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# Research Article
# Biology of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) Rearing on Artificial or Natural Diet in Laboratory

[1]Matheus Le Senechal Nunes, [1]Lara Leal Figueiredo, [1,2]Rízia da Silva Andrade, [1]Janayne Maria Rezende, [1]Cecilia Czepak and [1]Karina Cordeiro Albernaz-Godinho

[1]Integrated Pest Management Laboratory, Agronomy School, Federal University of Goiás, Goiânia, Brazil
[2]Metropolitan College of Anápolis, Anápolis, Goiás, Brazil

## Abstract

**Background and Objective:** Rearing insects in the laboratory is fundamental to solving problems related to basic and applied entomology. *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is considered as one of the most economically important pests for worldwide agriculture. Maintaining colonies of this insect in the laboratory allows advances in integrated pest management studies. The aim of this study was to evaluate different diets for *H. armigera* growth and successfully establish the growth of this lepidoptera-pest in laboratory conditions. **Materials and Methods:** The larvae of *H. armigera* were rearing in three diets. The egg and larva stages were kept in a heated room with a temperature of 28±2°C, UR of 60±10% and 14 h of photophase. Twenty-five couples per diet were individualized in cylindrical cages made of PVC pipe kept at 25±2°C, UR of 60±10% and 14 h of photophase. The data from larval stage were submitted to Kruskal-Wallis non-parametric analysis and the means were compared by Dunn method. Biological parameters of adults were submitted to one-way analysis of variance (ANOVA) followed by Tukey's test. The viability of the different development stages were compared by Fisher's exact test. The sex rate was submitted to the $x^2$. **Results:** The artificial diets presented a higher viability in the stages egg, larva, pre-pupa and pupa and lower development period in regard to the natural diet. The shortest larval period was recorded on the chickpea-based diet. The pre-pupal period was similar in the diets evaluated. The moths that developed on the natural diet showed a longevity that was significantly less than on the other two diets. The pre-oviposition, oviposition and incubation of eggs periods did not present a statistical difference. The population would increase daily by 6, 5 and 3% on the chickpea, bean and green bean diet. **Conclusion:** The present study indicated that the artificial diets were more adequate for *H. armigera* and the chickpea-based diet resulted in a shorter generation time of *H. armigera*.

Key words: Life table, cotton bollworm, reproductive parameters, growth indices, heliothinae

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Corresponding Author: Cecilia Czepak, Integrated Pest Management Laboratory, Agronomy School, Federal University of Goiás, Goiânia, Brazil Tel: +5562981298787



Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

*Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is considered as one of the pests with greatest economic importance for global agriculture. It is an exotic pest, with documented occurrence in Brazil in 2013[1-3] and it is currently disseminated in diverse Brazilian states causing considerable damages in the main crop systems of the country, such as soybean, beans, corn, cotton and tomato.

It is a polyphagous pest, whose larvae were already identified in more than 180 species of cultivated and wild plants, with high reproductive potential and high capacity for dispersion and survival[3,4]. Even in adverse conditions, it can complete multiple generations per year, finalizing its cycle from egg to adult in 4-6 weeks[5]. In addition, some populations have presented frequent cases of resistance to various pesticides and *Bacillus thuringiensis* (Bt) crops[6-8]. The set of characteristics previously described make it easy to understand the potential of *H. armigera* to cause economic harm. Around US $ 78 billion is the annual value of the crops that are exposed to this species[9]. This fact has worried producers, as well as the Brazilian and world scientific communities[3,10,11].

Rearing insects in the laboratory is fundamental to solving problems related to basic and applied entomology. The maintenance of insect colonies in the laboratory is essential to modern pest control strategies and thanks to the development of artificial diets means there has been the possibility to raise insects in great quantities[12,13]. This makes possible advances in integrated pest management studies. Therefore, to study an exotic and voracious pest like *H. armigera*, it is important to understand the pest, not only the biological characteristics of the pest in field conditions but also how to maintain it in laboratory colony.

Although it is possible to keep the insects uninterrupted throughout the year on natural food, excessive labor is necessary to manipulate the biological material and the vegetable species used in feeding the insects. One alternative is to use artificial diets, which in addition to providing the continuous maintenance of the insects in the laboratory, allows a decrease in the labor involved in rearing them[14].

Artificial diets based on diverse ingredients are used to maintain *H. armigera* in the laboratory: wheat germ[15], tapioca[16], beans[17], chickpeas and tomato paste[18-20], yeast and sucrose[21]. However, it is necessary to constantly upgrade insect rearing techniques, especially regarding artificial diets. The upgrade is intended to maintain the biological quality of the insects throughout the generations and adapt the environment of each laboratory.

