



General Entomology

Egg parasitoids of the cassava hornworm (*Erinnyis* spp.) associated to cassava in the Pará State, Brazil

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Abstract. The aim of this study was to assess the occurrence and identify the egg-parasitoid species of the cassava hornworm (*Erinnyis* spp.) in cassava plants (*Manihot esculenta* Crantz - Euphorbiaceae), facilitating their usage in the biological control as a management strategy in the Pará State. During February to December of 2016, cassava hornworm eggs were collected in the Cassava Germplasm Bank area of the *Embrapa Amazônia Oriental*, located at the city of Belém, State of Pará. In the laboratory, eggs were separated in viable and parasitized and were daily observed until the hatching of the caterpillars and emergence of the parasitoids. The caterpillars were reared until reaching the adult stage and the sex determined at the pupae stage. A total of 482 eggs were collected, with a higher occurrence at March, and 244 caterpillars were obtained with sex ratio of 0.52 with 191 specimens reaching adult stage. Among these adults, 95.81% were *Erinnyis ello* (Linnaeus) and 4.19% *Erinnyis alope* (Drury). A total of 1,087 parasitoids were obtained from 131 eggs and belong to four Hymenoptera families: Platygasteridae (*Telenomus dilophonotae* Cameron), Eulophidae (*Chrysonotomyia* sp. aff. *serjaniae*), Trichogrammatidae (*Trichogramma marandobai* Brun, Moraes & Soares) and Encyrtidae (*Ooencyrtus* sp.). The *Erinnyis* spp. eggs were mostly parasitized by *T. dilophonotae*, which was classified along with *T. marandobai* as constant. The occurrence of these natural enemies highlights the potential for natural biological control against *Erinnyis* spp.

Keywords: Biological control; *Manihot esculenta*; *Telenomus dilophonotae*; *Trichogramma marandobai*.

The cassava (*Manihot esculenta* Crantz – Euphorbiaceae) is cultivated in more than a hundred countries, usually by small producers, being one of the most important sources of energy in the tropics and having its roots and subsequent subproducts consumed by millions of people (FILGUEIRAS & HOMMA 2016; FAO 2018).

The Nigeria is the main producer, in terms of quantity (59 million ton), followed by Thailand (31 million) and having Brazil (17 million) occupying the fifth place in the world ranking (FAO 2018). In Brazil, the cassava is largely cultivated across the whole territory, mostly by small producers (FILGUEIRAS & HOMMA 2016). The Northern region made 35.2% of the national production in 2019, with the state of Pará outstanding as the largest cassava producer of the country, contributing with 21.2% of the national production and with 60% of the regional production (IBGE 2019).

The cassava hornworm (*Erinnyis* spp. - Lepidoptera: Sphingidae) is one of the most important pests of the cassava, due to its high capacity of consuming leaves during the larvae stage. It is a pest of sporadic occurrence, with the incidence varying a lot from area to area, usually occurring at the beginning of the rain or dry season (FARIAS & BELLOTTI 2006).

Erinnyis ello (Linnaeus) and *Erinnyis alope* (Drury) are distributed in countries of North, Central and South America. *E. ello* has a bigger importance in relation to *E. alope*, having a large variety of host plants, especially those belonging to

Euphorbiaceae (FARIAS & BELLOTTI 2006). In Brazil, in terms of Northern and Northeastern region, *E. alope* was related in farming areas of cassava, although specimens were also collected with light traps in areas which are not specifically cassava farms (SILVA & CARNEIRO 1986; SANTOS *et al.* 2014; VIEIRA *et al.* 2014).

The attack of the hornworm to cassava might result in the complete defoliation of the plant. However, the effects on the yield may vary according to the plant development, number of attacks, soil fertility and environmental conditions. When the attack happens in the first months of plant development might result in reduction of root efficiency and planting material, damages which may lead to death of the plants (FARIAS & BELLOTTI 2006).

