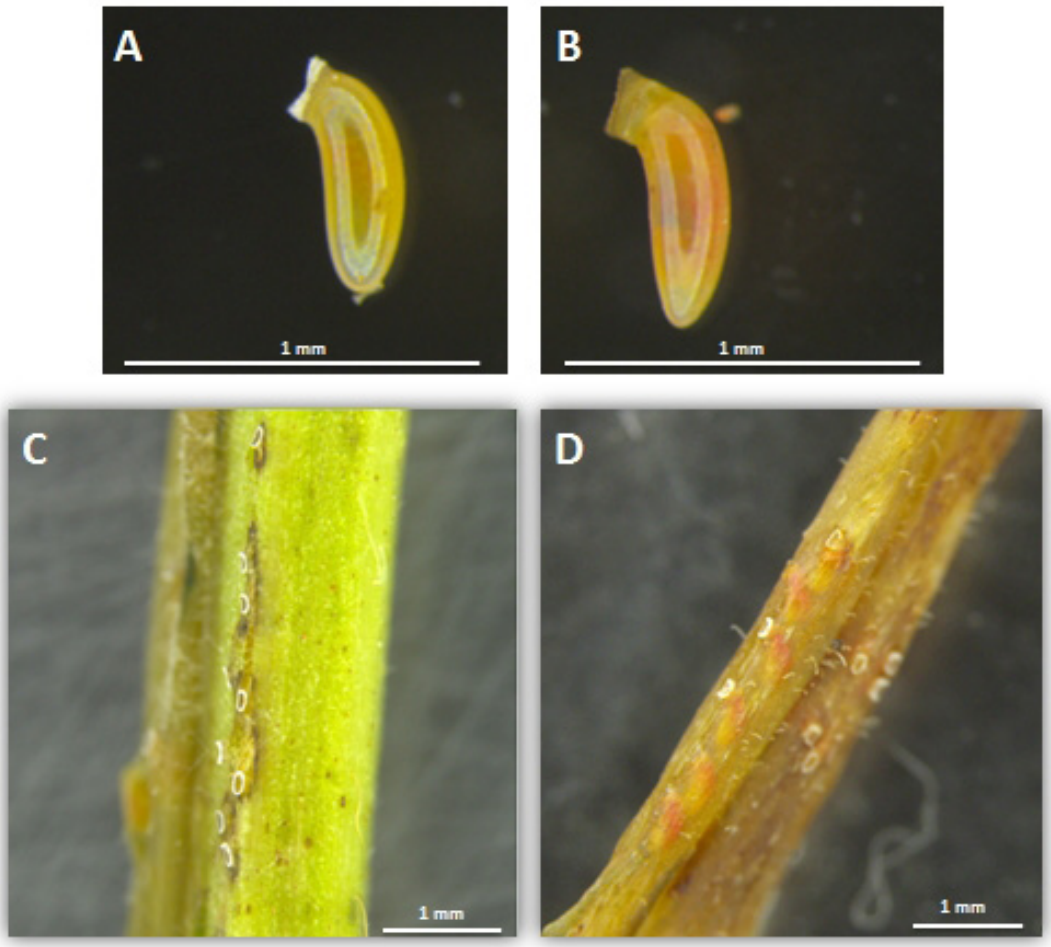

Figure 1 Eggs of *Microtechnites bractatus* oviposited on bean plants, *Phaseolus vulgaris* L. (A) egg recently oviposited and removed from the plant; (B) seven-day-old egg removed from the plant; (C) newly oviposited eggs; (D) eggs after seven days of oviposition.

Table 1
Mean values and standard deviation (minimum-maximum) (mm) referring to the morphological parameters of the five nymphal instars of *Microtechnites bractatus* (BL, body length; HL, head length; HW, head width; ID, interocular distance; AL, antenna length; I - IV, antennomeres; PL, pronotum length; PW, pronotum width; WPL, wing pads length; and PTL, posterior tibia length). Different letters in the lines indicate statistical differences between the insect instars (ANOVA, gl=5, p<0.05).

