Research, Society and Development, v. 11, n. 4, e5311426789, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i4.26789

Feeding preference of *Helicoverpa armigera* larvae (Lepidoptera: Noctuidae) on vegetative and reproductive organs of soybean plants

Preferência alimentar da lagarta *Helicoverpa armigera* em órgãos vegetativos e reprodutivos de soja

Preferencia alimentaria de la oruga *Helicoverpa armigera* en órganos vegetativos y reproductivos de soja

Received: 02/09/2022 | Reviewed: 02/21/2022 | Accept: 03/01/2022 | Published: 03/11/2022

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Abstract
Helicoverpa armigera is a pest insect with a large dispersal range, a high degree of polyphagy in the larval stage, and of difficult control. The pest's ability to survive due to the availability of food and persist in grain production systems present in several Brazilian regions allows it to infest a wide range of crops, including soybeans and invasive plants during the off-season. There is general knowledge that the larva prefers to feed on plant reproductive organs, but it is unknown if this is true for soybeans, a crop to which it is adapting following its recent recognition as a pest. Basic knowledge of herbivore feeding behavior is necessary to support control within the integrated pest management philosophy. The purpose of this study was to examine the preference behavior of *H. armigera* larvae in their first, fourth, and sixth instars for vegetative and reproductive organs of soybean plants. It is concluded that the larvae, regardless of instar, feed on all available vegetative and reproductive organs, but they have preferences based on their current developmental stage.
Keywords: Pest insects; Host selection; Choice.

Resumo
Helicoverpa armigera (Lepidoptera: Noctuidae: Heliothinae) é um inseto que se caracteriza pela grande capacidade de dispersão, elevado grau de polifagia na fase larval e, como praga, pela dificuldade de controle. A disponibilidade de alimento possibilita a sobrevivência e a permanência da praga nos sistemas de produção de grãos praticados em várias regiões brasileiras infestando um grande número de culturas, incluindo soja, e plantas invasoras nos períodos de entressafra. Existem informações genéricas que a larva prefere se alimentar de órgãos reprodutivos das plantas, mas não se sabe se isso é a realidade em soja, cultura à qual está se adaptando após sua recente constatação como praga. Informações básicas sobre o comportamento alimentar dos herbívoros são importantes para subsidiar o controle dentro da filosofia do manejo integrado de pragas. Esse trabalho teve como objetivo caracterizar o comportamento de preferência de lagartas de 1º, 4º e 6º ínstar de *H. armigera* entre órgãos vegetativos e reprodutivos das plantas de soja. Conclui-se que as lagartas, independentemente do ínstar, se alimentam de todos os órgãos vegetativos e reprodutivos disponíveis, porém apresentam preferência conforme o seu estádio de desenvolvimento.
Palavras-chave: Insetos-praga; Seleção hospedeira; Preferência.

Resumen
Helicoverpa armigera (Lepidoptera: Noctuidae: Heliothinae) es un insecto que se caracteriza por su alta capacidad de dispersión, alto grado de polifagia en estado larvario y, como plaga, es de difícil control. La disponibilidad de alimentos permite sobrevivir y permanecer en los sistemas de producción de plantas agrícolas en varias regiones brasileñas, incluyendo una gran cantidad de cultivos, incluyendo malezas en la temporada de soja. La información general es que la larva prefiere alimentarse de los órganos reproductores de las plantas, pero no se sabe que esto sea así en cultivo tras su reciente descubrimiento como plaga. La información básica sobre el comportamiento alimentario de los herbívoros es para apoyar el control dentro de la filosofía del manejo integrado de plagas. Este estudio tuvo como objetivo caracterizar el comportamiento de preferencia de larvas de *H. armigera* de 1°, 4° y 6° estadio entre los órganos vegetativos y reproductivos de las plantas de soja. Se concluye que el estadio, independientemente del estadio de desarrollo, se alimenta de todos los órganos vegetativos y reproductivos disponibles, pero tiene preferencia según su desarrollo evolutivo.
Palabras clave: Insectos plaga; Selección de hospedantes; Preferencia.

