Herpetogramma bipunctalis (Lepidoptera: Crambidae) biology and techniques for rearing on leaves of the blackberry (*Rubus* spp., Rosaceae)

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(With 3 figures)

Abstract

The larvae of the southern beet webworm *Herpetogramma bipunctalis* (Fabricius, 1794) damage the leaves of species in the plant genus *Rubus*. The present study investigated the biology of *H. bipunctalis* and developed a protocol for raising *H. bipunctalis* under laboratory conditions. On the basis of the biological data, we devised a life table. In order to develop the rearing procedures, we determined which oviposition substrate and blackberry cultivar were the most appropriate for larval development. The mean durations of the egg, larval, and pupal stages were 5.59 days, 26.37 days, and 13.37 days, respectively, and the corresponding survival rates were 80.83%, 49.07%, and 83.23%. The mean pupal weight was 0.0491 g for males and 0.0536 g for females. The mean life cycle (egg-to-adult) period was 45.33 days, and overall survival to adulthood was 33.01%. *H. bipunctalis* females laid a mean of 252.63 eggs over a mean of 13.60 days of oviposition; the mean pre-oviposition period was 2.67 days. Mean female and male life spans were 17.51 and 19.25 days, respectively, and the sex ratio was 0.51. The life-table data indicated that *H. bipunctalis* can reproduce 57.9 times per generation. Each cage contained one blackberry leaf placed on a paper towel. This method allowed us to obtain the greatest number of eggs. The larval stage was shorter for insects reared on leaves of the Guarani cultivar than for those reared on leaves of the Xavante cultivar (22.63 vs. 26.37 days). These basic data can aid in establishing strategies for the management of *H. bipunctalis* on blackberry farms.

Keywords: Lepidoptera, Crambidae, blackberry pests, life cycle, rearing practices.

Biologia e técnica de criação de *Herpetogramma bipunctalis* (Lepidoptera: Crambidae) em folhas de amoreira-preta (*Rubus* spp., Rosaceae)

Resumo

Lagartas de *Herpetogramma bipunctalis* (Fabricius, 1794) causam danos nas folhas da amoreira-preta (*Rubus* spp.). O objetivo deste trabalho foi estudar sua biologia e desenvolver uma técnica de criação, em condições de laboratório. Com os dados de biologia, foi elaborada uma tabela de vida de fertilidade. Para o estudo da técnica de criação, foram determinados o substrato para obtenção de ovos e a cultivar de amoreira-preta mais adequada para o desenvolvimento larval. A duração dos estágios de ovo, lagarta e pupa foram de 5,59, 26,37 e 13,37 dias, com sobrevivência de 80,83, 49,07 e 83,23%, respectivamente. O peso das pupas foi 0,0491 g para machos e 0,0536 g para fêmeas. O ciclo biológico (ovo-adulto) foi de 45,33 dias, com sobrevivência total de 33,01%. As fêmeas colocaram, em média, 252,63 ovos durante 13,60 dias, com um período de pré-oviposição de 2,67 dias. A longevidade média de fêmeas e machos foi de 17,51 e 19,25 dias, respectivamente, e a razão sexual, de 0,51. Por meio da tabela de vida de fertilidade, determinou-se uma capacidade de aumento de 57,9 vezes a cada geração. Gaiolas revestidas com papel toalha e com a presença de folhas de amoreira-preta propiciaram a obtenção de uma maior quantidade de ovos. A duração do estágio larval para os insetos alimentados com folhas de amoreira-preta cv. Guarani (22,63 dias) foi menor do que aqueles alimentados com folhas da cv. Xavante (26,37 dias). Estas informações são básicas e auxiliarão em futuros estudos, visando ao estabelecimento de estratégias de manejo de *H. bipunctalis* em amoreira-preta.

Palavras-chave: Lepidoptera, Crambidae, praga da amoreira-preta, ciclo de vida, técnica de criação.

1. Introduction

The plant genus *Rubus* includes many types of fruits, including the blackberry. The blackberry plant is a flowering shrub that thrives in temperate regions and is suitable for cultivation on small farms. In the state of Rio Grande do Sul (RS) in southern Brazil, blackberries are widely produced because production costs are low, the plants are hardy and easily managed, and little pest control is required (Antunes, 2002; Antunes et al., 2010). However, several pests can affect blackberry production in this region (Nava et al., 2007): *Eulechriops rubi* Hespenheide, 2005 (Coleoptera: Curculionidae), *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae), leaf beetles (Coleoptera: Chrysomelidae), leafcutter ants (Hymenoptera: Formicidae), and mites (Acari). In addition, the leaf-eating larvae of *Herpetogramma bipunctalis* (Fabricius, 1794) (Lepidoptera: Crambidae), commonly known as the southern beet webworm, have been observed on blackberry farms in southern RS. In 2010, the population size of *H. bipunctalis* in the state was the largest in March and April, and smallest in November (Diez-Rodríguez et al., 2010). Larvae of *H. bipunctalis* cause damage by feeding on blackberry leaves, which curl as a form of protection, resulting in early senescence (leaf drop) (Nava, DE. Personal communication).