	1st instar	2nd instar	3rd instar	4th instar	5th instar ♂	5th instar ♀	Mean	Standard deviation
	(n=20)	(n=20)	(n=20)	(n=20)	(n=20)	(n=20)	(n=120)	(n=120)
BL	0.71 ± 0.07a (0.58-0.82)	1.03 ± 0.04b (0.93-1.09)	1.12 ± 0.09c (0.99-1.28)	1.36 ± 0.09d (1.22-1.52)	1.74 ± 0.10e (1.56-1.92)	1.62 ± 0.08f (1.47-1.76)	1.26	0.36
HL	0.15 ± 0.04a (0.11-0.28)	0.17 ± 0.02ab (0.14-0.21)	0.19 ± 0.02bc (0.16-0.24)	0.21 ± 0.03cd (0.16-0.27)	0.26 ± 0.02e (0.22-0.29)	0.23 ± 0.04de (0.19-0.32)	0.20	0.05
HW	0.25 ± 0.01a (0.23-0.26)	0.31 ± 0.01b (0.29-0.34)	0.38 ± 0.01c (0.35-0.40)	0.46 ± 0.01d (0.43-0.47)	0.51 ± 0.01e (0.49-0.54)	0.53 ± 0.02e (0.48-0.56)	0.41	0.10
ID	0.19 ± 0.01a (0.16-0.22)	0.21 ± 0.01b (0.18-0.24)	0.24 ± 0.01c (0.22-0.26)	0.26 ± 0.02d (0.24-0.30)	0.28 ± 0.02e (0.25-0.31)	0.30 ± 0.02f (0.26-0.33)	0.24	0.04
AL	0.83 ± 0.03a (0.76-0.89)	1.00 ± 0.04b (0.93-1.08)	1.19 ± 0.05c (1.08-1.27)	1.51 ± 0.10d (1.23-1.65)	1.87 ± 0.04e (1.74-1.95)	1.87 ± 0.08e (1.70-2.03)	1.38	0.41
I	0.09 ± 0.01a (0.06-0.11)	0.10 ± 0.01ab (0.08-0.11)	0.11 ± 0.01bc (0.09-0.13)	0.14 ± 0.02d (0.09-0.16)	0.17 ± 0.02e (0.12-0.19)	0.16 ± 0.03e (0.10-0.18)	0.13	0.04
II	0.13 ± 0.01a (0.12-0.18)	0.18 ± 0.01b (0.15-0.21)	0.24 ± 0.02c (0.19-0.28)	0.37 ± 0.05d (0.29-0.43)	0.51 ± 0.04e (0.44-0.55)	0.50 ± 0.05e (0.38-0.58)	0,.32	0.15
III	0.17 ± 0.01a (0.15-0.18)	0.22 ± 0.02b (0.20-0.24)	0.29 ± 0.02c (0.24-0.33)	0.39 ± 0.04d (0.28-0.43)	0.52 ± 0.02e (0.48-0.58)	0.51 ± 0.03e (0.46-0.56)	0,.35	0.14
IV	0.29 ± 0.02a (0.26-0.33)	0.35 ± 0.02b (0.30-0.38)	0.40 ± 0.03c (0.33-0.44)	0.47 ± 0.04d (0.37-0.52)	0.53 ± 0.03e (0.48-0.57)	0.55 ± 0.03e (0.50-0.62)	0.43	0.10
PL	0.09 ± 0.01a (0.07-0.11)	0.15 ± 0.01b (0.13-0.17)	0.15 ± 0.03bc (0.10-0.20)	0.20 ± 0.03d (0.15-0.24)	0.24 ± 0.02e (0.20-0.28)	0.23 ± 0.02e (0.16-0.27)	0.18	0.06
PW	0.23 ± 0.01a (0.20-0.25)	0.33 ± 0.02b (0.30-0.37)	0.38 ± 0.02c (0,35-0,43)	0.46 ± 0.02d (0.42-0.50)	0.58 ± 0.02e (0.54-0.60)	0.57 ± 0.02e (0.54-0.61)	0.43	0.13
WPL	-	-	0.17 ± 0.01a (0.14-0.20)	0.37 ± 0.05b (0.30-0.45)	0.79 ± 0.05d (0.66-0.87)	0.53 ± 0.03c (0.48-0.59)	0.47	0.23
PTL	0.31 ± 0.01a (0.23-0.36)	0.43 ± 0.03b (0.39-0.50)	0.57 ± 0.03c (0.52-0.63)	0.76 ± 0.03d (0.71-0.84)	1.00 ± 0.06e (0.86-1.09)	0.98 ± 0.10e (0.72-1.11)	0.68	0.27