1. Introduction

Helicoverpa armigera larvae (Lepidoptera: Noctuidae: Heliothinae) feed on the most diverse organs, including apical shoots, leaves of various ages, and reproductive organs such as flowers and fruits, in countless species of plants, both cultivated and wild (Salvadori et al.,2013; Guedes et al., 2013). After being registered in Brazil (Czepak et al.,2013), they became a pest of the soybean crop (*Glycine max*), where they attack seedlings first and, later, leaves, legumes, and grains from emergence to harvest (Salvadori et al., 2013; Salvadori & Suzana, 2014; Suzana et al., 2015; Ávila et al., 2013).

According to the literature, the *H. armigera* larvae preferentially feed on various plant organs, particularly buds, inflorescences, fruits, and pods (Reed, 1965; Wang and Li, 1984). Considering the generalist habit of searching for food, lepidopterans decide what they will eat according to the supply and nutritional adequacy of the food (Babic et al., 2008). *H. armigera* larvae fed on leaves and reproductive organs of plant species showed variation in survival, duration and consumption and in the weight of pupae they originate (Suzana, 2015; Suzana et al., 2015). Despite these indications, it is unknown in what proportion this occurs in soybeans, depending on the stage of development of larvae and plants, which is relevant knowledge to support the establishment of management criteria for this pest. Information about larvae displacements through plant organs, depending on their development stage, is important for definitions in insecticide application technology, in order to better reach the target and increase control efficiency.

The purpose of this study is to characterize the feeding behavior of *H. armigera* larvae in the first (small), fourth (medium), and sixth (large) instars in terms of their preference to consume vegetative and reproductive organs of soybean plants.

2. Methodology

The study was carried out in the laboratory, in a climate-controlled chamber (25 ± 2 ºC, 60 ± 10% relative humidity and 12-hour photophase). Larvae of *H. armigera* from the laboratory kept in the artificial diet of Greene et al. (1976), and soybean plants (cv. BMX Ativa RR) grown in pots (8 liters), in a greenhouse were used in the experiment.

The larvae's food preference and consumption behavior were studied in vegetative and reproductive organs of soybean plants at two stages of development. Two preference tests were conducted in free-choice arenas with larvae from three stages: first, fourth, and sixth instars. The following vegetative organs were evaluated in one of the tests, which were removed from plants at stage V5 (according to the scale developed by Hanway & Thompson, 1985): a) Expanding leaves (EL), with 1.8 cm long leaflets in the process of opening; b) New leaves (NL), newly expanded, with leaflets 6.9 cm long and 5.4 cm wide, completely open after 3-5 days, light green in color; and c) Old leaves (OL), fully expanded with leaflets 10.4 cm long and 7.7 cm wide, completely open after 15-20 days, dark green in color. In the other test, in addition to the previously mentioned vegetative organs, the following reproductive organs were evaluated, taken from plants at stages R5.2 and R5.5 (according to

Hanway & Thompson, 1985): a) Legumes that are well-developed, with a length of 4 cm and grains that are in the early stages of development (LGE) and b) Well-developed legumes, 5.5 cm long, with fully developed grains (LGD).

The experiment was carried out in Petri dishes measuring 9 cm (1st and 4th instars) and 15 cm in diameter (6th instar), covered with filter paper moistened with distilled water. The organs under test were distributed equidistantly near the plate's edges, and the larvae were released in the center of the dishes at the start of the tests, either individually (4th and 6th instars) or in groups of ten (1st instar).

The number of larvae present in each organ was determined half an hour after the start of the test for the three instars, as well as after 24 hours for the first instar, 12 hours for the fourth instar, and 6 hours for the sixth instar, and total consumption was calculated at the end. The consumption of leaves and legumes was estimated in mass (mg) by weighing before and after feeding, with due correction for the variation in weight found in control plates containing only the organs under evaluation, without larvae.

The experiments were carried out in a randomized block design, with four replications (50 larvae/repeat). Data were subjected to analysis of variance and means were compared using the Tukey test (HSD or Kramer, $p<0.05$).

3. Results and Discussion

3.1 First instar larvae

In all evaluations performed, first instar larvae visited all types of leaves available, but with a higher frequency of choice for expanding leaves (EL) (Figure 1). Consumption was also higher in expanding leaves (EL) versus new leaves (NL) and old leaves (OL).

