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# Canibalism in the larval instars of *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae): temperature and food quantity

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**ABSTRACT.** Cannibalism is a frequent behavior in *Spodoptera frugiperda* (J. E. Smith, 1797) larvae either in the field or in a laboratory. The purpose of this work was to investigate the factors temperature and food quantity on the cannibal behavior in instars of this insect under laboratory conditions. The neonates were conditioned at temperatures of 22, 25, 28 and 31 ± 1°C until they reached the 3rd, 4th, and 5th instar. The number of 20 larvae were transferred to different gerbox® with the amount of food varying from 0, 5, 10, 15, and 20 g of artificial diet. Cannibalism was evaluated after 72 hours. In all the instars evaluated, the larvae showed cannibalistic behavior as a function of temperature and amount of food. The amount of 15 g of artificial diet is sufficient to feed the 3rd and 4th instar larval for 72h, regardless of temperature. For the 5th instar this amount is 10 g.

**Keywords:** artificial diet; cannibal behavior; fall armyworm.

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## Introduction

Cannibalism is defined as intraspecific predation (Fox, 1975). Some trophic groups of the insect orders are cannibals, which feed on tissues, cells or internal fluid of living animals, such as parasites and parasitoids, or freshly killed by a predator, for example, which manifest this behavior for feeding, population density regulation, and sexual copulation schemes (Fox, 1975; Montgomery, 1983; Marinoni, 2001; Elgar & Schneider, 2004; Richardson, Mitchell, Reagel, & Hanks, 2010; Aisenberg, Costa, & Gonzále, 2011).

Cannibalism is a frequent behavior in the *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) larvae both in the field and in a laboratory (Chapman et al., 2000; Sarmento et al., 2002; Bentivenha, Baldin, Montezano, Hunt, & Paula-Moraes, 2017). This behavior may be favored by food limitation and insufficient space, as well as by the search for supplemental water and/or nutrients (Joyner & Gould, 1985; Vangansbeke et al., 2014, Bentivenha, Baldin, Hunt, Paula-Moraes, & Blankenship, 2016; Bentivenha et al., 2017), but despite being frequent, it is not obligatory, as described by Da Silva and Parra (2013).

Other factors such as abiotic conditions (temperature, humidity), asynchrony with the host plant and quantities of vulnerable insects can also trigger cannibalism (Richardson et al., 2010). Under laboratory conditions, factors related to food (amount and arrangement), temperature, and developmental stage were investigated for *Helicoverpa armigera* (Hübner, 1809) (Lepidoptera: Noctuidae), showing that greater complexity of diet arrangement decreased cannibalism due to less favored encounters between larvae, and that in the third instar cannibalism intensified, especially at high temperatures and lack of food (Tang, Zhao, Xu, & Qui, 2016).

A study like this is needed for *Spodoptera frugiperda* where there is a need for individualization during *in vivo* maintenance under laboratory conditions due to cannibalistic behavior. Another example of the importance of knowing the cannibal behavior and the factors involved is for the *in vivo* production of entomopathogenic agents, where the mass production of the biological agent requires rearing and inoculation into the larvae as a host (Valicente, Tulher, & Barros, 2010). Due to the cannibalistic habit of the host insect, it is necessary to individualize the larvae, increasing costs and production time along the production chain (Chapman et al., 2000; Valicente et al., 2010; Da Silva & Parra, 2013).

Thus, for the development of these and other research studies, bioassays are carried out in groups of larvae, and it is important to know the cannibal behavior in function of the factors that can interfere in this habit of the insect. Therefore, the objective of this work was to investigate the factors of temperature and food quantity on cannibal behavior in three instars of *S. frugiperda* larvae under laboratory conditions.

## Material and methods

The experiments were conducted in the Entomology Sector of the *Núcleo de Desenvolvimento Científico e Tecnológico em Manejo Fitossanitário de Pragas e Doenças (NUDEMAFI)* located in the Center for Agricultural Sciences and Engineering of the *Universidade Federal do Espírito Santo*, Espírito Santo State, Brazil (CCAE/UFES), from June to August 2020.