The *H. bipunctalis* moth has a wingspan of approximately 22 mm and is yellowish-gray, with some darker spots and indistinct stripes on the forewings. Females of *H. bipunctalis* lay groups of 4-5 eggs on the abaxial surface of leaves. Last-instar *H. bipunctalis* larvae can be as long as 19 mm. They are translucent and yellow to greenish-gray, with dark heads. *H. bipunctalis* pupae are brown, 10 mm long, and can be found inside curled leaves or amid plant debris on the ground (King and Saunders, 1984).

Herpetogramma bipunctalis occurs from the United States to South America and is an important pest in the Caribbean region, where it causes damage to *Beta vulgaris* L. (Chenopodiaceae) and *Daucus carota* L. (Apidaceae). Species of the genus *Amaranthus* (Amaranthaceae) are among the principal hosts of *H. bipunctalis* (King and Saunders, 1984). In Bolivia and Peru, *H. bipunctalis* is a pest of *Chenopodium quinoa* Willd. (Amaranthaceae) (Rasmussen et al., 2003). In Uruguay, Biezanko et al. (1974) reported the presence of *H. bipunctalis* on other species of the family Amaranthaceae, such as *Atriplex hortensis* L. and *Chenopodium ambrosioides* L., as well as on *B. vulgaris*. *H. bipunctalis* has also been found on sugarcane (*Saccharum officinarum*, Poaceae) in Peru (Ayquipa and Cueva, 1979) and in Florida, U.S.A. (Hall, 1988).

In Brazil, *H. bipunctalis* has been observed on chard and beet crops (Silva et al., 1968). However, there are no reports of *H. bipunctalis* on blackberry bushes. Because there is little information regarding *H. bipunctalis*, the objectives of the present study were to gather information on the biology of the species to develop rearing procedures for obtaining eggs, and to determine the most appropriate substrate for larval development. Our aim was to make the insects available year-round in order to support studies on control methods.

2. Material and Methods

2.1. Rearing procedures

We collected larvae from bushes of the Xavante blackberry cultivar in the city of Pelotas, Brazil (31° 46' 19" S and 52° 20' 33" W). The insect was identified by Dr. Vitor Osmar Becker.

The larvae were reared in the laboratory under controlled conditions (temperature 25 ± 2 °C; relative humidity 70 ± 20%, in a 14-hours photophase). Larvae were placed in plastic containers (10 × 10 × 6 cm) and reared with leaves of the Xavante cultivar until they reached the pupal stage. The pupae were separated by sex and placed in individual glass tubes (2.3 × 9 cm). Males were identified by the presence of two sclerites on the ninth abdominal segment. After emergence, the moths were placed in polyvinyl chloride tubes (10 × 10 cm) (one male and one female per tube). The tubes were lined with paper towels. Each tube contained food for the adults (10% honey solution, supplied on cotton dental rolls in small receptacles) and a substrate for oviposition (a Xavante leaf in a container with water). The paper towels lining the tubes also served as the oviposition substrate. The paper towels were replaced daily, and the food was replenished as necessary.

2.2. Biology on the Xavante blackberry cultivar

We assessed the following biological parameters for pupae (n = 161): duration of the pupal stage, survival rates, and weight at 48 hours (by sex). In order to evaluate the adult stage, we formed couples aged ≤ 48 hours (n = 30). We determined the life span for each sex, the fecundity and the duration of the pre-oviposition and oviposition periods. We counted the eggs laid on paper towels and those laid on blackberry leaves. For eggs laid on the leaves, we counted the numbers laid on the abaxial and adaxial surfaces. We also determined the duration of and survival rates for the embryonic period, using eggs collected from the third clutches of 15 couples (n = 605). To determine the duration and survival rates for the larval stage, we placed newly hatched larvae (n = 100) in 20 Petri dishes (9.5 cm diameter, 5 larvae/dish), each containing two Xavante leaves. The larvae were observed daily. We devised a life table based on the data for the duration of the egg-to-adult development period, egg-to-adult viability, sex ratio número de fêmeas/número de fêmeas + número de machos, pre-oviposition period, number of eggs laid per day, and daily mortality for males and females in order to determine the intrinsic rate of increase (Silveira Neto et al., 1976).

