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BIOLOGY AND PREFERRED OVIPOSITION SITE OF THE *MAHANARVA INDENTATA* FROGHOPPER (HEMIPTERA: CERCOPIDAE) ON SUGARCANE

Vinicius De Vicente Chaves[1], Guilherme Vieira Pimentel[1], Alejandro Hipólito Pabón Valverde[1], Lidiane Aparecida Silva[1], Márcio Henrique Pereira Barbosa[1,*] and Luiz Alexandre Peternelli[2]
[1]Department of Agronomy, Universidade Federal de Viçosa, 36570-000, Viçosa, Minas Gerais State, Brazil

[2]Department of Statistics, Universidade Federal de Viçosa, 36570-000, Viçosa, Minas Gerais State, Brazil

*Corresponding author; E-mail: barbosa@ufv.br

Abstract

The froghopper *Mahanarva indentata* (Walker) (Hemiptera: Cercopidae) is an economically important pest of sugarcane in Brazil. With the purpose of complementing various biological studies of the froghopper species associated with sugarcane, this research was developed to describe the morphology, and development of the eggs, nymphs and adults of *M. indentata*, as well as aspects of its reproductive biology and oviposition site preference. The study was conducted in a greenhouse of the Department of Agronomy, Federal University of Viçosa (UFV). Sugarcane plants of the cultivar, 'RB867515', at 60 days after planting were used to feed the insects. The information was obtained at 26 ± 2 °C, 70% ± 10% RH and 14:10 h L:D. Mean egg development lasted 36 days with 95% of egg viability. The nymphal phase with 5 instars lasted an average of 62 days with 65.84% survival. Adult males and females lived 12 and 20 days respectively. The durations of the pre-oviposition, oviposition and post-oviposition periods were 9.57 ± 0.80 days, 7.78 ± 1.14 days and 2.60 ± 0.57 days, respectively. Average fecundity was 38.2 eggs per female and total generation time (adult - egg) was 99 days. All adult females showed an oviposition preference for the abaxial surface of the sugarcane leaf and specifically for the basal portion of the leaf where 72% of the eggs were deposited. The newly acquired information on *M. indentata* reinforces the need to define differences between the various *Mahanarva* species related to their biology and management, since this is a very important pest of the sugarcane production in Brazil.

Key Words: embryonic development, fecundity, froghopper, longevity, *Mahanarva fimbriolata, Mahanarva posticata,* nymphs, *Saccharum*

Resumo

A cigarrinha *Mahanarva indentata* (Walker) (Hemiptera: Cercopidae), destaca-se como praga de importância econômica para os canaviais do Brasil devido sua ampla distribuição nos Estados do Paraná, São Paulo, Minas Gerais e Bahia. Com o propósito de complementar os estudos da biologia de espécies de cigarrinhas associadas à cana-de-açúcar, desenvolveu-se essa pesquisa para descrever a morfologia, as durações das fases de ovo, ninfa e adulto além dos aspectos da biologia reprodutiva e da preferência por sítio de oviposição da *M. indentata.* O bioensaio foi conduzido em casa de vegetação do Departamento de Fitotecnia da Universidade Federal de Viçosa (UFV). Plantas de cana-de-açúcar da variedade RB867515 com 60 dias após o plantio foram utilizadas para alimentação dos insetos. Os dados foram obtidos sob temperatura de 26 ± 2 °C, UR 70% ± 10% e fotoperíodo de 14 horas. A duração da fase de ovo foi de 36 dias e viabilidade de 95%. A fase de ninfa, com cinco instares, teve duração média de 62 dias e viabilidade de 65,84%. As longevidades de machos e de fêmeas foram de 12 e 20 dias respectivamente, com período de pré-oviposição de 9 dias e oviposição de 7 dias. A fecundidade média foi de 38,2 ovos/fêmea e a duração do ciclo de vida (ovo-adulto) de 99 dias. As fêmeas não exibiram flexibilidade pelos substratos de oviposição, com 100% de preferência pela face abaxial das folhas de plantas de cana-de-açúcar. Quanto ao sítio de oviposição na folha, detectou-se a preferência pela região basal com 72% dos ovos. Na região intermediária encontraram-se 28% dos ovos, enquanto que no ápice da folha não houve oviposição. Novas informações a partir desta espécie expande nossa compreensão da necessidade de delinear diferenças comuns entre as espécies de *Mahanarva* com relação à sua biologia, diante da importância que essa praga apresenta na cultura da cana-de-açúcar no Brasil.