### Mass Production and Maintenance of *Spodoptera frugiperda*

The population of *Spodoptera frugiperda* was established from pupae from the Laboratory of Biological Control of Embrapa Maize and Sorghum (Sete Lagoas, Minas Gerais State, Brazil). The rearing and maintenance of *Spodoptera frugiperda* were carried out under standardized conditions in a climate-controlled room at a temperature of 25 ± 2°C, relative humidity of 60%, and photoperiod of 12 hours. The adults were kept in PVC cages and fed using absorbent cotton soaked in 10% sucrose solution. For oviposition, white paper sheets were used to cover the inside of the cages and removed every two days for the removal of the eggs. The eggs were kept in transparent plastic jars until the hatching of the larvae. They were transferred with the help of a soft-bristle brush to plastic containers (100 mL) containing an adapted artificial diet based on beans, wheat germ, brewer's yeast, and carrageenan (Souza, Ávila, & Caballero, 2001), where they remained for five days. Then approximately 40 larvae were transferred to a gerbox® acrylic container (dimensions 11 x 11 x 3 cm) and after 10 days were individualized in a gerbox® (3 cm diameter) until pupation. With the emergence of the adults, they were transferred to the rearing cages, continuing the cycle.

### Bioassay

In the present study, we investigated the influence of temperature and amount of food on cannibalism in three instars of *Spodoptera frugiperda*. For the bioassay, newly hatched *Spodoptera frugiperda* larvae from laboratory mass rearing were placed in climate-controlled chambers at 22, 25, 28, and 31 ± 1°C, 12 hours photoperiod, relative humidity (RH) 70 ± 10%. When they reached the instar for the bioassay setup, in this case, the 3$^{rd}$, 4$^{th}$, and 5$^{th}$ instar, the larvae in groups of 20 individuals gerbox$^{-1}$ (dimensions 11 x 11 x 3 cm) were transferred according to the amount of food in 0, 5, 10, 15, and 20 g. The food source was the artificial diet identical to that used for mass rearing. Figure 1 illustrates this layout scheme of food distribution and quantity.

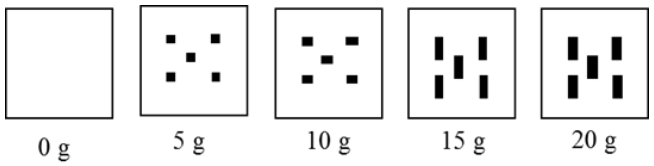


**Figure 1.** Schematic of the distribution layout and amount of food used in the cannibalism bioassay of *Spodoptera frugiperda* at each instar and temperature.

The experimental design was entirely randomized with four repetitions. Each instar was evaluated as a function of food availability and temperature, thus making 20 treatments in each instar evaluated in a factorial scheme (4 temperatures x 5 food quantities).

Cannibalism was evaluated after 72 hours, quantifying the number of live insects. For the statistical analysis, an absolute value was assigned to these factors, i.e., a qualitative value, because the intention is to evaluate the cannibal behavior in each treatment, and not to obtain a maximum or minimum value through regression in which the values are treated as quantitative. The R statistical program was used to perform the analyses (R Core Team, 2020).

## Results and discussion

The cannibalism of *Spodoptera frugiperda* larvae showed a significant interaction between the amount of artificial diet and temperature in the instars evaluated, proceeding with the unfolding of the factors (Table 1). For the third instar larvae, except for the temperature factor within the levels of 15 and 20 g of food which were not significant, the others showed significance (Table 2). Larvae cannibalism was higher in the absence of food and with increasing temperature, reaching 86.25% at 31°C (Table 2). A similar result for the amount of food was found for *Helicoverpa armigera* after 56 hours of evaluation, reaching 51.67% mortality at this same temperature (Tang et al., 2016). The absence of food or supplementation of water and/or nutrients can be a consequence for individuals to cannibalize themselves (Joyner & Gould, 1985; Vangansbeke et al., 2014).