When legumes at the beginning of grain development (LGE) and legumes with fully developed grains (LGD) were offered alongside the vegetative organs, a similar result in terms of frequency of choice was obtained (Figure 2). Despite the ability to choose between vegetative and reproductive organs, the consumption evaluation confirmed the preference of first instar larvae for expanding leaves. However, it is noteworthy the fact that first instar larvae already feed on legumes as well as new and old leaves.

3.2 Fourth instar larvae

The larvae did not show a clear preference for any of the vegetative organs offered at this stage (Figure 3). They were initially evenly distributed across the food types (0.5 h), but with a predominance of non-choice behavior (NC). The presence of larvae was greatest in expanding leaves (EL) and old leaves (OL) 12 hours after infestation but did not differ from non-choice. Consumption occurred in all leaves, but it was greater in expanding leaves (EL) than in new (NL) or old leaves (OL).

When reproductive organs were offered alongside vegetative organs (Figure 4), there was no preference for choice among fourth instar larvae in the first evaluation (0.5 hours), with non-choice predominating. In the assessment 12 hours after infestation, the highest presence was found in old leaves (OL) and legumes at the start of grain development (LGE). All organs were visited with minor variations in frequency, and a significant non-choice behavior was also observed. Consumption of expanding leaves (EL) and legumes was higher both at the start of grain development and with developed grains (LGD). As with the first instar larvae (small larvae), the fourth instar larvae (medium size) consumed legumes as well.

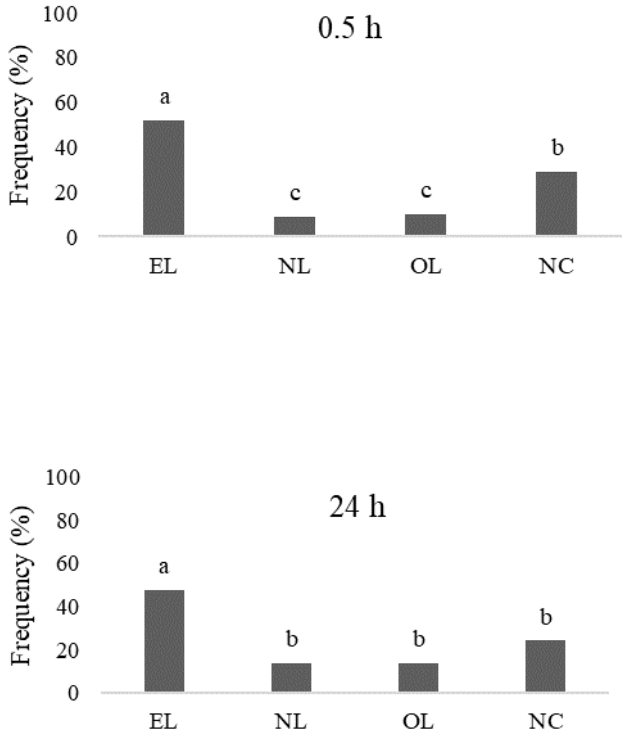
3.3 Sixth instar larvae

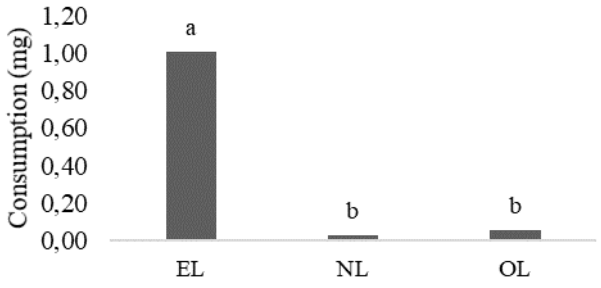
At this stage, when only vegetative organs were offered, the larvae visited all types of leaves without discrimination in both evaluations (0.5 and 6h) (Figure 5). Initially, 43% of them did not select any of the available options, a trend that

continued in the 6-hour assessment after the evaluations began. The consumption result revealed that the food is divided among leaves of various ages, with no clear predominance in any of them.

When reproductive organs were offered alongside vegetative organs, the sixth instar larvae did not show preference at first (0.5h), visiting all organs without distinction, and the non-choice behavior was the most common (44%) (Figure 6). The preferred food in the following evaluation (6h) was legumes with fully developed grains (LGD). As with the vegetative organs test, all organs were consumed in this test. Expanding leaves (EL) and legumes with fully developed grains (LGD), on the other hand, were consumed in greater proportions.