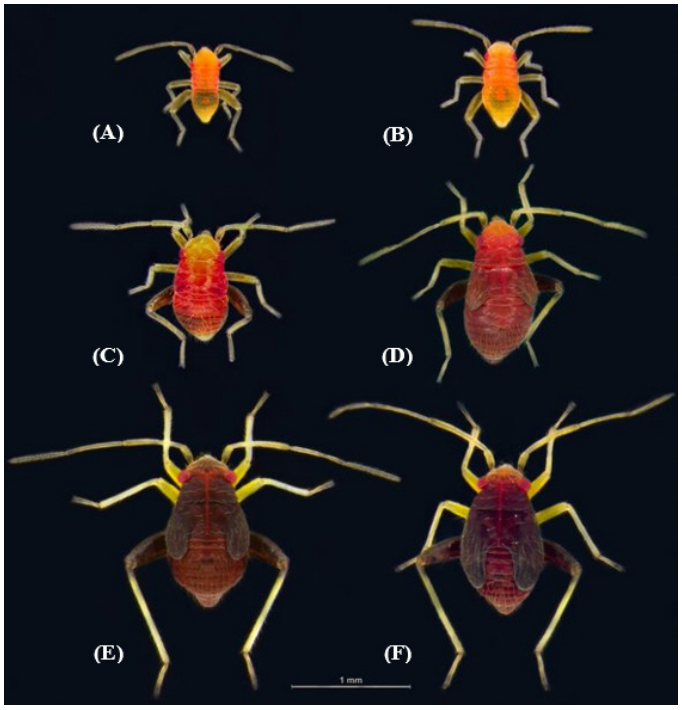

Figure 2 Five nymphal instars of *Microtechnites bractatus*: (A) first instar; (B) second instar; (C) third instar; (D) fourth instar; (E) fifth instar reduced wing pads for adult brachypterous (female); (F) elongated fifth-instar wing pads for adult macropterous (male).

average AL/BL ratio of 0.97. Antenomers IV, III, II, and I measuring 0.35, 0.22, 0.18, and 0.10 mm, respectively. Short pronotum with a PW/PL ratio of 2.21. Legs yellow-brown, with darker femurs on hind legs. Posterior tibiae of 0.42 mm, larger than in the first instar (ANOVA, Tukey, gl=5, p<0.001). Abdomen with sparse, erect, and yellowish bristles. Dorsal abdominal opening of scent glands inconspicuous.

Instar 3 (Table 1, Fig. 2C): oval body, yellowish reddish, with accentuated segmentation. Sparse, erect, and yellowish bristles, simple in appearance and not branched. Total body length, on average, 1.12 mm. Triangular head pointed between the antennas. Wider than long, with an average HW/HL ratio of 2.02. Light red eyes and interocular distance of 0.24 mm, different from the other instars (ANOVA, Tukey, gl=5, p<0.001). ID/HW ratio of 0.62, different from other instars (ANOVA, Tukey, gl=5, p<0.001). Light yellow antennae with yellow-brown antennae terminations. As long as the body, with an AL/HL ratio of 1.07 on average. Antenomers with segments IV, III, II, I measuring 0.40, 0.29, 0.24, 0.11 mm, respectively. Pronotum measuring 0.15 mm in length, similar to nymph 2 (ANOVA, Tukey, gl=5, p=0.971). Wider than long, with an average PW/PL ratio of 2.56. Meso and metathoracic segments with posterior bilateral projections, consisting of the wing pad. Reddish-brown wing pads reaching the second abdominal segment, measuring 0.17 mm, on average and WPL/BL ratio of 0.15, significantly lower than in subsequent instars (ANOVA, Tukey, gl=5, p<0.001). Legs yellowish-brown, with femurs darker in the hindquarters. Tibia measuring 0.57 cm in length, on average. Abdomen with sparse, erect, yellow bristles. Dorsal abdominal opening of odorous glands inconspicuous.