**Table 1.** Analysis of variance of the percentage of cannibalism in the third, fourth, and fifth instar larvae of *Spodoptera frugiperda* fed different amounts of artificial diet under the temperature of 22, 25, 28, 31 ± 1°C. Photophase: 12 hours and RH: 70 ± 10%.

| Analysis of variance | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | 3rd Instar | | 4th Instar | | 5th Instar | |
| | GL | QM | Pr>Fc | QM | Pr>Fc | QM | Pr>Fc |
| Temperature | 3 | 2 | 4.61E-13 | 4 | 3.79E-04 | 4 | 3.19E-04 |
| Amount of food | 4 | 3 | 0.00E+00 | 5 | 0.00E+00 | 5 | 0.00E+00 |
| Temperature * Amount of food | 12 | 5 | 3.89E-11 | 3 | 6.10E-03 | 2 | 5.68E-03 |
| Waste | 60 | 4 | | 2 | | 3 | |
| Total | 79 | 1 | | 1 | | 1 | |
| CV(%) | 28.77 | | | 43.69 | | 25.61 | |

**Table 2.** Percentage of cannibalism in the third instar larvae of *Spodoptera frugiperda* fed different amounts of artificial diet under the temperature of 22, 25, 28, 31 ± 1°C. Photophase: 12 hours and RH: 70 ± 10%.

| Cannibalism (%) - 3rd instar larvae | | | | |
|---|---|---|---|---|
| Food | Temperature* | | | |
| | 22°C | 25°C | 28°C | 31°C |
| 0 g | 42.50 Ca | 70.00 Ba | 75.00 Ba | 86.25 Aa |
| 5 g | 2.50 Bb | 30.00 Ab | 2.50 Bb | 23.75 Ab |
| 10 g | 1.25 Bb | 11.25 Ac | 1.25 Bb | 18.75 Ab |
| 15 g | 6.25 nsb | 7.50 nsc | 1.25 nsb | 6.25 nsc |
| 20 g | 3.75 nsb | 2.50 nsc | 0.00 nsb | 2.50 nsc |

*Means followed by the same capital letter in the row and lower case in the column do not differ at the 5% probability level by the Scott-Knott test. ns Not significant for the temperature within each level of the amount of food.

In addition, cannibalism can improve the fitness of arthropods in natural environments by providing access to essential nutrients, and in some cases, this behavior is dependent on available food. One example was observed in females of *Amblydromalus limonicus* (Garman & McGregor) (Acari: Phytoseiidae) that exhibited cannibalistic behavior of their own eggs when adult females were fed with eggs of *Ephestia kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae), eggs of *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) or pollen of *Typha angustifolia* L. (Typhaceae). However, when adult females were fed on *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae), and *Carpoglyphus lactis* (Linnaeus, 1767) (Acari: Carpoglyphidae) it was not necessary to cannibalize their own eggs (Vangansbeke et al., 2014). Despite its advantages for the fitness of the insect, cannibalism can also be harmful, when the cannibals end up being injured by their prey or infected with pathogens (Chapman et al., 1999; Tang et al., 2016; Van Allen et al., 2017).

In the present study, as the amount of food increased, as observed in the treatments with 15 and 20 g of artificial diet, there was no interference of temperature for cannibalism. We can infer for this instar larval from this result that, provided there is an amount of food, the temperature factor does not influence cannibal behavior, as shown in Table 2. The temperature is an important factor for the development of *Spodoptera frugiperda*, larvae reared on sweet corn kernels showed the optimal range for egg, larval and egg-to-adult development was between 26 and 30°C (Du Plessis, Schlemmer, & Van Den Berg, 2020). Was also found that the optimal temperature for the fastest development of the larval stage and lowest mortality was 30°C and the minimum threshold of 12.12°C (Du Plessis et al., 2020). In field conditions, this factor determines its highest pest potential, especially in tropical climates, precisely because they have a wide temperature range and consequently reach a greater distribution throughout the territory (Parra et al., 2021).