2.3. Type of oviposition substrate

The experiment was carried out in order to determine the best substrate to obtain eggs of *H. bipunctalis*. We tested five different oviposition substrates: paper towel alone; plastic alone; paper towel + leaf of the Xavante cultivar; plastic + leaf of the Xavante cultivar and leaf of the Xavante cultivar alone. For each of the oviposition substrates, we placed 13 pairs aged ≤ 48 hours in individual polyvinyl chloride tubes (10 × 10 cm). The tubes were lined with either paper towels or plastic, and the adults were fed a 10% honey solution through capillary action supplied on cotton rolls in small receptacles. Each leaf was placed in a container with water. The number of eggs was counted every 48 hours with the aid of a stereomicroscope. The oviposition substrates and the food were replaced every 48 hours. The experiment was performed in a fully randomized manner. The data were submitted to analysis of variance, and if this was significant, the means were compared by the Tukey test (P ≤ 0.05).

2.4. Larval development on two blackberry cultivars

We used leaves of the blackberry cultivars Xavante (without prickles) and Guarani (with prickles) in order to assess the duration and survival rates for the larval stage. Newly hatched larvae were placed in Petri dishes (9.5 cm diameter, 5 larvae/dish), each containing two leaves of one cultivar. For each cultivar, we used 20 Petri dishes, for an overall total of 100 larvae. The food was replaced as needed, and the larvae were observed daily. The experiment was performed in a fully randomized manner. The data were submitted to analysis of variance, and the means were compared by the Tukey test (P ≤ 0.05).

3. Results and Discussion

3.1. Biology on Xavante blackberry cultivar

The mean duration of the egg stage was 5.59 ± 0.13 days, and the egg-stage survival rate was 80.83%. From the 7,579 eggs laid, 6,704 (88.45%) were laid on the blackberry leaves and 875 (11.55%) were laid on the paper towels, a significant difference (F = 34.77, df = 1; p < 0.0001). Out of the 6,704 eggs laid on the blackberry leaves, 5,984 (89.26%) were laid on the abaxial surface of the leaves and 720 (10.74%) on the adaxial surface, also a significant difference (F = 35.99; df = 1; p < 0.0001; see Figure 1). According to King and Saunders (1984), *H. bipunctalis* eggs are normally laid on the leaf abaxial surface. Therefore, the laying of eggs on the adaxial surface of leaves might be related to the protocol used to rear *H. bipunctalis* under laboratory conditions, where the insects were confined in cages. The eggs on the abaxial surface of the leaves were laid between the leaf veins, whereas those on the adaxial surface were laid on the leaf veins. This is probably a way of protecting the eggs from predation and parasitism, as well as from the effects of climatic factors in order to maintain the population density of *H. bipunctalis* in equilibrium with other organisms.

The mean duration and survival rate for the larval stage were 26.37 ± 0.29 days and 49.07%, respectively; and the mean duration and survival rate for the pupal stage were 13.37 ± 0.19 days and 83.23%, respectively. The life cycle (egg-to-adult development time) was 45.33 days, and egg-to-adult viability was 33.01%. Mean pupal weight was 0.0491 ± 0.0007 g for males and 0.0536 ± 0.0008 g for females, differing significantly between the sexes (F = 16.76; df = 1; p < 0.0001). Because females are responsible for reproduction, they acquire more nutrients than males do during the larval stage, and therefore have higher body weights (Slansky Junior and Scriber, 1985).

The mean pre-oviposition period was 2.67 ± 0.18 days, and the mean oviposition period was 13.60 ± 1.48 days. During oviposition, *H. bipunctalis* females laid a mean of 252.63 eggs each. Mean male and female life spans were 19.25 ± 1.65 days and 17.51 ± 1.42 days, respectively; the sex ratio was 0.51.

Calculation of the net reproductive rate indicated that *H. bipunctalis* can reproduce 57.9 times per generation under laboratory conditions, i.e., has a high biotic potential. The mean *H. bipunctalis* generation time (the time elapsed between the birth of the parents and that of their offspring) was 51.21 days. The intrinsic rate of increase for the species was 0.079, and the finite rate of increase (the number of females added to the population divided by the number of females that will generate females) was 1,082. These results indicate that over 51.21 days (the mean *H. bipunctalis* generation time), approximately 57.9 females will be born to the reproducing females, under laboratory conditions. The rate of population increase peaked on day 49, the point at which the age-specific fecundity and age-specific survival rate coincided (see Figure 2).