Therefore, the aim of this study was to evaluate different diets for *H. armigera* growth and successfully establish the growth of this lepidoptera-pest in laboratory conditions.

## MATERIAL AND METHODS

**Obtaining the insects and stock rearing:** *Helicoverpa armigera* larvae were collected from a commercial soybean crop on Mutum Farm (Palmeiras de Goiás, Goiás, Brazil, 16°39'29" S and 49°56'13" W) and kept in a plastic container containing parts of the soybean plants to keep them fed and then be sent to the Integrated Pest Management Laboratory in the Agronomy School at the Federal University of Goiás, Goiânia, Goiás, Brazil. After the screening to select healthy individuals, the larvae were kept on an artificial diet modified from Greene *et al.*[22], until they completed the larval stage. The larval stages were kept at a temperature of 28±2°C, UR of 60±10% and 14 h of photophase.

Newly emerged male and female moths were transferred to cylindrical cages made of PVC (20 cm of diameter×21 cm of height), lined internally with A4 sulfite paper, closed in the upper part with voile fabric and in the lower part with a disposable plate lined with paper towel and fed with an aqueous solution of honey at 10% (in water) (Fig. 1). As an oviposition substrate, feminine napkins (Carefree® daily protection-without perfume) were placed inside the cage as observed in Fig. 1 (25±2°C, UR of 60±10% and 14 h of photophase). Samples of the adults were sent to the Ministry of Agriculture, Livestock and Food Supply (MAPA/LANAGRO-GO) for molecular identification and confirmation of the species.

The postures were collected every 48 h and incubated in plastic containers with 400 mL capacity. After hatching, the larvae were transferred to plastic cups (100 mL capacity) containing an artificial diet where they remained until the beginning of the third instar. Then, in order to avoid cannibalism the larvae were individualized in plastic cups (50 mL capacity), covered by an acrylic lid containing 10 mL of artificial diet and maintained until the pupa stage.

The pupae were withdrawn from the diet, separated by sex and placed in cages where they remained until the emergence of the adults. With the subsequent generations, the biological parameters of *H. armigera* were evaluated on a natural and artificial diet.

**Biology on artificial and natural diet:** Artificial diets based on beans, an artificial diet based on chickpeas (Table 1) and a natural diet using green beans (*Phaseolus vulgaris* L.) were

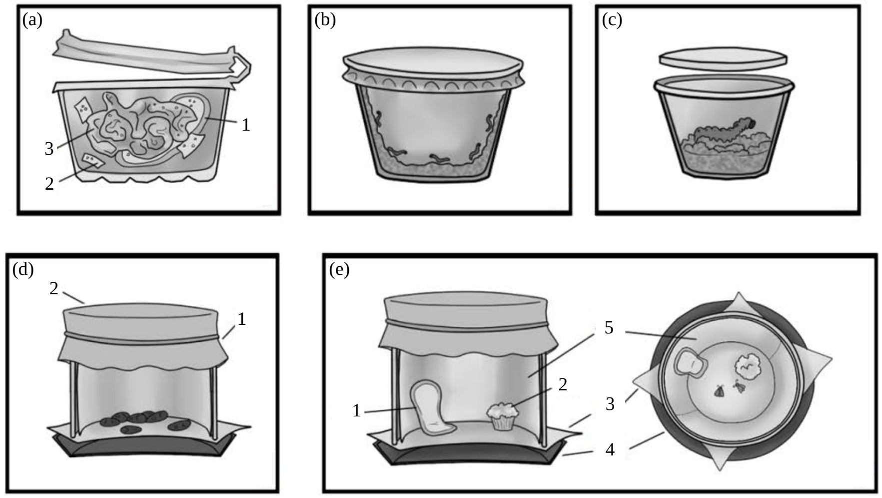


Fig. 1(a-e): Methodology to maintain the *Helicoverpa armigera* populations on an artificial diet in the laboratory, (a) Container containing postures; 1: Oviposition substrate (feminine napkins), 2: Fragments of filter paper moistened with water, 3: Postures in the voile, (b) Plastic pot with lid, 100 mL capacity, containing artificial diet and newly emerged larvae, (c) Plastic cup with acrylic lid (50 mL capacity) containing artificial diet and second larva instar, (d) Cages for pupae; 1: Rubber elastic, 2: Voile cloth and (e) Cages for adults; 1: Feminine napkin used as oviposition substrate, 2: Cotton moistened with honey solution at 10% to feed the adults, 3: Paper towel lining the cages, 4: Plastic plate, 5: Sulphite paper used in the lateral lining of the cages