Instar 4 (Table 1, Fig. 2D): oval body, reddish-brown to yellowish in the region of the head and end of the abdomen. Sharp targeting. Sparse, erect, and yellowish bristles, simple in appearance and not branched. Total body length, on average, 1.36 cm. The triangular head is wider than long with an average HW/HL ratio of 2.23 and has prominent bright red eyes. ID/HW ratio of 0.57, lower than in previous instars (ANOVA, Tukey, df=5, p<0.001) and similar to later instars (ANOVA, Tukey, df=5, p<0.05). Light yellow antennae with brown antennae endings, longer than the body, with an average AL/BL ratio of 1.12. Antenomers IV, III, II, I measuring 0.47, 0.39, 0.37 and 0.14 mm, respectively. Pronotum measuring 0.20 mm in length, larger than nymph 3 (ANOVA, Tukey,

gl=5, p=0.0001). PW/PL ratio of 2.42, on average. Wing paddark brown, reaching the third abdominal segment and measuring 0.37 cm, different from the other instars (ANOVA, Tukey, gl=5, p<0.001). WPL/BL ratio of 0.27, different from other instars. Fore and median legs yellow. Hind legs with light yellow tibias and tarsi with brown ends, hind femurs brown to yellow-brown. Hind tibiae measuring 0.76 cm on average. Abdomen with sparse, erect, yellow bristles. Dorsal abdominal opening of odorous glands inconspicuous.

Instar 5 (Table 1, Figs. 2E and F): evident dimorphism in the wing pads in individuals that generate adult brachypterous and adult macropterous. Dark reddish-brown body, with erect yellow hairs, sparse throughout the body and denser on the abdomen. Sharp targeting. Oval body in short wing pads (SWP) is thin and elongated in individuals with long wing pads (LWP). Shorter body length in SWP subjects (1.62m) than in LWP subjects (1.74 mm (ANOVA, Tukey, gl=5, p=0.0002). Head in a triangular shape. Long, similar in brachypterous and macropterous in terms of width (ANOVA, Tukey, gl=5, p=0.05) and length (ANOVA, Tukey, gl=5, p=0.08). Prominent dark red eyes. Larger interocular in LWP individuals (ANOVA, Tukey, gl=5, p=0.0001). Similar ID/HW ratio for SWP and LWP (ANOVA, Tukey, gl=5, p=0.849). Brown apical, with similar length and appearance in SWP and LWP individuals (ANOVA, Tukey, gl=5, p=0.999). Pronotum greater in length and width compared to fourth-instar nymphs (ANOVA, Tukey, gl=5, p=0.0001), and similar among fifth-instar dimorphic nymphs (ANOVA, Tukey, gl=5, p=0.978). Wing pads darker than the body, of greater length in individuals that generate macropterous (ANOVA, Tukey, gl=5, p=0.0001), reaching the third abdominal segment in individuals that generate brachypterous fifth abdominal segment in individuals that generate macropterous. The fore and middle legs are light yellows. Hind legs with yellow tibias and tarsi and dark brown femurs. Posterior tibiae is equal in individuals that generate brachypterous and macropterous (ANOVA, Tukey, gl=5, p=0.782). Abdomen with sparse, erect, yellow bristles. Dorsal abdominal opening of inconspicuous scent glands.

Biology and life table

The nymph and adult biological parameters of *M. bractatus* were influenced by the plant that served as food for the individuals.

Nymph survival was influenced by the substrate (Mantel-Cox, df=4, p<0.05). It remained above 80% in white clover, alfalfa, and beans, reaching 50% in potatoes and less than 30% in wheat (Fig. 3). In surviving individuals, the mean duration of each instar and the nymphal stage did not vary between treatments (Kruskal-Wallis; df=4, p>0.05) (Table 1). The nymphal period of *M. bractatus* was, on average, 12 to 13 days (Table 2).

Due to the low survival rate of the nymphal stage of individuals fed on wheat, the biological parameters of the adult and egg stages were not analyzed for insects reared on this plant. Therefore, fertility and longevity data and the fertility life table were analyzed only for individuals raised on white clover, alfalfa, beans, and potatoes.

Longevity in *M. bractatus* ranged between 9 and 16 days, with an average of 12.9 days. Males and females showed similar longevity in white clover, potatoes, and alfalfa (Mantel-Cox, df=3, p>0.05), while in beans, the longevity in females were higher than in males (Mantel-Cox, df=3, p=0.000). White clover plants provided greater longevity when compared to other feeding substrates (Mantel-Cox, df=3, p>0.05) (Table 3).