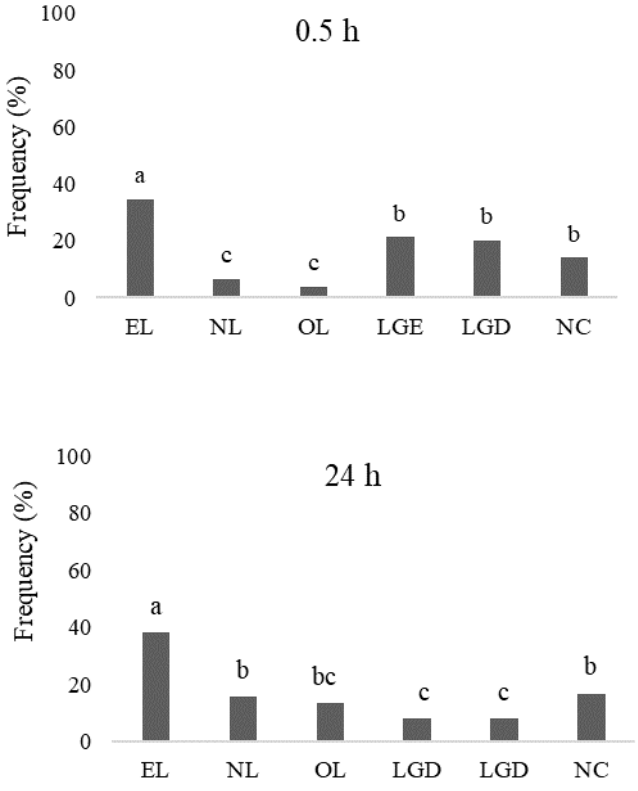
Figure. 1. Frequency of *Helicoverpa armigera* 1st instar larvae in expanding leaves (EL), new leaves (NL), old leaves (OL), and no choice (NC), at 0.5 and 24 hours after the start of the test with option of choice, and consumption after 24 hours, in soybean plants (cv. BMX Ativa RR), in the laboratory (25 ± 2 ºC, 60 ± 10 % RH and 12 hours of photophase). Note: the same letter on the columns indicates statistical equality by the Tukey-HSD test ($p<0.05$).

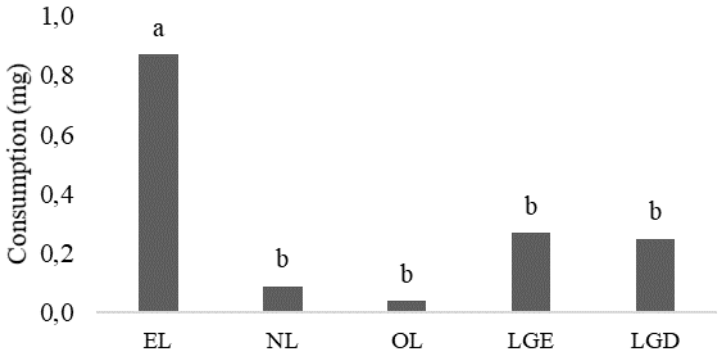




Source: Authors' data (2018).