Palavras-Chave: desenvolvimento embrionário, fecundidade, cigarrinha, longevidade, *Mahanarva fimbriolata, Mahanarva posticata,* ninfas, *Saccharum*

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Several species of froghopper or spittlebugs (Hemiptera: Cercopidae) are general pests of sugarcane and gramineous forage plants in the Neotropical region (Thompson 2004; Carvalho & Webb 2005). *Mahanarva bipars* (Walker) and *Mahanarva andigena* (Jacobi) occur on sugarcane in South America (Rodríguez & Peck 2007). Among froghoppers associated with the sugarcane in Brazil, 3 *Mahanarva* species are prominent because of the economic importance of their damage; these are: *M. fimbriolata* (Stål), *M. posticata* (Stål) and *M. indentata* (Walker) (Mendonça 1996; Dinardo-Miranda 2008). The damage caused by *M. indentata* is similar to that of *M. fimbriolata* and *M. posticata*. High infestations of *M. indentata* cause large economic loss because this pest causes necrosis of the leaves, shortening of the internodes of the plant and reduction up to 50% on the sucrose content (Pinto et al. 1993; Mendonça 1996).

The temperature conditions (26 °C–30 °C) and high humidity (80% RH) favor the development of froghoppers in the field. Dinardo-Miranda (2003) reported that in the state of São Paulo (SP), southeastern Brazil, the life cycle of *M. fimbriolata* begins in Sep/Oct with the nymphal populations reaching their climax in Dec/Jan and decreasing significantly in Apr with the end of the rains and the beginning of winter. In the state of Minas Gerais (MG), southeastern Brazil, the *M. indentata* is more abundant from Oct to Mar, which corresponds to the period with higher temperatures and more rainfall (Barbosa et al. 1980; Mendonça et al. 1996).

Nymphs of *M. fimbriolata* develop in the soil on adventitious roots, and can also occur in the culm of the plant (Dinardo-Miranda 2008), unlike *M. indentata* that develops in the cartridge, where it remains until adult emergence (Mendonça et al. 1996). Population fluctuations of froghoppers can vary significantly because of the oviposition site and the farming practices used in sugarcane plantations. *Mahanarva posticata* lays its eggs in the leaf sheaths (Mendonça et al. 1996), whereas *M. fimbriolata* lays its eggs on the soil surface and over the sugarcane stubble (Dinardo-Miranda 2003).

According to Dinardo-Miranda (2008), *M. fimbriolata* has become a pest in the state of São Paulo at the end of the 1990's decade when mechanical harvesting of sugarcane without first removing leaves by fire was developed. In this harvest system, the accumulation of the leaves contributes to maintaining the humidity of the soil, which favors the population growth of this species. Moreover, removal of the leaves with fire contributed to the destruction of a froghoppers' eggs. Current harvesting practices can also promote increases in the populations of other species of froghoppers associated with sugarcane.

Although *M. indentata* is a pest of economic importance in the sugarcane plantations of Brazil, progress in developing population management measures for it have been limited by the lack of basic biological information. Studies on the morphology and biology of this insect are very important, since such essential information is relevant to pest management, predicting potential damage, and elucidating population dynamics. The aim of this study was to elucidate the biology and preference of oviposition sites on various substrata of *M. indentata* by quantifying female fecundity, and the durations of the eggs, nymphs, and adults.

Materials and Methods

Insects

The research was conducted in a greenhouse of Agronomy Department, of Federal University of Viçosa (UFV), from Oct 2010 to Mar 2011. *Mahanarva indentata* froghoppers were collected by net in sugarcane plantations of the Sugarcane Research and Improvement Center, located in the municipality of Oratórios (S 20° 24' 14" W 42° 48' 44"), Zona da Mata region, state of Minas Gerais, Brazil. Specimens were sent to G. S. Carvalho (Pontific Catholic University of Rio Grande do Sul, Porto Alegre, Brazil) who confirmed the taxonomic identification of the froghoppers species. The froghopper adults were placed in cages made with delaine fabric (80 × 100 × 50 cm) together with sugarcane plants for feeding, mating and oviposition. This work was conducted in a greenhouse at 26 ± 2 °C, 70% ± 10% RH and 14:10 h L:D by means of the techniques of Garcia et al. (2007).

