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THE BIOLOGY OF *DIATRAEA FLAVIPENNELLA* (LEPIDOPTERA: CRAMBIDAE) REARED UNDER LABORATORY CONDITIONS

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Abstract

Aspects of the biology of the sugarcane pest *Diatraea flavipennella* (Box 1931) (Lepidoptera: Crambidae), locally named *broca-pequena da cana-de-açúcar*, reared and maintained under laboratory conditions and fed on an artificial diet have been investigated. The larval stage, which involved 7 instars, continued for a mean period of 34.87 d. Each instar could be characterized by the size of the cephalic capsule, which increased 1.28-fold on average between instars. The mean duration of the pupal stage was 12.75 d. The pupae exhibited sexual dimorphism in that the females were larger than the males, while the latter exhibited a genital pore that was absent in the females. In adult insects, the female/male ratio was 1:1.3. Adult females were on average 28.73 mm in size while the mean value for adult males was only 20.80 mm. Females commenced oviposition on the first d of their adult life and were able to oviposit until d 6. On average each female produced 431.05 eggs during her lifetime, although the majority of eggs were deposited during the first 2 d after emergence.

Key Words: artificial diet, sugarcane borer, biological aspects, lepidoptera, *Diatraea flavipennella*, Crambidae

Resumen

Aspectos da biologia da broca-pequena da cana-de-açúcar, *Diatraea flavipennella* (Lepidoptera: Crambidae), foram investigados em condições de laboratório (26 ± 1°C; 80 ± 10% U.R.; fotoperíodo de 12h) e alimentada em dieta artificial. O estágio larval apresentou um período médio de 34,87 dias e 7 instares, sendo cada estádio separado pela largura da cápsula cefálica, a qual aumentou a cada ínstar numa razão de 1,28. O estágio pupal durou um período médio de 12,75 dias. As pupas apresentaram dimorfismo sexual, onde as fêmeas foram maiores do que os machos, os quais exibiram um poro genital, ausente em fêmeas. A razão sexual entre adultos foi de 1:1,3. As fêmeas adultas apresentaram-se maiores que os machos, com envergadura, em média, de 28,73 cm e 20,80 cm para machos. A oviposição iniciou-se no primeiro dia de vida da fêmea e estendeu-se até o sexto dia, com uma média geral de 431,05 ovos/fêmea, apresentando uma maior produção de ovos nos dois primeiros dias de vida.

Translation provided by the authors.

Within the world economy, sugarcane (*Saccharum officinarum*) constitutes one of the most important crops in terms of annual production and as a major source of employment. Sugarcane biomass is the raw material for the production of alcohol (for beverages and fuel) and animal feed, as well as for sugar and various derived products. However, the production of sugarcane is not straightforward by virtue of the considerable problems caused by numerous pests that can devastate the crop and diminish the yield. Insects of the genus *Diatraea* (Lepidoptera: Crambidae) cause the most damage to sugarcane crops resulting in significant losses of revenue.

Commercially available pesticides are, unfortunately, not efficient for the control of *Diatraea* spp. on sugarcane for a variety of reasons mainly associated with the continuous presence of the host plant in the field throughout the whole year, the simultaneous occurrence of mature and immature forms of the insect, and the feeding habits of the insect. An alternative strategy is that of integrated pest management (IPM), which involves biological control of the insect together with a range of tactics including manual collection of the larvae, introduction of resistant varieties of sugarcane, and the use of pheromone baits. So far, IPM has been the most efficient method of controlling *Diatraea* spp. infestation.

In a number of regions of Brazil, *Diatraea flavipennella* (Box, 1931), popularly known as *broca pequena da cana-de-açúcar*, is considered to be

the main sugarcane pest. This insect can not only kill a plant directly by damaging the apical buds, but it can also cause indirect damage through infiltration of the larvae into the culms, leading to the ingress of phytopathogenic organisms into the plant (Mendonça 1996). The duration of complete metamorphosis of *D. flavipennella* is very irregular and depends on numerous factors such as the climate and the host plant (Guagliumi 1972/73).