The biologic control of the cassava hornworm can be done by other insects which might be either parasitoids or predators of eggs and larvae. Regarding the parasitoids, members of the Encyrtidae, Platygasteridae (Scelionidae) and Trichogrammatidae are traditional references in the literature as parasitoids of cassava hornworm eggs. In this context, the genus *Trichogramma* stands out for parasitizing eggs of several Lepidoptera species (FARIAS & BELLOTTI 2006; JOHNSON 1990; OLIVEIRA *et al.* 2010; SANTOS *et al.* 2014; VIEIRA *et al.* 2014).

In the Legal Amazon, there are records of *Trichogramma* in the States of Amazonas, Mato Grosso and Roraima, with the species *Trichogramma marandobai* Brun, Moraes & Soares,

Trichogramma pretiosum Riley and *Trichogramma lasallei* Pinto. Despite knowing some species which occurs in the Amazon area, there are no information about their host association and usage in biological control (QUERINO & ZUCCHI 2016). To determine the parasitoid species which parasitize the hornworm eggs may favors the implementation of the biological control as a management strategy. Therefore, the aim of this study was to assess the occurrence of parasitoids in eggs of the cassava hornworm associated to cassava plants, at the municipality of Belém, State of Pará, Brazil.

MATERIAL AND METHODS

The study was made from collects of cassava hornworm eggs at Cassava Germplasm Bank (CGB) of the *Embrapa Amazônia Oriental* (1°26'28,0" S - 40°26'44,2" W), located at the municipality of Belém, State of Pará, Brazil, from February to December / 2016. The metropolitan region of Belém has tropical climate, predominantly hot and humid, fitting the climate types "Af" of the Köppen classification and B4rA'a' of the Thornthwait classification, with annual mean temperature of 26 °C, relative humidity of 84% and annual precipitation of 2,981.9 mm (PACHECO et al. 2011). The CGB area was composed by different genetic material, totalizing 180 accesses, with up to nine identified plants per access (clone), with spacing of 1 m x 1 m, kept under proper care (clear off of the leaves to control invaders and fertilizing).

In field, visual observation of eggs at the adaxial surface of the leaves were made, in intervals of up to 30 days. The leaves with eggs were detached from the plant, without hurting the access, and thus were kept in plastic bowls to transport to the laboratory.

At the lab, eggs were separated under a stereomicroscope according to their color: green and yellow considered as viable and black as possibly parasitized. To each egg, a portion of leaf was cut and individualized along the egg inside acrylic flasks of 3 cm diameter and 6 cm high, containing filter paper and sealed with plastic wrap (Lusafilme-R105®). The eggs were kept under environmental conditions (means of 27 °C temperature and 72% relative humidity) and were daily observed until hatching of the caterpillars and emergence of the parasitoids.

The caterpillars were individualized in plastic recipients of 11.5 cm diameter and 8 cm high, with a filter paper at the interior and with the cover having a whole sealed with voile tissue to allow for gas exchange. The larvae were daily fed with cassava leaves until pupae stage, when the sexing was made based on the genital opening. Afterwards emergence, the adult moths were identified by comparison with specimens deposited at the *Coleção Entomológica* of the *Embrapa Amazônia Oriental* (CPATU-ENTO), based on morphological characters.

The parasitoids emerged were collected under stereomicroscope with a brush with fine setae, quantified and stored in recipients containing 70% alcohol. The sexing was based on morphological characters (antenna, abdomen or reproductive organ). The specimens were identified according to QUERINO & ZUCCHI (2011) for Trichogrammatidae, HANSSON (2004) for Eulophidae and NOYES (2010) for Encyrtidae. For Platygastridae it was used JOHNSON (1984, 1990) and comparison with material deposited at the C.A. Triplehorn Insect Collection, from Ohio State University (Columbus, Ohio, USA).

The sex ratio and the constancy were calculated as in SILVEIRA NETO et al. (1979). The parasitoid families were classified according to the collect frequency: constant (C > 50% of the collects), accessory (C between 25% and 50%) and accidental

(C < 25%).

Parasitoid samples were deposited at the CPATU-ENTO and at the *Coleção de Insetos Entomófagos "Oscar Monte" do Instituto Biológico*, at Campinas, State of São Paulo, with exception of Trichogrammatidae, under the reference number IB-CBE-624.