Sugarcane Plants

'RB867515' sugarcane plants were used in this study because of the high levels of *M. indentata* nymphal and adult infestation observed on this cultivar. In the greenhouse, the 'RB867515' sugarcane culms were reproduced vegetatively on polyethylene trays (28 cm × 42 cm × 7.5 cm) with organic plant growth medium. The plants were kept on these trays until 60 days after planting when they had a height of about 30 cm and had 5 totally expanded leaves. Subsequently, these seedlings were moved to experimental units made of PVC pipes (6 cm diam × 6.5 cm length), wrapped with acetate (6 cm diam × 50 cm height) pierced with approximately 100 holes (1 mm diam) to allow ventilation. The acetate was fastened onto the PVC pipes by means of rubber bands placed on its edges. In order to guarantee total sealing of the experimental unit in order to prevent the escape of the insects, another PVC pipe with a lid was put on the top part of the acetate. The sugarcane seedlings of the experimental units served as a source of food and sites for oviposition.

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Egg Morphology

Egg experiments were conducted under controlled conditions of hatching (25 °C and 100% RH). The eggs were placed in Petri plates (6 cm diam, 2 cm height), wrapped with filter paper moistened with distilled water. The experiment was completely randomized with 5 repetitions, each with 50 eggs/Petri dish. Morphological measurements were done (length and width) of the egg development phases by stereomicroscope.

Morphology of Nymphs

The nymphs were raised on sugarcane plants in the greenhouse (26 ± 2 °C, 70 ± 10% RH, and photoperiod of 14:10 h L:D). Sugarcane plants propagated vegetatively, i.e., cuttings of the cultivar 'RB867515' were planted in experimental units made with PVC pipes. At 60 days after planting, each plant was infested with an insect. Nymphs of the first instar that had just hatched were placed on the base of the plant. According to Kuenzi & Coppel (1985) and Peck (1998), the change from one instar to another could be determined by direct observation of presence of exuviae on the nymphs. A total of 237 nymphs were evaluated by stereomicroscope with a micrometric ocular. For each instar, we determined the width of the head capsule (longest distance between the compound eyes) and the length of the body (maximum distance from the front end of the head to the end of the abdomen).

Fecundity and Morphology of the Adults

In order to determine longevity and fertility, teneral adults were placed on sugarcane plants placed in experimental units each made of PVC pipe. In order to calculate the fecundity (number of eggs/female), the oviposition period was considered to be the time between the first and the last observed ovipositon. Thirty five pairs were assembled each one with 1 male and 1 female, and each day the number of live and dead adults were recorded. The morphological measurements (width of the head capsule, width of the body, length of the body with and without the wings) were made after death for both genders by a stereomicroscope with a micro-metric ocular.

Egg, Nymph and Adult Biology

Aspects of the insect's biology evaluated were as follows: egg – duration of the developmental period phases in the embryo and egg viability; nymph – duration of the nymphal phases; adult – time before oviposition, during oviposition and post oviposition, fecundity and longevity.

Preferences for Substrates and Oviposition Sites

In order to establish the preference of the *M. indentata* female for the oviposition substrate, 3 options were considered: uncovered soil, surface of the plant stem and surface of the plant leaf. Sixty-day sugarcane plants were used. Each plant was infested with a pair of teneral adults placed in cages made with PVC pipes previously described. After 20 days of infestation, the eggs on each substrate were collected. The eggs were extracted from the soil by flotation in a 30% saline solution described by Lapointe et al. (1989). The substrates were directly observed in those cases in which the eggs were found on the stem and leaf surfaces. As the leaf eggs were regained, it was noticed the pattern on the plant leaf according to Kuijiper method: leaf + 1, leaf 0, leaf -1 and the cartridge leaf (Cheavegatti-Gianotto 2011), as well as the location of the eggs on the leaves: basal, intermediate and top area.

Statistical Analysis

All the experiments were completely randomized. The results were subjected to an analysis of the variance. Means were separated by Tukey's test at the level of 5% of significance. The t-test was used to compare the body size between males and females. Analyses were performed with the R software (R Core Team 2012).