Studies concerning the morphology, physiology, and biology of insect pests are very important since they provide valuable insights into aspects of pest management including damage potential, population dynamics and fluctuation, growth rate, and spatial distribution. Such knowledge permits the establishment of appropriate control measures. However, most studies have focused on overall understanding of the genus *Diatraea*. The objective of the present investigation was, therefore, to examine the specific biology of *D. flavipennella* through determination of defined parameters including the number of ovipositions per female, number of eggs per oviposition, viability and incubation time of eggs, development of larvae and pupae, male/female ratio, and longevity of adults.

Materials and Methods

Initiation and Maintenance of the Insect Population

Larvae of *D. flavipennella* were obtained from infested sugarcane plants located in commercial plantations in the State of Alagoas, Brazil, and transported to the Laboratório de Química Entomológica at the Universidade Federal de Alagoas. Eggs, pupae, and adults were maintained in the laboratory at 22 ± 1°C, 70 ± 10% relative humidity and 12 h photoperiod, while larvae were maintained at 26 ± 1°C, 80 ± 10% relative humidity and 12 h photoperiod. Larvae received an artificial diet developed by Hensley & Hammond (1968) and modified in collaboration with the Laboratório de Assistência Fitossanitária e Controle Biológico (FITOSSAN Maceió—AL, Brazil), according to the following description: ascorbic acid (7.0 g); agar-carrajeenate (26.0 g: 12.0 g), vitamin solution (60 mL), sucrose (162 g), and sugarcane culms in powder (40 g). Adult insects were fed with 10% sucrose solution. All of the described experiments were conducted with insects that had been reared and maintained under laboratory conditions.

Incubation of Eggs, Emergence of Larvae, Pupae and Adults, and Viability of the Immature Forms

The number of eggs in each of 10 newly deposited egg masses was determined with use of a Wild Leica model M3B stereomicroscope. Each egg mass was placed in a separate glass tube (8.5 length × 2.5 cm diam) containing artificial diet and observed daily until larvae emerged. Newly emerged larvae (n = 110; 0 to 24 h old) were placed individually into acrylic dishes (1.5 cm depth × 6.0 cm diameter) containing artificial diet and observed daily. The following aspects relating to the development of larvae were recorded: occurrence of pupation, presence of a cephalic capsule, the size of the cephalic capsule (measured with a stereomicroscope containing an ocular micrometer), and the number of dead larvae. Pupae (n = 140) originating from the egg masses mentioned above, were grouped by sex, measured with a calliper and maintained in acrylic dishes (1.5 cm depth × 6.0 cm diameter) until emergence of adult insects. The following aspects relating to the development of pupae were recorded daily: occurrence of metamorphosis, the number of males and females that emerged, and the number of dead pupae.

Longevity and Reproduction of Adult Insects

Twenty four newly emerged adults originating from the egg masses mentioned above were placed in pairs (1 male with 1 female) in glass cages (15 cm × 30 cm × 20 cm). Each group was observed daily and the numbers of dead males and females were recorded. Measurements of adult size were performed at this stage.

Measurements of Adult Size

Twenty two pairs (1 male with 1 female) of newly emerged adults were placed in PVC tubes (10 cm length × 10 cm diameter) that had been lined with greaseproof paper. The paper lining was removed each day and the number of eggs present was determined.

Statistical Analysis

All experiments were conducted in a randomized design. The results concerning the sizes of male and female pupae and the longevity of male and female adults were submitted to analysis of variance, and differences between mean values of each sex were determined by Tukey's test at the 5% probability level.

Results

Number of Eggs per Oviposition, Incubation Period, and Viability of Eggs

Females of *D. flavipennella* deposited egg masses containing between 3 and 58 (mean 33.2 ± 2.53 SEM) elliptical-shaped, milky-white eggs per oviposition. The eggs gradually became dark yellow in color as the embryos matured, and eventually turned black at the stage when the larvae