Based on the bioassay, 15 g of artificial diet is adequate to prevent cannibalism of the 3rd instar caterpillars, regardless of the temperature evaluated. However, we will note that when considering the temperature: 22 and 28°C the amount of 5 g of diet is sufficient for feeding; 10 g for 25°C and 15 g for 31°C (Table 2).

Concerning the 4th instar larvae, similar results to the previous instar were observed for the food quantity factor, i.e. the absence of food favors the increase in cannibal behavior. However, 5 and 10 g of diet at 25°C and 5 g of diet at 28°C equaled the percentage of cannibalism in the absence of food (Table 3).

**Table 3.** Percentage of cannibalism in the fourth instar larvae of *Spodoptera frugiperda* fed different amounts of artificial diet under the temperature of 22, 25, 28, 31 ± 1°C. Photophase: 12 hours and RH: 70 ± 10%.

| Cannibalism (%) – 4th instar larvae | | | | |
|---|---|---|---|---|
| Food | Temperature* | | | |
| | 22°C | 25°C | 28°C | 31°C |
| 0 g | 70.00 Aa | 38.75 Ba | 33.75 Ba | 65.00 Aa |
| 5 g | 10.00 Bb | 21.25 Aa | 17.50 Aa | 28.75 Ab |
| 10 g | 3.75 Bb | 18.75 Aa | 1.25 Bb | 17.50 Ab |
| 15 g | 1.25 $^{ns}$b | 8.75 $^{ns}$b | 5.00 $^{ns}$b | 6.25 $^{ns}$c |
| 20 g | 0.00 $^{ns}$b | 5.00 $^{ns}$b | 1.25 $^{ns}$b | 3.75 $^{ns}$c |

*The upper-case letters in the row and lower case letters in the column, do not differ at 5% probability level by Scott-Knott test. $^{ns}$Not significant, regarding the unfolding of the amount of food within each temperature level. Data transformed into arc sine √(x/100).

For this same instar, in the condition of food amounts of 0, 5 and 10 g the temperature significantly influenced the cannibal behavior. In the absence of food (0 g) the temperature extremes evaluated, 22°C and 31°C, presented the highest values of cannibalism, 70 and 65%, respectively. In the 5 g quantity, this interference in temperature is observed from 25°C on, varying from 17.50 to 28.75%. And for 10 g the temperatures of 25 and 31°C stood out, with an average of around 18% of cannibalism. Furthermore, the cannibal behavior shown in the treatments with 15 and 20 g of artificial diet was not interfered with by temperature (Table 3).

For the fourth instar, 5 g of diet is enough for 20 larvae to feed at 22°C and 31°C. For the temperatures of 25 and 28°C, this amount varies from 15 g and 10 g, respectively (Table 3). Other authors have found that the arrangement of food on the rearing container also interferes with this behavior, where cannibalism is minimized with the use of more complex grouping in the arrangement of the artificial diet as if they formed "artificial barriers", there are difficulties for the encounter between the larvae increasing survival rates (Tang et al., 2016). Given this, there is a possibility of minimizing cannibalism in mass rearing adjusting this methodology, regarding temperature combination and food distribution arrangements.

Although frequent, both in the field and in the laboratory, cannibalism seems not to be mandatory as described by Da Silva and Parra (2013), pointing out a group rearing method with 90% larval survival. Thus, research studying the factors that involve this cannibalistic habit provides discussions about the subject and adds knowledge to solve problems in biofactories, for example, or even leads to points for new research.

As observed in the previous instars, in the fifth instar, the absence of food and the increase in temperature also intensified cannibalism, reaching 72.50% at 31°C (Table 4). The temperature of 25°C was excluded from the analysis because it showed the presence of pre-pupae during the evaluation, and because the pre-pupal stage leaves vulnerable to cannibalism. In a study of cannibalism in another Noctuidae species, such as *Helicoverpa zea*, it was found that at the moment of instar change the larvae became more susceptible to attack (Dial & Adler, 1990).