Results

Eggs

Mahanarva indentata eggs (Fig. 1A) went through 4 distinguishable phases. The first phase (S1) was described by the appearance of the line of the hatching on the chorion, on the front side of the egg. The formation of the dark elliptic area below the line of the hatching and the appearance of a red spot near the front side of the egg, distinguished the second phase (S2). An external characteristic of the third phase (S3) was the opening of the chorion along the line of hatching, which left the operculum exposed. During the fourth phase (S4), the embryo exhibited red ocelli and abdominal spots near the front and the back of the egg, respectively. Eggs were light yellow when laid becoming orange shortly before the time of hatching.

During the egg phase, externally visible alterations of color and size of the embryo occurred, which resulted in increases in length and width of 30.8% and 32.8%, respectively, from S1 to S4 (Table 1). The eggs showed an average viability (± SE) of 94.8 ± 1.27% in an average period of hatching of 36.43 ± 1.00 days, with a range of 25 to 54 days. SI had the longest duration (17.23 ± 0.19 days), which constituted 47.3% of the total duration (Fig. 2).

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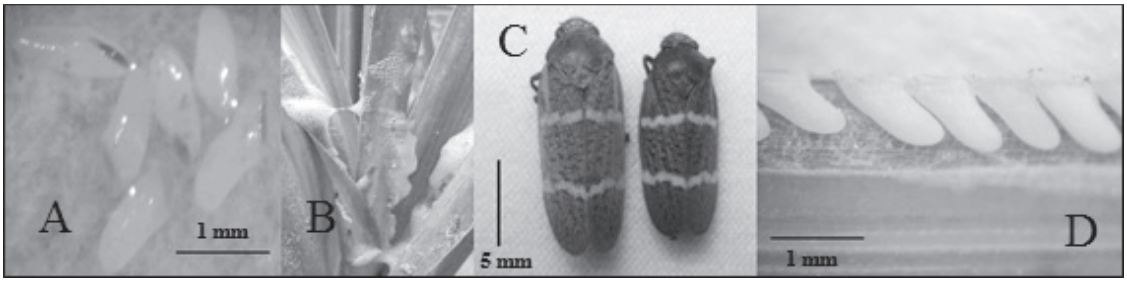


Fig. 1. Life cycle stages of *Mahanarva indentata*. (A) Eggs. (B) Nymphs. (C) Adults, dorsal view: female (left.) and male (right). (D) Eggs inserted into the sugarcane leaf.

Nymphs

The yellow nymphs remained inside the internal part of the cartridge of the sugarcane plants, during the entire cycle, and they were always enclosed in a dense foam (Fig. 1B). The change from one phase to the other was marked by molting, i.e., shedding of the exuvium, and we determined the instar number by the width of the head capsule (Fig. 3). The width of the head capsule and the length of the body of nymphs increased 78.9% and 82.1 % from the 1st to the 5th instar, respectively (Table 2). The average time of the total nymphal phase was 62.62 ± 2.46 days, with the 5th instar having the longest duration of 20.62 ± 1.40 days, which is 33% of the nymphal period (Fig. 4).

Adults

Mahanarva indentata adults displayed sexual dimorphism expressed by the larger size of the female in all of the morphological characters evaluated (Table 3). The character which showed the greatest difference was head capsule width, which was 11.6% greater in females. The sex ratio was 1:1. The males displayed dark brown wings with 2 transverse orange stripes, while the females displayed light brown wings and two transverse orange stripes color similar to the male insects (Fig. 1C). The average life span of adults was 15.63 ± 0.87 days, being 19.97 ± 1.62 and 12.30 ± 0.50 days for females and males, respectively. The durations of the pre-oviposition, oviposition and post-oviposition periods were 9.57 ± 0.80 days, 7.78 ± 1.14 days and 2.60 ± 0.57 days, respectively. The average fecundity was 38.21 ± 5.09 eggs/female. The duration of the *M. indentata* cycle, (egg to adult) was 99.05 ± 1.73 days.