Females of *M. bractatus* started the oviposition period between the third and ninth days after emergence, with an average pre-oviposition period of 5 days. On average, the oviposition period had an average duration of 7 days (1-23 days) and a post-oviposition period of 2 days (0-5 days). Host plants influenced the number of eggs laid per day and fecundity. Adults feeding on white clover laid more eggs per day (Kruskal-Wallis; df=3, p<0.05) and had higher fecundity (Kruskal-Wallis; df=3, p<0.05). Furthermore, eggs deposited by these females were more viable than those from females kept in alfalfa, beans, or potatoes (Kruskal-Wallis; df=3, p<0.05). The incubation period had an average duration of 10 days in all treatments, ranging from 9 to 13 (Kruskal-Walis; df=3, p>0.05) (Table 3).

The plants on which the insects fed did not influence the mean generation time (T), the intrinsic population growth rate (Rm) or the population doubling time (Dt) (Table 3). However, the net reproduction rate (R_0) and the finite population increase ratio (λ) showed a statistical

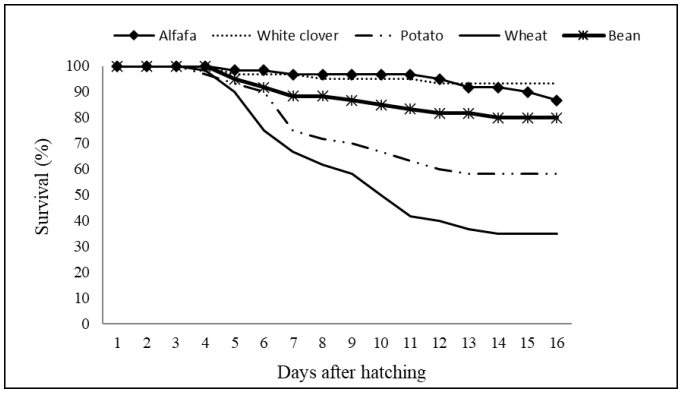

Figure 3 Average survival of *Microtechnites bractatus* nymphs fed with alfalfa, white clover, potato, wheat, and beans under laboratory conditions (25 ± 2°C, photoperiod 12L:12D). *Lines followed by different letters differ from each other by the paired Mentex-Cox test (Log Rank) (df=1, p<0.05).

Table 2
Mean duration ± standard deviation (days) of instars of *Microtechnites bractatus* nymphs, fed on white clover, alfalfa, beans, potatoes, and wheat (25 ± 2ºC, photoperiod 12L:12D).

Treatments	Duration (days)					
	I Instar	II Instar	III Instar	IV Instar	V Instar	Total*
White clover	3 ± 0.18a* n=60	2 ± 0.29a n=60	2 ± 0.22a n=58	2 ± 0.26a n=57	3 ± 0.49a n=57	12 ± 0.65a n=56
Alfafa	3 ± 0.40a n=60	2 ± 0.45a n=60	2 ± 0.57a n=58	2 ± 0.54a n=58	3 ± 0.59a n=56	13 ± 0.82a n=52
Beans	3 ± 0.30a n=60	2 ± 0.40a n=59	2 ± 0.34a n=54	2 ± 0.42a n=53	3 ± 0.63a n=51	12 ± 2.50a n=48
Potatoes	3 ± 0.44a n=60	2 ± 0.42a n=57	2 ± 0.56a n=49	2 ± 0.70a n=40	3,5 ± 0.79a n=38	13 ± 1.10a n=34
Wheat	3 ± 0.79a n=60	2 ± 0.68a n=52	2 ± 0.86a n=40	2 ± 0.84a n=31	3 ± 0.71a n=23	13 ± 1.36a n=21

**Means followed by the same letters in the column do not differ statistically (Kruskal-Wallis, df=4, p>0.05).*

Table 3
Biological parameters (Mean±standard deviation, minimum and maximum value) of adults and eggs of *Microtechnites bractatus* fed with white clover, alfalfa, beans, and potatoes under laboratory conditions (25 ± 2ºC, photoperiod 12L:12D).