Climatic data (temperature, relative humidity and precipitation) were obtained at the *Laboratório de Agrometeorologia* of the *Embrapa Amazônia Oriental*, at Belém, State of Pará.

RESULTS

Out of the total 16 collects, 482 hornworm eggs were obtained, a mean of 30 eggs per collect. The highest number of eggs was verified at the collects made at March the fourth and the 22th, as well as at the April 8th, representing 20.75%, 20.95% and 23.24% of the total, respectively (Table 1). The highest occurrence of hornworm eggs at March coincided with the highest monthly precipitation (465.3 mm) during collection period and with the reduction of the precipitation in April (Figure 1).

Table 1. Number of *Erinnyis* spp. (Lepidoptera: Sphingidae) parasitized, viable and unviable collected in cassava plants. Belém, State of Pará, 2016.

Collection date	Number of eggs			Total
	Parasitized	Viable	Unviable*	
17/Feb	1	2	0	3
25/Feb	5	6	1	12
04/Mar	22	53	25	100
22/Mar	41	39	21	101
08/Apr	43	44	25	112
26/Apr	5	6	0	11
12/May	7	5	3	15
02/Jun	3	11	3	17
21/Jun	2	7	1	10
12/Jul	4	8	3	15
02/Aug	2	7	4	13
29/Aug	0	0	0	0
19/Sep	0	1	0	1
26/Oct	1	21	1	23
17/Nov	5	20	4	29
06/Dec	5	14	1	20
Total	146	244	92	482

*Without caterpillar hatching, dry or with dark mass within.

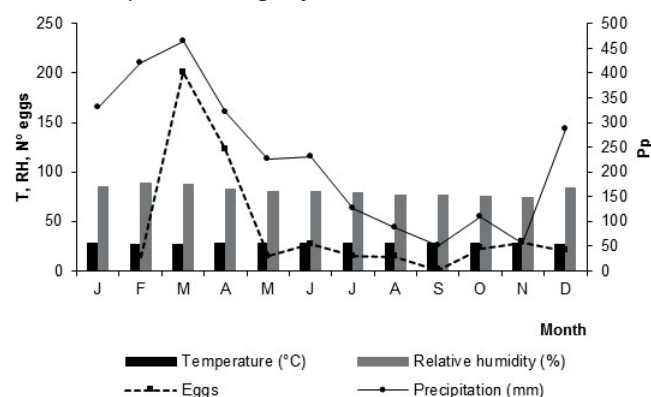


Figure 1. Climatic conditions and number of collected *Erinnyis* spp. eggs in cassava plants. Belém, State of Pará, 2016.

Out of the total collected eggs, 50.62% were viable, 30.29% were parasitized and 19.09% unviable (no caterpillar hatching, eggs with dark mass inside or the eggs were dry).

A total of 244 caterpillars were obtained, of which 83.61% reached pupae stage. The mortality at caterpillar stage was



Figure 2. *Erinnyis ello* (A) and *Erinnyis alope* (B).

of 16.39%, while at pupae stage was of 6.37%. The sex ratio considering specimens in pupae stage was of 0.52. Out of the 191 specimens which reached adult stage, 95.81% were identified as *E. ello* and 4.19% as *E. alope* (Figures 2A, B). In 87.5% of the collects, the presence of *E. ello* was observed whilst the presence of *E. alope* was only observed during June, August, September and December, with low incidence.

Parasitized hornworm eggs were observed in 14 collections, with exception of the second half of August, when no eggs were found, and at September, which only one viable egg was collected. The highest number of parasitized eggs occurred at March and April, period in which a higher incidence of cassava hornworm eggs was observed.

A total of 1,087 parasitoid eggs were obtained from 131 eggs, distributed in four families: Platygastridae (*Telenomus dilophonotae* Cameron), Eulophidae (*Chrysonotomyia* sp. aff. *serjaniae*), Trichogrammatidae (*T. marandobai*) and Encyrtidae (*Ooencyrtus* sp.). Dead parasitoids which were still in development stage were observed in 10.27% of the 146 parasitized eggs, thus impeding their identification. The species of Platygastridae and Trichogrammatidae were classified as constant, Eulophidae as accessory and Encyrtidae as accidental.