Substrate and Oviposition Site Preference

Female strongly preferred the leaves of the sugarcane as the oviposition substrate because 100% of the eggs were recovered from them. The eggs laid were inserted into the plant tissue obliquely in rows from 1.5 to 3 cm long on the central vein of the abaxial surface of the leaf (Fig. 1D). On the -1, 0 and +1 leaves, at the upper part of the plants, 93% of the eggs were recovered, whereas the remaining 7% were found on the cartridge leaf. The females strongly preferred the basal portion of the leaf, where 72% of the eggs were deposited, only 28% were deposited on the intermediate portion and none were deposited on the top portionof the leaf. (Fig. 5).

Discussion

The morphological characteristics of the *M. indentata* eggs during the 4 phases of development were similar to those described by Peck et al. (2004a) for *Aeneolamia flavilatera* Urich froghopper's eggs. The average egg dimensions were 1.56 mm in length and 0.58 mm in width, similar to those recored for this species by Pinto et al. (1993). The average size of the *M. indentata* eggs was about 20% greater than those of *Mahanarva andigena* (Rodríguez & Peck 2007). The

Table 1. Lengths and widths (mm; Mean ± SE, N = 250) of the embryo of *Mahanarva* indentata during the four phases of embryonic development.

	Phase of Embryonic Development			
Characteristics	S1	S2	S3	S4
Length	1.19 ± 0.005 a	1.65 ± 0.005 b	1.70 ± 0.007 c	1.72 ± 0.006 c
Width	0.47 ± 0.01 a	0.52 ± 0.003 b	0.66 ± 0.004 c	0.70 ± 0.003 d

In each row, averages followed by different letters are significantly different (α = 0.05), according to Tukey's test.

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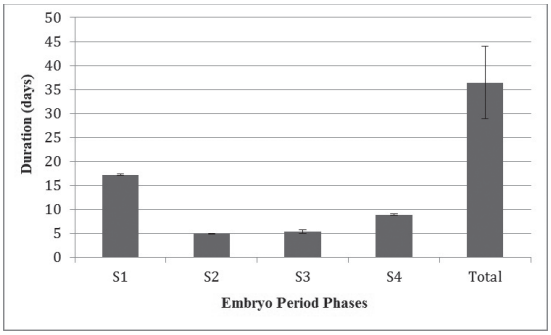


Fig. 2. Duration (days) of the phases of embryonic development of *Mahanarva indentata* (n = 250).

viability of the eggs averaged 90%, similar to that previously reported by Pinto (1993), and close to the 93.5% viability of *M. posticata* eggs (Barbosa 1986) and greater than the 81.0% of *M. fimbriolata* eggs (Garcia et al. 2006).

The average time for *M. indentata* eggs to hatch was 36.4 days, and a similar duration as quoted by Barbosa et al. (1980) for the same species. However, the duration for the egg phase found in this study was expressively longer than the one recorded for the eggs of the *M. posticata* with 17 days (Barbosa 1986, Mendonça et al. 1996), for *M. fimbriolata* with 21 days (Mendonça et al. 1996) and for *M. andigena* with 16.4 days (Rodríguez & Peck 2007).

According to several reports (Koller & Honer 1993; Sujii et al. 1995), the range of the duration for eggs to hatch of most froghoppers, in conditions appropriate for the embryonic development, is from 15 to 60 days. The range found for *M. indentata* in this study was from 25 to 54 days.

The morphological characteristics of the 5 *M. indentata* instars were similar to those described by Kuenzi and Coppel (1985) for *Clastoptera arborina* Ball froghopper nymphs. Head capsule width allowed us to identify the 5 instars, which in accordance with Dyar's law, showed a regular geometric progression of 1.47-fold between successive instars. Pinto et al. (1993) reported that the entire nymph phase of *M. indentata* is passed between the leaves of the sugarcane – unlike *M. posticata*, *M. andigena* and *M. bipars* (Mendonça et al. 1996; Peck et al. 2004b; Rodriguez & Peck 2007) nymphs, which are also located on the plant leaves. Nevertheless nymphs of the latter species remained on the cartridge area only until the second or third instar, when they descended to the leaf sheath to complete their nymphal development.