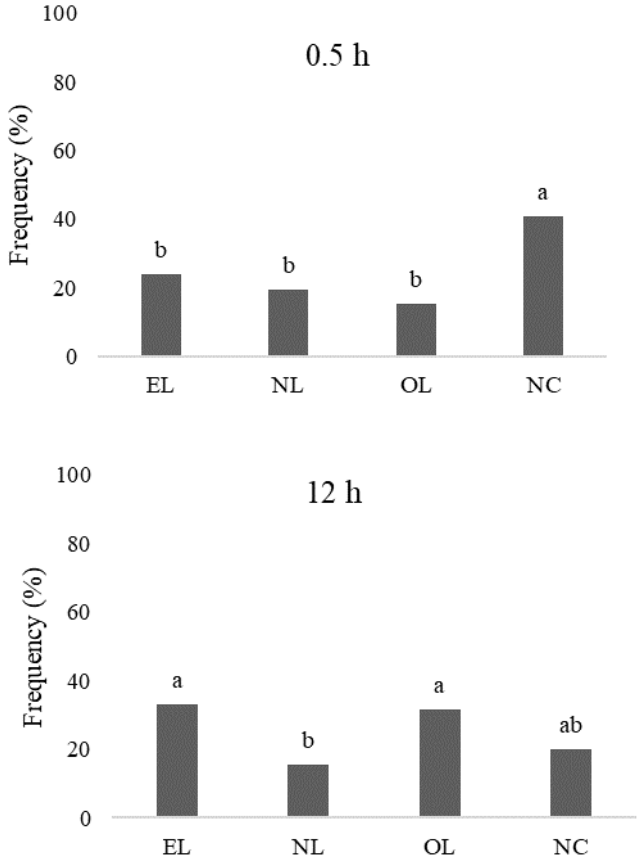
Figure 2. Frequency of *Helicoverpa armigera* 1st instar larvae in expanding leaves (EL), new leaves (NL), old leaves (OL), legumes in early grain development (LGE), legumes with fully developed grains (LGD), and no choice (NC), at 0.5 and 24 hours after the start of the test with option of choice, and consumption after 24 hours, in soybean plants (cv. BMX Ativa RR), in the laboratory (25 ± 2 ºC, 60 ± 10 % RH and 12 hours of photophase). Note: the same letter on the columns indicates statistical equality by the Tukey-Kramer test ($p<0.05$).

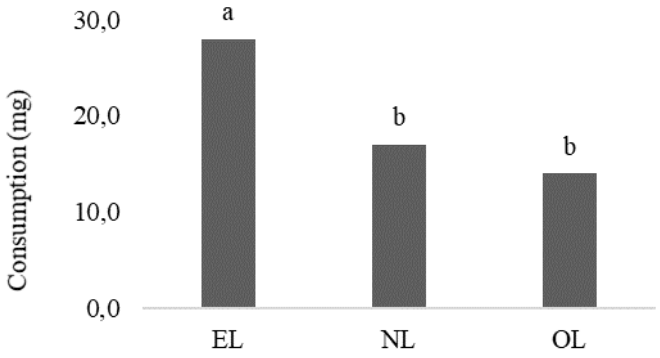




Source: Authors' data (2018).

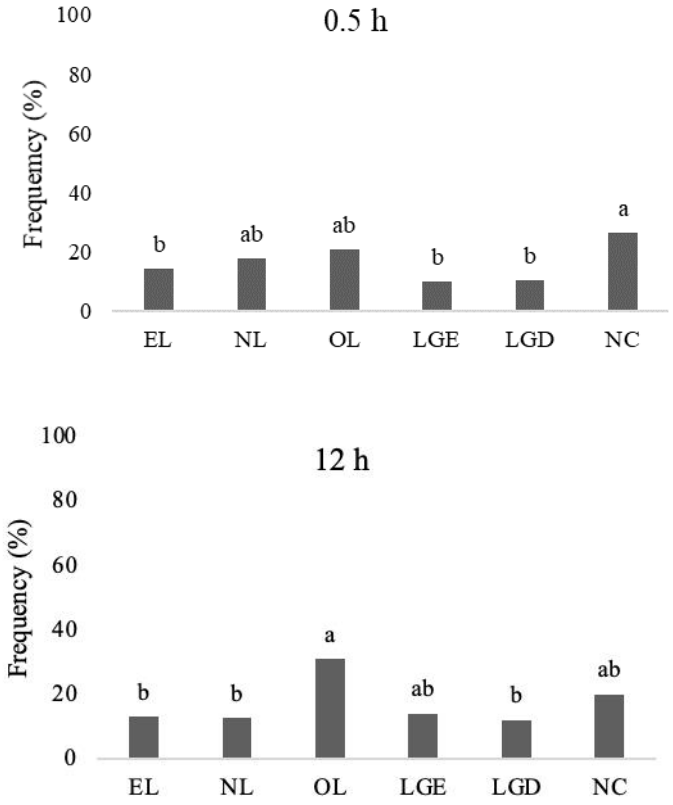
Figure 3. Frequency of *Helicoverpa armigera* 4th instar larvae in expanding leaves (EL), new leaves (NL), old leaves (OL), and no choice (NC), at 0.5 and 12 hours after the start of the test with option of choice, and consumption after 12 hours, in soybean plants (cv. BMX Ativa RR), in the laboratory (25 ± 2 ºC, 60 ± 10 % HR and 12 hours of photophase). Note: the same letter on the columns indicates statistical equality by the Tukey-HSD test (p<0,05).