The highest number of hornworm parasitized eggs was done by *T. dilophonotae* (54 eggs, mean of 5.04 individuals per egg), followed by *Chrysonotomyia* sp. aff. *serjaniae* (51 eggs,

mean of 7.16), *T. marandobai* (25 eggs, mean of 18.88) and *Ooencyrtus* sp. (1 egg, 2 individuals per egg). The sex ratio was of 0.84 to Platygastridae, 0.79 to Eulophidae, 0.92 to Trichogrammatidae and 1 to Encyrtidae.

The collected specimens of *Telenomus* differed from what is established to *T. dilophonotae*, as they have preocellar pit (Figures 3A, B); furthermore, the males had 11 antennomeres (Figure 4). For *T. dilophonotae*, the preocellar pit should be absent and males should have 10 antennomeres (JOHNSON 1990). However, the examination of the *T. dilophonotae* holotype deposited at the Natural History Museum (United Kingdom, London) allowed for the confirmation that the preocellar pit is present at this species; due to its tiny proportions, it is likely that this structure went unnoticed. Regarding the male antennomeres number, we considered more important that his number is lower than 12, a normal and plesiomorphic condition to males of Telenominae. This way, the material was identified as *T. dilophonotae*. Regarding to the Eulophidae obtained, they were provisorily named as *Chrysonotomyia* sp. aff. *serjaniae*, as their identification is not yet certain.

DISCUSSION

In the present study, the highest number of collected eggs happened during March and April, suggesting a higher incidence of adults during this period (rain season). The results obtained are in agreement with the ones reported