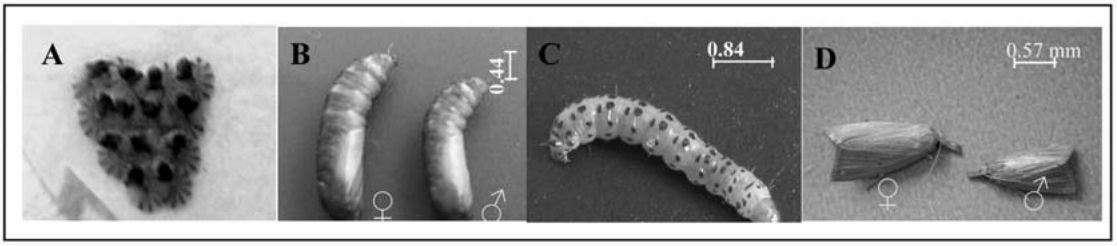

Fig. 1. Stages of the life cycle of *Diatraea flavipennella* reared and maintained under laboratory conditions: A—an egg mass (160-fold increased); B—pupae; C—a larva; and D—male and female adults.

emerged (Fig. 1A). The viability of the eggs per mass varied between 54 and 100% (mean 87.8%), and the average incubation period was 8.35 d (± 0.17) (Table 1).

Development and Viability of Larvae and Pupae

The larvae of *D. flavipennella* were yellowish in color with brown spots that did not appear to form any uniform pattern along the dorsal surface of the insect (Fig. 1B). The cephalic capsule was yellow or brownish color. The mean duration of the larval stage was 34.87 d (± 0.41) and the average viability of larvae was 75.46% (Table 1). Assuming that metamorphosis from one instar to another is indicated by the release of the cephalic capsule, the total number of instars was determined to be 7 (Fig. 2). The mean durations of the instars (Table 2) varied between 6.20 ± 0.37 d (1st instar) and 2.89 ± 0.65 d (7th instar), while the average sizes of the cephalic capsules ranged between 0.32 ± 0.01 mm (1st instar) and 1.50 ± 0.04 mm (7th instar). The mean size of the last instars was 26.0 ± 0.4 mm although some were as large as 32 mm.

The average duration of the pupal stage (Fig. 1C) was 12.75 d ± 0.42 and the mean viability was 77.63% (Table 1). Male and female pupae had 8 tergites and could be identified from the difference in the external genitalia because males had a distinct pore that was absent in females. The size of the pupae varied between 12 and 21 mm, with a mean value of 16.13 ± 0.17 mm; however, female pupae were larger (mean 17.90 ± 0.22 mm) than males (mean 14.77 ± 0.11 mm), and the difference was significant ($P < 0.05$).

Longevity of Adult Insects and Number of Eggs Deposited per Female

The female/male ratio in adult insects was 1:1.3. Adults were milky white in color and varied in size from 18-33 mm, with average dimensions of 28.73 (± 0.25) mm for females and 20.80 (± 0.86) mm for males. The wings were striated and the central part of the frontal wings bore a black spot (Fig. 1D). The average life span of adult insects was 9.17 ± 0.69 d. The lifespan of male insects was not different statistically ($P > 0.05$) from female insects. Females began to oviposit on d 1 after emergence and continued until the d 6. The maximum production of eggs occurred during the first 2 d (Fig. 3). The lifetime-number of eggs laid by the females varied between 96 and 585, with an average of 431.05 ± 30.28.

Discussion

Diatraea flavipennella was able to complete its life cycle successfully within a population reared and maintained under experimental conditions. The number of eggs laid by adult females of this

Table 1. The duration and viabilities of eggs, larvae, and pupae, and life span of adults of *Diatraea flavipennella* reared on an artificial diet regime.

Phase	Number of specimens examined (*n*)	Duration of stage (d)	Viability (%)
Eggs	110 (masses)	8.35 ± 0.17	87.80
Larvae	110	34.87 ± 0.41	75.46
Pupae	140	12.75 ± 0.42	77.63
Adults	24	9.17 ± 0.69	—

Mean values ± SEM are shown.

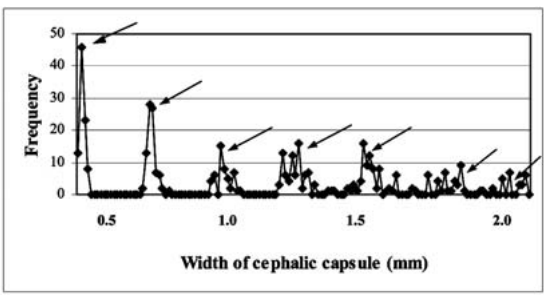

Fig. 2. Frequency distribution of the sizes of cephalic capsules during the larval stage of *Diatraea flavipennella*. The 7 instars were characterized by the most frequently occurring sizes of cephalic capsules (arrowed).