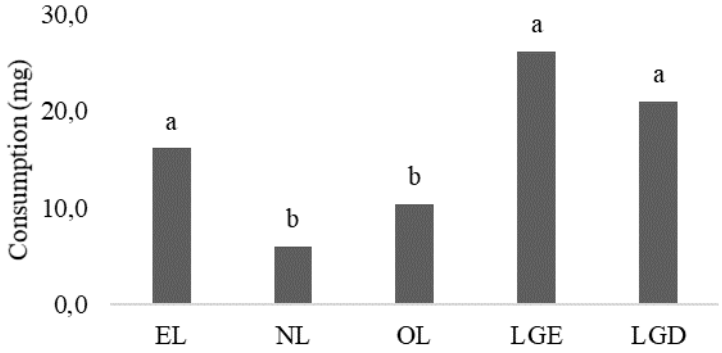




Source: Authors' data (2018).

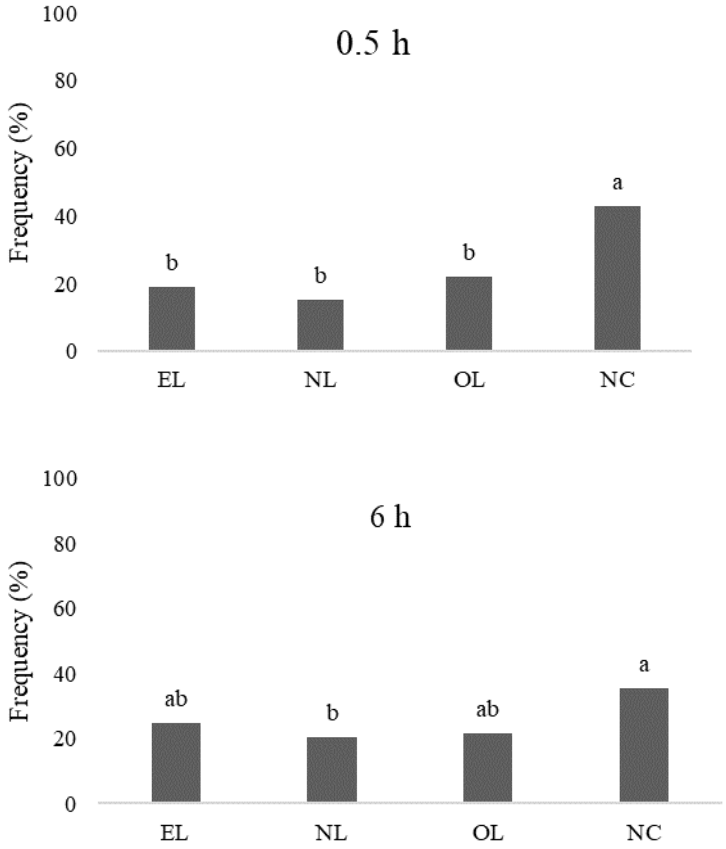
Figure 4. Frequency of Helicoverpa armigera 4th instar larvae in expanding leaves (EL), new leaves (NL), old leaves (OL), legumes in early grain development (LGE), legumes with fully developed grains (LGD), and no choice (NC), at 0.5 and 12 hours after the start of the test with option of choice, and consumption after 12 hours, in soybean pants (cv. BMX Ativa RR), in the laboratory (25 ± 2 ºC, 60 ± 10 % HR and 12 hours of photophase). Note: the same letter on the columns indicates statistical equality by the Tukey-Kramer test ($p<0.05$).