Treatments	Longevity (days)			Fecundity***	No of eggs per day***	Viability*** (%)	Incubation period*** (days)
	♀	♂	Mean**				
White clover	15.9 ± 0.9 (7-28)	16.4 ± 1.2^{ns} (5-29)	16.1 ± 0.7a (5-29)	53.0 ± 5.8a (0-134)	9.40 ± 0.7a (1-30)	88a	10 ± 0.1a (9-13)
Alfafa	11.8 ± 0.8 (3-23)	12.1 ± 1^{ns} (3-24)	11.9 ± 0.6b (3-24)	29.0 ± 4,6b (0-87)	7.42 ± 0.7a (1-24)	72b	10 ± 0.1a (9-12)
Beans	13.9 ± 1.0 (5-25)	9.1 ± 0.7* (5-19)	11.5 ± 0.7b (5-25)	12.5 ± 6.1c (0-137)	5.64 ± 0.7b (1-24)	80ab	10 ± 0.2a (10-13)
Potatoes	12.2 ± 1.0 (3-27)	11.5 ± 1^{ns} (4-27)	11.9±0.7b (3-27)	10.5 ± 3.9c (0-76)	4.04 ± 0.5b (1-15)	76ab	10 ± 0.2a (9-14)

**Indicates the statistical difference between males and females in the same treatment (Mantel-Cox, df=1, p<0.05). ^{ns}indicates non-significant differences between sexes in the same treatment. Means followed by the letter in the column do not differ statistically. **Mantel-Cox, df=3, p<0.05. ***Kruskal-Wallis, df=3, p<0.05*

Table 4
Average generation duration (T) of *Microtechnites bractatus*, net reproduction rate (R_0), intrinsic population growth rate (rm), finite increase ratio (λ) and doubling time (Dt). Values in parentheses indicate parameters estimated by Bootstrap. (25 ± 2°C, photoperiod 12L:12D).

Treatments	Parameter				
	T	R_0	rm	λ	Dt
White clover	32.17 (29.62-35.00) a	26.77 (14.90-43.58) a	0.102 (0.079-0.126) a	1.11 (1.09-1.13) a	6.9 (5.53-8.79) a
Alfafa	31.59 (29.39-34.21) a	15.14 (7.53-31.17) ab	0.086 (0.059-0.113) a	1.09 (1.06-1.12) ab	8.06 (6.16-11.66) a
Beans	33.24 (30.82-36.12) a	11.81 (7.43-17.13) ab	0.074 (0.056-0.090) a	1.07 (1.06-1.09) ab	9.33 (7.69-12.21) a
Potatoes	32.06 (29.55-34.65) a	7.64 (3.98-14.66) b	0.063 (0.041-0.089) a	1.07 (1.04-1.83) b	10.93 (8.05-17.97) a

Life-table parameters and the respective confidence intervals calculated by bootstrap, confidence intervals followed by the same letter do not differ (significance 95% from 10,000 simulations).

difference between the white clover and potato treatments. In turn, alfalfa and bean plants did not differ from the other treatments (Table 4).

Discussion

Many species of Miridae are reported in plants grown in the Neotropical region, and their occurrence has been increasingly frequent in agricultural environments (Ferreira et al., 2001; Nogueira et al., 2019; Ferreira et al., 2021). Studies involving the morphology of immature stages can provide the early identification of insects and the rapid adoption of management strategies.

In the present study, the immature stages of *M. bractatus* showed variations in color and size. Eggs vary in color throughout embryonic development. In *M. bractatus*, the egg morphology is similar to that seen in other species of the group (Hinton, 1981; Ma et al., 2002; Perdikis and Lykouressis, 2002; Pires et al., 2010), elongated and slightly curved, with a yellowish color that becomes reddish during embryonic development (Figs. 2A and B). They are endophytically deposited and remain partially exposed with the operule exposed (Figs. 2C and D), as occurs in *Campyloneura virgula* (Herrich-Schaeffer) (Cobben, 1968). For the eggs of *M. bractatus*, the present study corroborates the observations of Beyer (1921) and Capinera (2001), who report the eggs of *Halticus citri* (syn. n *M. bractatus*) with a yellowish-white color, with measurements similar to those verified here.