The duration of the *M. indentata* fifth instar was prolonged, similar to that of *M. posticata*

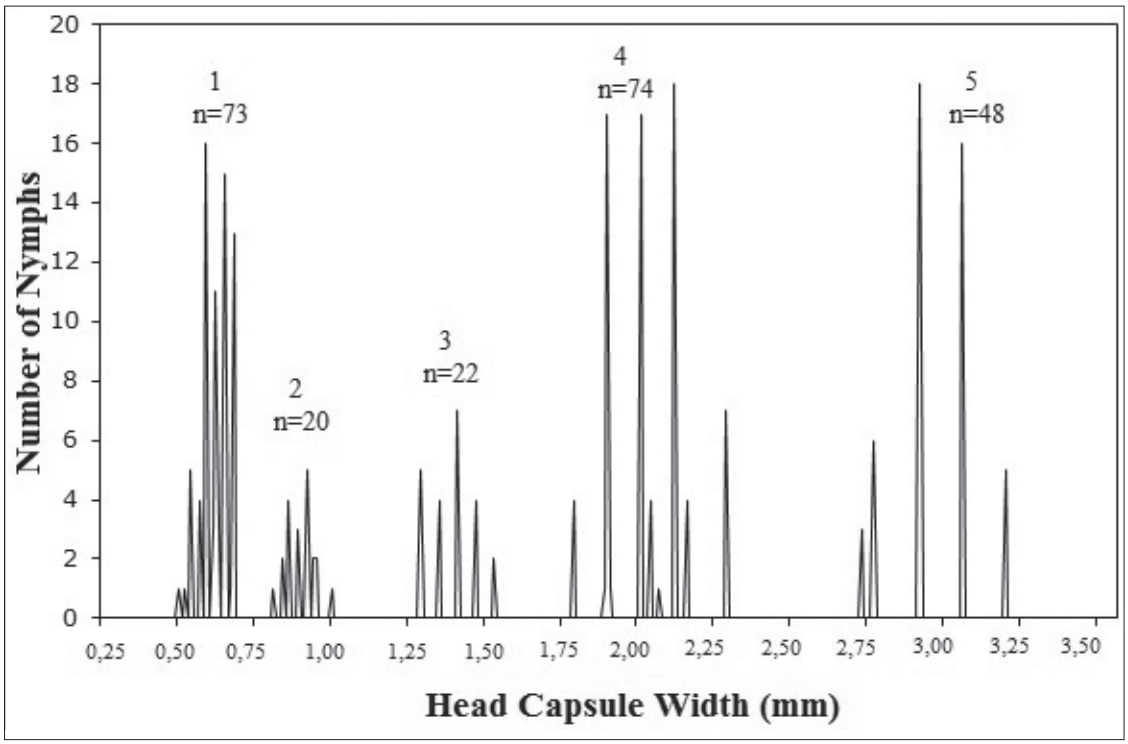


Fig. 3. Frequency distributions of the head capsule widths of *Mahanarva indentata* during the 5 instars of development.

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TABLE 2. WIDTH OF THE HEAD CAPSULE AND LENGTH (MM) OF THE BODY OF EACH OF THE FIVE *MAHANARVA INDENTATA* INSTARS (MEAN ± SE, *N* = 20 TO 70).

Instar	Head Capsule Width	Length body
I	0.61 ± 0.005 a	2.71 ± 0.04 a
II	0.89 ± 0.01 b	4.16 ± 0.13 b
III	1.39 ± 0.01 c	6.74 ± 0.14 c
IV	2.02 ± 0.01 d	10.16 ± 0.18 d
V	2.90 ± 0.01 e	15.20 ± 0.27 e

In each column, averages followed by different letters are significantly different ($\alpha = 0.05$), according to Tukey's test.

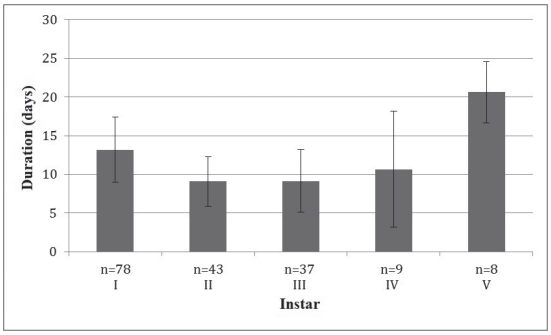


Fig. 4. Durations (days; means ± SE) of the 5 nymphal stadia of M*ahanarva indentata*.