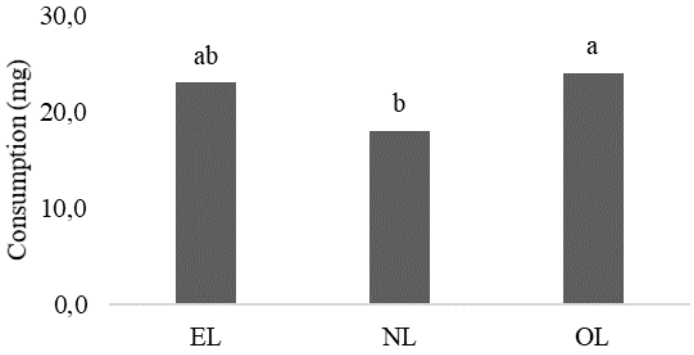




Source: Authors' data (2018).

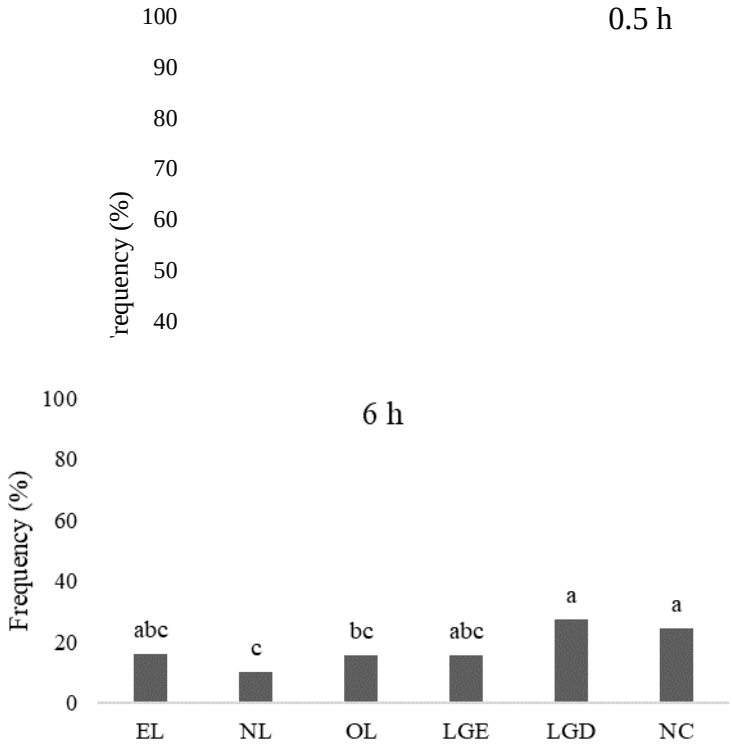
Figure 5. Frequency of *Helicoverpa armigera* 6th instar larvae in expanding leaves (EL), new leaves (NL), old leaves (OL), and no choice (NC), at 0.5 and 6 hours after the start of the test with option of choice, and consumption after 6 hours, in soybean plants (cv. BMX Ativa RR), in the laboratory (25 ± 2 ºC, 60 ± 10 % HR and 12 hours of photophase). Note: the same letter on the columns indicates statistical equality by the Tukey-Kramer test (p<0,05).

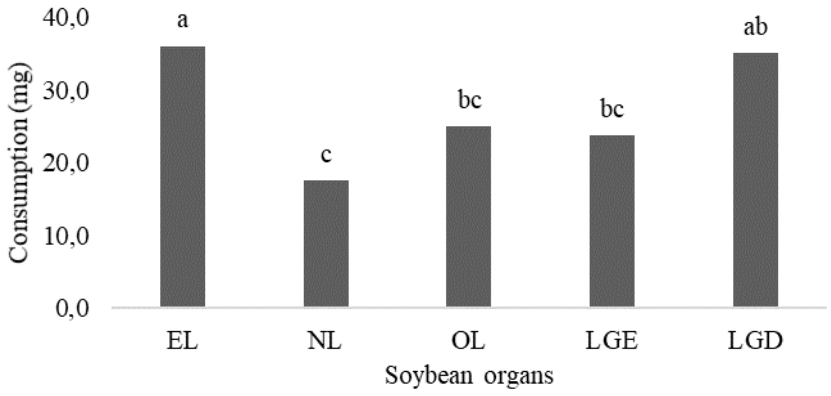




Source: Authors' data (2018).