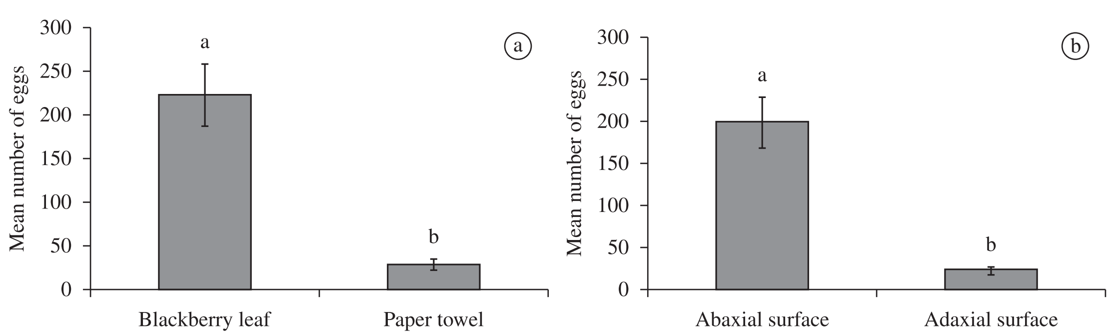

Figure 1. Mean number of eggs laid by *Herpetogramma bipunctalis* on blackberry leaves and paper toweling (a), and mean number of eggs laid on the abaxial and adaxial surfaces (b), at a temperature of 25 ± 2 °C and relative humidity 70 ± 20%, in a 14-hours photophase. Means followed by the same letter in a column did not differ statistically from each other by the Tukey test, at the 5% probability level.

3.2. Type of oviposition substrate

The number of eggs laid on the paper towels + leaf of the Xavante cultivar was significantly larger than the number of eggs laid on any of the remaining oviposition substrates (F = 15.73; df = 4; p < 0.0001; see Figure 3). This showed that the leaves in the cages played an important role as a stimulus for *H. bipunctalis* oviposition. This requirement for a plant part has been observed for other lepidopteran larvae. Nava et al. (2005) observed that for *Stenoma catenifer* Walsingham (Lepidoptera: Elachistidae), the presence of avocado fruit was essential for obtaining eggs on the paper towels covering the cages. This requirement has also been demonstrated for *Heliothis virescens* (Fabr., 1781) (Lepidoptera: Noctuidae), since the placement of a cotton leaf in the upper part of the cage increased oviposition on the voile and paper lining the cages (Moreti and Parra, 1983). Thus, the blackberry leaves probably provided a chemical stimulus for oviposition of *H. bipunctalis* on the paper towels, which facilitates handling of the eggs.

According to Ramaswamy (1988), the physical stimulus provided by the oviposition substrate constitutes an important signal for female lepidopterans. However, for *H. bipunctalis*, the physical stimulation is most likely linked to the chemistry, because of the reduced number

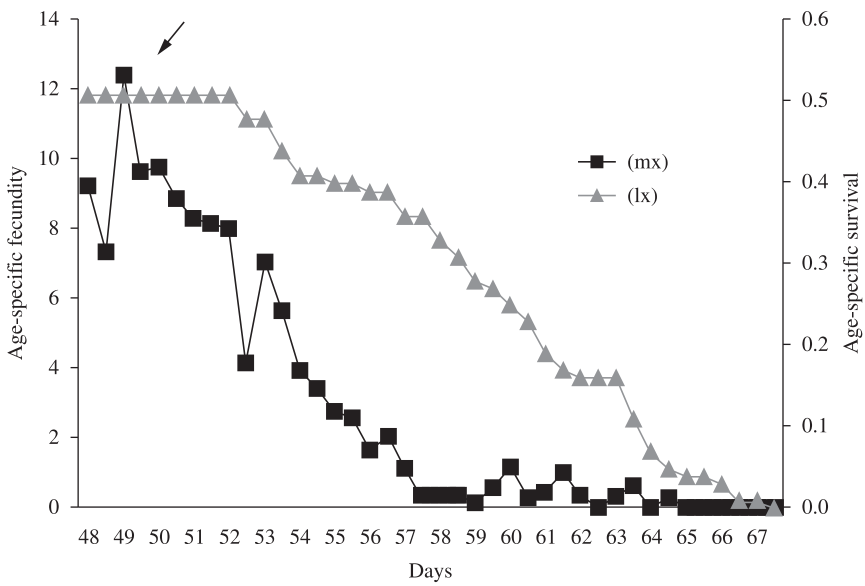

Figure 2. Age-specific fecundity (m_x) and age-specific survival rate (l_x) of *Herpetogramma bipunctalis* reared on leaves of the Xavante blackberry cultivar, at a temperature of 25 ± 2 °C and relative humidity 70 ± 20%, in a 14-hours photophase. The arrow indicates the maximum rate of increase.