Nymphs of *M. bractatus* differ among instars in terms of color, segmentation, and morphometric parameters. Along the instars, the color of the nymphs evolves from pinkish yellow to dark reddish-brown, and the segmentation becomes accentuated. Erect and pale bristles are present in all instars, although denser in the fifth instar nymph. The legs are yellow in the first instar, maintaining the color throughout development, although the posterior femurs and tibia ends are brown in the later instars. The reddish body coloration, from the second instar, does not coincide with that described by Beyer (1921) and Capinera (2001), who describe the nymphs of *Halticus citri* (syn. n. *M. bractatus*) with green body coloration light to dark green. Such variations may be due to the substrate on which the insect feeds (Schwertner et al., 2002; Matesco et al., 2009) and, although the works of those authors have not described the plant that served as food for the nymphs, it is suggested that further studies are needed to relate the feeding with the color patterns of *M. bractatus* nymphs. In the present study, the immatures used for the morphological description were fed beans and did not seem to vary in color compared to those fed with alfalfa, white clover, potatoes, and wheat.

In several studies, the separation of instars in Miridae is based on external characteristics, such as the length of antennal segments, wings, and the presence of spots or appendages on the body (Perdikis and Lykouressis, 2002). Capinera (2001) and Beyer (1921) separated the five instars of *H. bractatus* and *H. citri* [syn. n. *M. bractatus*], respectively, by the length of the nymphs. However, in addition to biological studies, other measurements obtained in this study may provide greater precision for the morphometric characterization of immatures cause pointed out differences between the instars, like the body length, head width, intraocular distance, antenna, and antenomer length, pronotum length and width, tibia length and the relationship between the interocular distance and the head width and wing pads and body length.

In the fifth instar nymph, dimorphism in the wing pads is evident in *M. bractatus*, which is also related to the shape and size of the body. Individuals that generate brachypterous adults have greater body length and a rounded apical portion of the abdomen. In turn, individuals that generate macropterous adults are smaller, with a tapered and elongated abdomen. In addition to total length, interocular distance, wing pads size, and the ratio of wing pads to body length are significantly different among fifth-instar dimorphic individuals. These observations are being recorded for the first time for *M. bractatus* and may represent an additional way to differentiate male and female individuals. Regarding the length of wing pads, in the present study, this characteristic was related to the sex of adults since nymphs with short wing pads generated only females and nymphs with long wing pads generated only males. Despite this, it is not yet possible to affirm sexual dimorphism in fifth-instar nymphs, as Tatarnic and Cassis (2012)

indicate the occurrence of both females and males in both brachypterous and macropterous. In immature Miridae, few characters can help in the early separation of the sexes, and sexual dimorphism is associated with the presence of sensilla in the antennae of fifth-instar nymphs in *Lygus lineolaris* (Palisot de Beauvois) (Chinta et al., 1997). However, the characterization of sexual dimorphism would be of great value for biological and behavioral studies. It is necessary to identify the sex of individuals early and isolate them before sexual maturation, such as studies on sexual behavior, for example.

Plants offered to *M. bractatus* affected the biological parameters of survival in the nymphal stage, fecundity, egg stage viability, net reproduction rate, and finite rate of population increase. Insects were able to develop, reproduce and complete development in plants, except in wheat which provided mortality above 70% in the nymphal stage, originating deformed and unviable adults. On the other hand, white clover provided superior insect performance in most biological parameters evaluated.

In the nymphal period, survival was significantly higher in individuals fed on the three Fabaceae (white clover, alfalfa, and beans) in relation to the other plants (potatoes and wheat). The duration of instars and nymphal stage was not influenced by host plants for those who survived. In the adult phase, insects feeding on white clover were more fertile than in other plants, demonstrating greater suitability of this legume as a host of *M. bractatus*. In terms of population, the factors affected by the host plants were the net reproduction rate and finite increase ratio, which were higher in white clover than in potatoes. Thus, *M. bractatus* populations would have greater reproductive and population growth potential if fed with white clover, for example.