(Barbosa 1986) and *M. andigena* (Rodríguez & Peck 2007). The average nymphal period of *M. indentata* was 62.6 days, similar to the average of 61 days observed by Mendonça et al. (1996), which was much longer than that of other froghopper pest of sugarcane, i.e., 37.1 days, 39.7 days and 48.3 days for *M. fimbriolata*, *M. posticata* and *M. andigena*, respectively (Barbosa 1986; Rodriguez & Peck 2007), but close to the 60-day duration recorded for the *M. bipars* (Gómez et al. 2007).

Mahanarva indentata adults were sexually dimorphic in color and body size. The female live longer life than males, and underwent a sexual maturation phase prior to the oviposition period. The average fecundity of *M. indentata* was 38.2 eggs/female – much less than the average fecundity of 342.1 eggs/female *M. fimbriolata* reported by Garcia et al. (2006).

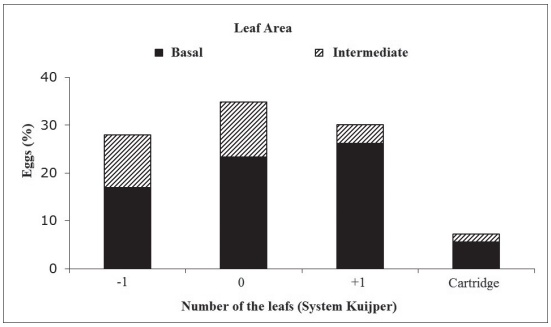


Fig. 5. Percentages of *Mahanarva indentata* eggs laid on the basal and intermediate portions of the leaves -1, 0, and +1 and the cartridge of the sugarcane cultivar, 'RB867515'.

Under our study conditions, we determined that the duration of the life cycle of *M. indentata* was 99 days, considerably longer than 60 days of *M. fimbriolata* (Garcia et al. 2006), 62.4 days of *M. posticata* (Barbosa 1986) and 64.8 days of *M. andigena* (Rodríguez & Peck 2007). The prolonged life cycle duration of *M. indentata* allows only 2 generations during the rainy period, from Oct to Mar in the state of Minas Gerais (Barbosa et al. 1980). In contrast *M. fimbriolata*, with life cycle duration of 60 days, has 3 generations during rainy period in the state of São Paulo (Garcia 2006; Dinardo-Miranda 2008).

Mahanarva indentata females preferred sugarcane leaves as their oviposition site. Generally the oviposition site is related to the place where the nymphs will develop. This study showed that *M. indentata* lays its eggs exclusively on sugarcane leaves and its nymphs remain on these leaves during the entire nymphal phase. On the other hand, *M. fimbriolata* lays its eggs mainly on the soil, and its nymphs feed on the plant's roots (Garcia et al. 2006), whereas *M. posticata* lays its eggs on the sheath of the leaves and its nymphs feed on the surface of the culm (Mendonça et al. 1996). In contrast, *M. andigena* and *M. bipars* lay their eggs on sugarcane stubble and on the soil. However, after the hatching, the nymphs move to the plant sheath (Rodríguez & Peck 2007; Gómez et al. 2007).

As *M. indentata* lays its eggs on the leaves, Mendes et al. (1988) proposed that burning sugarcane stubble for harvest should be considered as a method of cultural control in areas under

TABLE 3. MORPHOLOGICAL CHARACTERS (MM) OF ADULT *MAHANARVA INDENTATA* MALES AND FEMALES.

Sex	Head Capsule Width	Body Length With Wing	Body Length Without Wing	Body Length
Male	2.26 ± 0.01 a	11.21 ± 0.01 a	9.88 ± 0.20 a	4.42 ± 0.04 a
Female	2.53 ± 0.02 b	12.56 ± 0.14 b	10.73 ± 0.17 b	5.00 ± 0.06 b

Means in the columns followed by different letters are significantly different ($\alpha = 0.05$), by the t-test.

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this froghopper's attack, as well as to diminish the spread of *M. indentata* to other sugarcane regions. Nevertheless, the existing prohibition of burning the sugarcane in many states of Brazil, can facilitate increases in the froghopper's populations, and this can transform it into a pest of great importance in Brazil, much like *M. fimbriolata*.

Acknowledgments

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