Figure 6. Frequency of *Helicoverpa armigera* 6th instar larvae in expanding leaves (EL), new leaves (NL), old leaves (OL), legumes in early grain development (LGE), legumes with fully developed grains (LGD), and no choice (NC), at 0.5 and 6 hours after the start of the test with option of choice, and consumption after 6 hours, in soybean plants (cv. BMX Ativa RR), in the laboratory. (25 ± 2 ºC, 60 ± 10 % HR and 12 hours of photophase). Note: the same letter on the columns indicates statistical equality by the Tukey-Kramer test ($p<0,05$).





Source: Authors' data (2018).

4. Discussion

When the presence of larvae from different instars was compared in a test that only included vegetative organs, the presence of first instar larvae in expanding leaves remained constant, which defines the structure of preference for the larvae in this instar. Larvae in their fourth and sixth instars, on the other hand, display a lower frequency of choice in expanding leaves, 24 to 33% and 19 to 29%, respectively, at 0.5 and 12/6 h, but with a high consumption. A similar behavior was observed in a test that included both vegetative and reproductive organs, in which the presence of first instar larvae in expanding leaves ranged between 35 to 38% from 0.5 to 24 hours. However, the fourth and sixth instar larvae did not exhibit a greater frequency in expanding leaves, although consumption was higher, demonstrating the preference for such structure.

When reproductive structures were offered in addition to leaves, the preference of first instar larvae for legumes stands out, albeit with a low frequency, ranging from 41 to 16% from the first 0.5 hours to the first 24 hours. At the same time, the presence of fourth instar larvae in legumes increased slightly from 21 to 26%, while the presence of sixth instar larvae increased significantly from 18 to 43%.

The preference of first and fourth instar larvae for leaves, particularly expanding leaves, demonstrated in this study may be related to the fact that these foods are more tender, less fibrous, and thus more palatable and nutritionally valuable (Fitt, 1989). The observed food preference may also be associated to the development of the larvae' jaws and the variation of secondary compounds in the soy organs, the presence of which, or even the concentration of which, can have an attractive or repellent effect (Zalucki et al., 2002).

Sixth instar larvae showed no obvious preference behavior between vegetative and reproductive organs of soybean plants, which can be attributed to digestive enzymes present in the final instars (Patankar et al., 2001; Cunha, 2016), allowing for lower selectivity. Furthermore, it is likely that this behavior is related to the increased mobility of fourth instar larvae, as well as the growing demand for foods of higher nutritional quality for their biological performance. The preference for organs in full development may be a larval strategy to avoid consumption of organs that have already developed and have a higher concentration of defense compounds; thus, the preference may not necessarily indicate that the organ has the highest nutritional quality for the insect's development.

The search for shelter, nutritional adequacy, and the attractiveness of different parts of the plant can all explain *H. armigera* larvae behavior and food consumption. The species is known for performing better in some parts of the plant than others (Hmimina, 1988; Sison and Shanower, 1994). The constant movement between plant organs indicates that, under natural conditions, H. armigera larvae seek the best for their biological performance, such as more nutritious tissues or less chemically defended tissues (Perkins et al., 2013).

Larvae of *Heliothis virescens* (Lepidoptera: Noctuidae: Heliothinae) and *H. armigera* feed on the upper vegetative parts, i.e., new leaves, in their first three instars, and then move on to the reproductive parts, which include flowers, fruits, and seeds, in their fifth instar (Bortolotto et al., 2014; Rogers and Brier, 2010).

In all tests, regardless of the organs offered and the size of larvae, no plant organ failed to be visited and consumed. Thus, consumption becomes decisive to conclude on the acceptance of food by the insects. In this sense, there was no significant disparity between choice and consumption behavior, such that, in general, the most preferred organs were also the most consumed, as was the case with expanding leaves and legumes.

5. Conclusion

H. armigera larvae, regardless of instar, feed on all vegetative and reproductive organs of soybean plants that are available to them, but they have preferences based on their stage of development. First instar larvae prefer to feed on expanding leaves; fourth instar larvae maintain a preference for feeding on expanding leaves and incorporate legumes into their diet, particularly tender ones; and sixth instar larvae consume any type of organs, vegetative and reproductive, but feed on expanding leaves and legumes in greater proportion, both at the beginning of development.

To better understand food preference in vegetative and reproductive organs of soybean, more detailed research on the activity of larval digestive enzymes is needed.

Acknowledgments

To the Coordination for the Improvement of Higher Education Personnel (Capes) for granting the scholarship.

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