**Table 4.** Percentage of cannibalism in the instar larvae of *Spodoptera frugiperda* fed different amounts of artificial diet under the temperature of 22, 28, 31 ± 1°C. Photophase: 12 hours and RH: 70 ± 10%.

| Cannibalism (%) – 5th instar larvae | | | | |
|---|---|---|---|---|
| Food | Temperature* | | | |
| | 22°C | 25°C | 28°C | 31°C |
| 0 g | 41.50 Ca | - | 58.75 Ba | 72.50 Aa |
| 5 g | 15.00 Bb | - | 21.25 Ab | 25.00 Ab |
| 10 g | 1.25 $^{ns}$c | - | 2.50 $^{ns}$c | 6.25 $^{ns}$c |
| 15 g | 0.00 $^{ns}$c | - | 2.50 $^{ns}$c | 1.25 $^{ns}$c |
| 20 g | 0.00 $^{ns}$c | - | 2.50 $^{ns}$c | 1.25 $^{ns}$c |

*The upper case letters in the row and the lower case letter in the column, do not differ at 5% probability level by Scott-Knott test. $^{ns}$Not significant for the unfolding of the amount of food within each temperature level. NOTE: The temperature of 25°C was excluded because it showed the presence of pre-pupa.

An interesting result can be observed from the availability of 10 g of artificial diet whose temperature was not significant and this amount is enough to minimize cannibalism in this instar (Table 4). For *Helicoverpa*

*armigera,* there was a need for more food for the caterpillars tested to complete development at high temperature (Tang et al., 2016). In addition to temperature and food quantity, cannibal behavior can be influenced by the nutritional quality of the food and the larval instar (Chapman et al., 1999; Sigsgaard, Greenstone, & Duffield, 2002; Richardson et al., 2010; Vangansbeke et al., 2014).

In other studies, it was found that when *Helicoverpa zea* larvae of the same instar were gathered together, cannibalism took longer to occur than when the larvae were not uniform, about 96 h for the fifth instar larvae and only 6 h in groups with different instars (Dial & Adler, 1990). The same behavior was observed for *Spodoptera frugiperda*, in which cannibalism values were higher when interactions occurred between larvae at different developmental stages than at the same larval stage (Bentivenha et al., 2017). In addition, for *Spodoptera frugiperda,* it was found that cannibalism was higher among the fifth and sixth instar caterpillars than among the first instars (Chapman et al., 1999).

The knowledge of cannibal behavior and the factors involved is important for the in vivo production of entomopathogenic agents (Valicente et al., 2010). One of the main biological control methods employed for the control of the larvae is the use of entomopathogenic viruses, especially SfMNPV (*Spodoptera frugiperda multiple nucleopolyhedrovirus*), whose the mass production of the etiological agent requires rearing and inoculation into the larvae as a host and mass rearing of insects in the laboratory. There is a need for individualization during the in vivo maintenance of *Spodoptera frugiperda* under laboratory conditions, which increases costs and production time along the production chain (Chapman et al., 2000; Valicente et al., 2010; Da Silva & Parra, 2013; Tang et al., 2016).

Behavioral studies involving cannibalism, *Spodoptera frugiperda* larvae and *in vivo* production of baculoviruses are currently being carried out mainly with the intention of enabling large-scale production (Elvira, Williams, & Caballero, 2010; Da Silva & Parra, 2013; Valicente, Tuelher, Pena, Andreazza, & Guimarães, 2013). Thus, the present study contributes to the direction of this and other research that requires information about the factors that may interfere with this insect habit under laboratory conditions.

## Conclusion

The third instar, the 22 and 28°C 5 g of artificial diet is sufficient for 72 hours of feeding of 20 larvae; 10 g for 25°C and 15 g for 31°C.

The fourth instar, 5 g of diet was sufficient of 20 larvae to feed for 72 hours at 22 and 31°C. For temperatures of 25 and 28°C, this amount varied from 15 g and 10 g, respectively.

The amount of 15 g of artificial diet declined cannibalism of the 3$^{rd}$ and 4$^{th}$ instar larvae in 72 hours, regardless of the temperature evaluated. For the 5$^{th}$ instar larvae, this amount is 10 g.

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