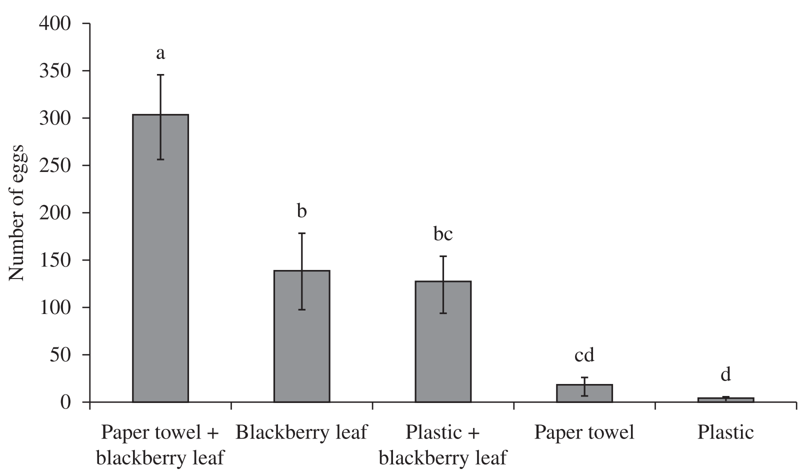

Figure 3. Mean number of eggs of *Herpetogramma bipunctalis* in cages containing different substrates, at a temperature of 25 ± 2 °C and relative humidity of 70 ± 20%, in a 14-hours photophase. Means followed by the same letter in a column did not differ statistically from each other by the Tukey test, at the 5% probability level.

of eggs laid in the absence of leaves, on both the paper towels and the plastic. Therefore, in order to obtain the largest possible number of eggs, in addition to providing blackberry leaves, it is necessary to provide loosely woven paper towels as a physical stimulus (Figure 3), which would provide protection for the eggs.

Paper towels and plastic have been shown to be efficient oviposition substrates for *Phidotricha erigens* Raganot (Lepidoptera: Pyralidae) and *Platynota rostrana* (Walker) (Lepidoptera: Tortricidae), respectively (Nava et al., 2006).

3.3. Larval development on two blackberry cultivars

For the *H. bipunctalis* larvae reared on leaves of the Guarani cultivar, larval-stage duration and viability were 22.63 ± 0.26 days and 50.98%, respectively. Therefore, *H. bipunctalis* larvae reared on leaves of the cultivar with prickles (Guarani) reached full development more rapidly than did those reared on leaves of the cultivar without prickles (Xavante) (26.37 ± 0.29 days), and the difference between the two groups of larvae was significant ($F = 92.39$; $p < 0.000$; $df = 1$). According to Parra et al. (2009), food quality is related to physical characteristics, allelochemicals, and nutritional components that influence the ability of insects to consume and digest food. Meneguim et al. (2010), studying the mulberry *Morus* sp. and the silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae), found that nutrient content can vary among mulberry cultivars. In that study, *Bombyx mori* larvae were fed leaves of the hybrid cultivar SK4 or of the standard cultivar Miura. Leaves of SK4 were found to have a higher nutrient content and were therefore better for feeding the larvae, providing good digestibility and a higher growth rate, as well as a relatively low consumption rate and metabolic cost. A study evaluating the morpho-anatomical features of leaves of different mulberry cultivars fed to domestic silkworms found that higher amounts of idioblasts, cystoliths, and epidermis, as well as a lower proportion of parenchyma, were the least desirable characteristics (Okamoto and Rodella, 2006). Leaves of the loquat *Eriobotrya japonica* Lindl., another member of the family Rosaceae, have unicellular non-glandular trichomes on both surfaces (abaxial and adaxial), as well as idioblasts containing calcium oxalate crystals, mucilage, and phenolic compounds (Souza et al., 2003). Therefore, it is possible that different factors are involved in the nutrition and consequent development of *H. bipunctalis* larvae. Consequently, studies investigating the chemical components and morpho-anatomical features of leaves of the Guarani and Xavante blackberry cultivars are warranted.

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