M. bractatus is a polyphagous species and many economic important plants are been recorder as host plants, such as potato (*Solanum tuberosum*) (Henry, 1983; Capinera, 2001), tomato (*Solanum lycopersicum*) (Capinera, 2001; Ferreira et al., 2015; Nogueira et al., 2019), eggplant (*Solanum melongena*) (Henry, 1983; Capinera, 2001), tobacco (*Nicotiana tabacum*) (Henry, 1983; Nogueira et al., 2019) pepper (*Capsicum* spp.) (Capinera, 2001; Nogueira et al., 2019), crotalaria (*Crotalaria* spp.) (Ribeiro et al., 2020), cucumber (*Cucumis sativus*) (Capinera, 2001; Nogueira et al., 2019), pumpkin (*Curcubita* spp.) (Capinera, 2001; Nogueira et al., 2019), alfalfa (*Medicago sativa*) (Henry, 1983; Ferreira et al., 2015; Nogueira et al., 2019), beans (*Phaseolus vulgaris*) (Henry, 1983; Capinera, 2001; Nogueira et al., 2019; Ribeiro et al., 2020) clover (*Trifolium* spp.) (Henry, 1983; Capinera, 2001; Ferreira et al., 2015; Nogueira et al., 2019) pea (*Pisum sativum*) (Capinera, 2001), oats (*Avena sativa*) (Henry, 1983; Nogueira et al., 2019), wheat (*Triticum* spp.) (Henry, 1983; Nogueira et al., 2019), corn (*Zea mays*) (Henry, 1983; Nogueira et al., 2019), barley (*Hordeum vulgare*) (Henry, 1983; Nogueira et al., 2019), sugar beet (*Beta vulgaris*) (Capinera, 2001), cabbage (*Brassica oleraceae*) (Capinera, 2001; Nogueira et al., 2019), lettuce (*Lactuca sativa*) (Capinera, 2001), cotton (*Gossypium* spp.) (Henry, 1983; Nogueira et al., 2019) among others. Through to this large number of host species, *M. bractatus* has a great potential to become an agricultural pest. Understanding the relationships between herbivorous insects and their host plants contribute in the comprehension of dynamics of insect behavior concerning their habitat, feeding habits, and other correlations with their hosts (Tscharntke and Brandl, 2004). This information provides important ecological knowledge and clarifies the impact that insects can have on crops (Nogueira et al., 2019). The plants tested here are of great agricultural importance and allows the properly development and reproductive of *M. bractatus*. This is a matter of concern, since their feeding can prevent the growth of alfalfa and white clover, and the plants can dry out under severe attacks (Beyer, 1921; Wheeler, 2001). In addition, severe attacks by *M. bractatus* destroyed production areas of tomatoes on the west coast of Mexico (Morrill, 1925).

Among the host plants of *M. bractatus*, Fabaceae are predominant in occurrence records (Nogueira et al., 2019), showing better resources for the survival of immature stages and greater fecundity in adults (Table 3). Potatoes (Solanaceae) and wheat (Poaceae) caused high immature mortality, although the survivors reached adulthood at a similar time to the other treatments. In the adult stage, although in smaller numbers, insects fed with potatoes developed and generated descendants. Therefore, this implies that even resulting in smaller populations, mirid can survive in winter crops such as wheat or potato in the absence of more favorable hosts. Considering the polyphagous habit of *M. bractatus*, which is listed for more than 45 plant species (Capinera, 2001; Schuh, 2013; Nogueira et al., 2019; Ferreira et al., 2021), further biological studies are needed to understand the dynamics of insect-plant relationships.

Our results contribute to knowledge in methods and techniques for creating and evaluating biological data of mirid species in the laboratory. Moreover, the morphological and morphometric description of nymphs can subsidize new studies about *M. bractatus*. In addition, the fertility life table provided (Table 3) is the first one made for this specie. It is a fundamental tool for studying population ecology as it allows population growth dynamics over generations to be estimated, summarizing insect populations' survival and reproductive potential on different hosts and environmental conditions (Panizzi and Parra, 2009; Akköprü et al., 2015; Ning et al., 2017).

Acknowledgments

The authors are grateful to Arodi Prado Favaris for the support in obtaining insect images and morphometry data. The authors also thank the anonymous reviewers for their helpful comments.

Funding

This research was supported by the National Institute of Science and Technology – Semiochemicals in Agriculture [FAPESP and CNPq – Grants #2014/50871-0 and #465511/2014-7, respectively]. L.K.R was sponsored by Coordination for the Improvement of Higher Education Personnel [CAPES Finance Code #001].

Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

Project Administration and funding acquisition, CN; Methodology, Validation, and original draft preparation, LKR and CN; Methodology and validation, CAL and ACJ; Review and Editing, ACJ, JTVR, and JMSB; Statistical Analysis, ACJ; Insect identification, formal analyses and review, PSFF.

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