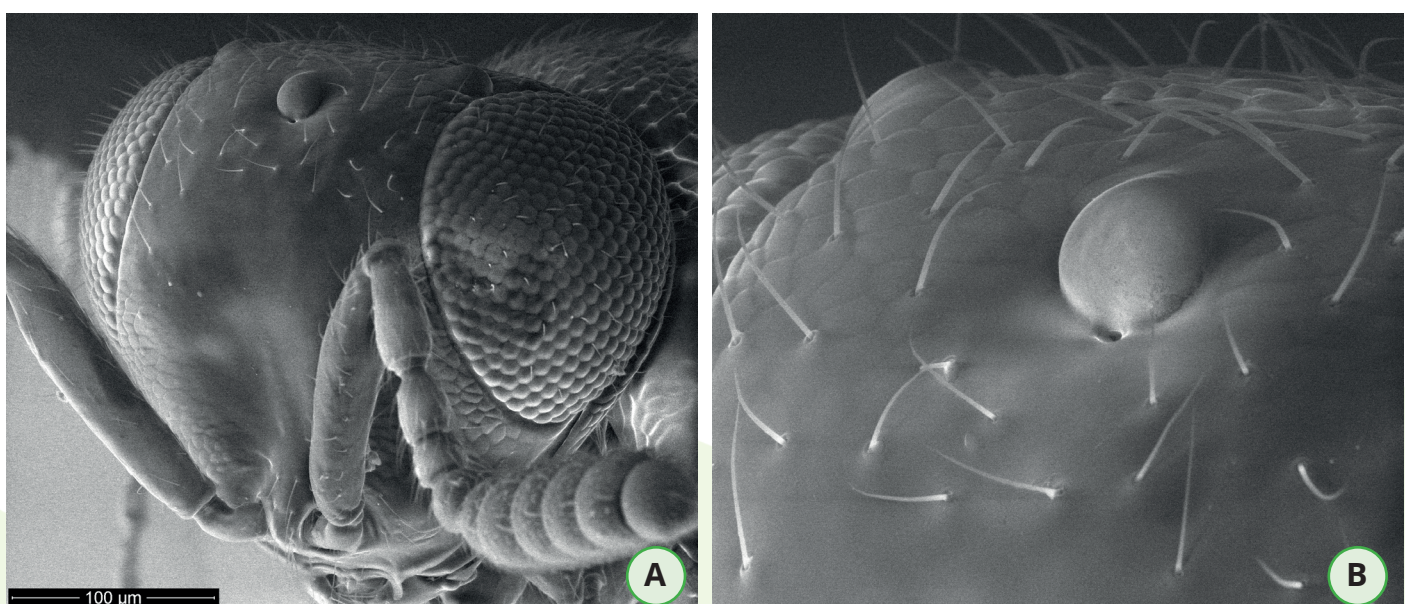


Figure 3. *Telenomus dilophonotae*, female, anterolateral view. A. Head. B. Preocellar pit.



Figure 4. *Telenomus dilophonotae*, male both antennae.

in the literature, regarding cassava hornworm species *E. ello* and *E. alope* (FARIAS & BELLOTTI 2006), with highlight to the first species, which represented 95.81% of adult specimens. Both species were previously related in cassava at the State of Pará with higher frequency from October to April, with *E. ello* occurring in small populations and *E. alope* with rare encounters (SILVA & CARNEIRO 1986).

From the parasitoid species of cassava hornworm eggs found in this study, the ones belonging to *Telenomus* are considered to be the most important due to its specificity in targeting *E. ello* as host, such as *T. dilophonotae* and *T. monilicornis* (= *sphingis*) Ashmead (FARIAS & BELLOTTI 2006). According to JOHNSON (1990), the species of *Telenomus* that parasitized eggs of *E. ello* are *T. connectans* Ashmead, *T. dilophonotae* and *T. monilicornis*.

In relation to *Chrysonotomyia* sp. aff. *serjaniae*, the specimens are morphologically similar to some species of the *planiseta* group of the genus *Chrysonotomyia*, as they have two pairs of mesoscutal setae and two digital spines. However, these parasitoids do not necessarily belong to *Chrysonotomyia*; more investigations regarding the Eulophidae genera are needed to confirm the proper classification of these specimens. The eulophid *Euplectrus floryae* (Schauff) was reported parasitizing larvae of *E. ello* in cassava at the city of Dourados, at the Mato Grosso do Sul State, with a mean of 4 to 8 individuals per larvae (BELLON et al. 2013).

In Brazil, species of *Trichogramma* were reported parasitizing eggs of *E. ello* (*Trichogramma atopovirilia* Oatman & Platner, *Trichogramma manicobai* Brun, Moraes and Soares, *T. marandobai*, *T. pretiosum*) and of *E. alope* (*T. marandobai*) (OLIVEIRA et al. 2010; QUERINO & ZUCCHI 2011; SANTOS et al. 2014; VIEIRA et al. 2014).

In the legal Amazon, there are records of *T. marandobai* at the State of Amazonas, *T. lasallei* at the State of Roraima and *T. pretiosum* at the State of Mato Grosso, however, only *T.*

marandobai was reported parasitizing eggs of *E. ello* in cassava (RONCHI-TELES & QUERINO 2005; VIEIRA et al. 2014; QUERINO & ZUCCHI 2016). The mean number of *T. marandobai* specimens per egg (18.8) obtained in the present study was inferior to the verified by SANTOS et al. (2014), a number of 28 individuals per egg.

Species of *Ooencyrtus* (Encyrtidae) are parasitoids of eggs from other insects, mostly belonging to Hemiptera or Lepidoptera (MEDEIROS et al. 1998; SILVA et al. 2016).

The occurrence of these natural enemies highlights the potential of biological control of *Erinnyis* species, with the enlargement of the geographical record distribution of these parasitoids. The absence of insecticide usage at the studied area might have contributed to the maintenance of favorable conditions to parasitoids, considering that out of 482 collected eggs, 146 were parasitized, representing about 30% of eggs free from hatching of hornworm caterpillars, as the parasitism occurred in the stage which the pest did not cause damage to the plants. These results strengthen the importance of biologic control, as the preservation and rising of the natural enemies populations might have been favored by the agroecosystem (FONTES et al. 2020).

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