TABLE 2. DURATION OF EACH INSTAR AND SIZE OF THE CEPHALIC CAPSULE DURING THE DEVELOPMENT OF LARVAE OF *DIATRAEA FLAVIPENNELLA*.

Instar	Duration of instar (d)	Size of cephalic capsule (mm)
1st instar	6.20 ± 0.37	0.32 ± 0.01
2nd instar	5.80 ± 0.94	0.46 ± 0.01
3rd instar	4.93 ± 0.40	0.61 ± 0 02
4th instar	5.56 ± 1.04	0.91 ± 0.03
5th instar	4.95 ± 0.45	1.22 ± 0.03
6th instar	4.54 ± 0.72	1.44 ± 0.04
7th instar	2.89 ± 0.65	1.50 ± 0.04

Mean values ± SEM are shown.

species was similar to that found for *D. saccharalis* (Holloway et al. 1928). The 7 instars detected during the larval stage of *D. flavipennella* were within the range expected for the order Lepidoptera, which is normally 5 to 6 but which can vary between 3 and 11 owing to intrinsic and extrinsic factors for each species (Parra & Haddad 1989). Variations in the number of instars have been previously reported for a number of species including *D. saccharalis*, *Lacanobia oleracea*, *Delterollyta majuscule*, and *Copitarsia incommoda* (Guagliumi 1972/73; Corbitt et al. 1996; Acatitla-Trejo et al. 2004; Nava et al. 2004). The size of the cephalic capsule varied with each instar and increased between instars by a mean ratio of 1.28 in agreement with Dyar (1980), who reported that the size ratio between instars can vary between 1.1 and 1.9. The size of the cephalic capsule can thus be used as a precise indication of each instar.

The duration of the pupal stage in *D. flavipennella* was longer than that previously reported for *D. saccharalis* (Holloway et al. 1928; Guagliumi 1972/73). The pupae of *D. flavipennella* exhibited sexual dimorphism that was characterized mainly by differential size in which the females were significantly larger than the males. This feature is typical of insects of the order Lepidoptera (Slansky & Scriber 1985), and has been observed in other species of the same genus, i.e., *D. saccharalis* (Holloway et al. 1928) and *D. grandiosella* (Chippendale & Sorenson 1997).

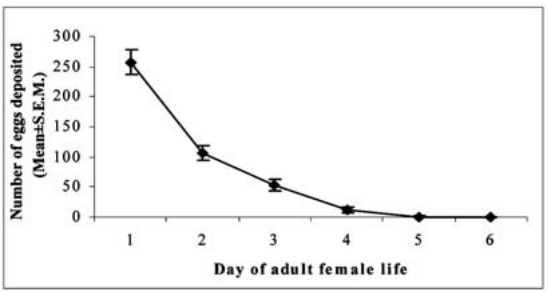

Fig. 3. Mean (± SEM) numbers of eggs deposited per d by individual females of *D. flavipennella* during their adult life span.

Adult females of *D. flavipennella* were able to oviposit for 6 d, although most eggs were deposited during the first 2 d of adult life. In contrast, oviposition in *D. saccharalis* is reported to last for only 4 d (Holloway et al. 1928).

The viabilities of larvae, pupae, and adults of *D. flavipennella* were found to be satisfactory, providing this species with a high reproductive potential and permitting the facile maintenance of an insect population both under laboratory and natural conditions. These findings are very similar to those previously reported for *D. saccharallis* (Filho & Lima 2001). However, for *D. flavipennella*, the periods necessary for the development of eggs, larvae, and pupae subjected to an artificial diet were different from those reported for insects fed on natural diet (Guagliumi 1972/73).

The present results contribute to the understanding of the biology of *D. flavipennella* and will be of value in the context of further studies concerning the reproductive behavior, survival rate, feeding habits, and pheromone production in this species, as well as in the establishment of biological control programs for this detrimental pest.

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