Table 1: Ingredients of the artificial diets used as food for *Helicoverpa armigera* modified from Greene *et al.*[22]

| Ingredients of the diet | Diet A (Bean) | Diet B(Chickpea) |
|---|---|---|
| Common beans (g) | 75 | - |
| Chickpea (g) | - | 75 |
| Wheatgerm (g) | 60 | 60 |
| Soyprotein (g) | 30 | 30 |
| Casein (g) | 30 | 30 |
| Brewer's yeast (g) | 37.5 | 37.5 |
| Agar (g) | 22.5 | 22.5 |
| Ascorbic acid (g) | 3.96 | 3.96 |
| Sorbic acid (g) | 1.98 | 1.98 |
| Tetracycline (g) | 124 | 124 |
| Nipagin (mg) | 3.3 | 3.3 |
| Water (mL) | 1200 | 1200 |
| Vitamin complex* (mL) | 9.9 | 9.9 |

*Vitamin solution extract from Parra[28]: Dry part (niacinamide 1.00 g, calcium pantothenate 1.00 g, riboflavin 0.50 g, thiamine 0.25 g, pyridoxine 0.25 g, folic acid 0.10 g, biotin 0.02 mg), Liquid part (vitamin B12 (1000 mg $mL^{-1}$) 2.00 mL), mix the dry and liquid parts in 1000 mL distilled water to prepare the vitamin solution

evaluated from October-December, 2015 at the Integrated Pest Management Laboratory, Federal University of Goiás. For the evaluation, the newly emerged larvae were individualized, from the stock rearing, with 150 caterpillars on each artificial diet and 250 caterpillars on the natural diet. Because it is a natural diet, the green beans were obtained from an organic provider, without the use of insecticides and changed every two days.

The egg and larva stages were kept in a heated room (Model EL011, Eletrolab) with a temperature of 28±2°C, UR of 60±10% and 14 h of photophase. The caterpillars were observed daily until reaching the pupa stage. The pupae obtained were weighed at 24 h of age and were separated by sex according to Butt and Cantu[23] and during emergence 25 couples per diet were individualized in cylindrical cages made of PVC pipe kept at 25±2°C, UR of 60±10% and 14 h of photophase.

The variables analyzed were: viability of all the stages, development period from egg to pupa, longevity, pre-oviposition and oviposition periods. Based on the biological data obtained from each female, fertility life tables were created. The average number of eggs per female (mx) on each oviposition date (x) was calculated, considering the total females, the accumulated survival index of the females (lx) during the oviposition period and the number of descendants that reached age x in the following generation (lx.mx).

Based on the information from the life tables the following variables were estimated: Net reproduction rate (Ro): estimate of the average number of females generated per female during the oviposition period and that will be born in the next generation; Interval between generations (T); Intrinsic growth rate (rm); Finite rate of increase ($\lambda$).

**Data analysis:** For statistical analysis of the data the software BioEstat 5.3 was used[24]. The data with normal distribution were submitted to one-way ANOVA and the averages were compared by the Tukey's test[25] ($p<0.05$). Non-normal distribution data were submitted to Kruskal-Wallis non-parametric analysis and the means were compared by Dunn method ($p<0.05$). The viability of the different development stages were compared by Fisher`s exact test (bilateral alpha = 0.01). The sex rate was submitted to the Chi-Square test[26].

## RESULTS

**Larva:** In regard to the duration of the larval stage, there was a significant difference ($p<0.05$) among the diets evaluated, with 14.54 days for the green bean diet and 13.05 and 11.67 days for the artificial bean and chickpea diets, respectively (Table 2). The viability of the larval stage, the survival percentage of individuals raised on the artificial diets was higher than the survival of the individuals kept on the green bean diet (Table 3).

**Pre-pupa and pupa:** The pre-pupal period was similar in the diets evaluated, with two days in the green bean, 1.92 days in the bean diet and 1.82 days in the chickpea diet (Table 2). The pupal period presented a significant difference ($p<0.05$) in the green bean diet when compared to the other two diets (Table 2). In the natural diet this period lasted around two days more for females and three days more for males when compared to the artificial diets. Among the artificial diets there was no observed difference. Asynchrony was observed in the emergence of the adults, with the females emerging before the males (Table 2). The number of viable pupae in the artificial chickpea diet provided a higher percentage than the other diets and again the natural green bean diet resulted in the lowest number of individuals (Table 2). The weight of the pupae was 30% less on the natural diet when compared to the weight of the pupae on the artificial diets (Table 2). The females weighed more than the males, however a significant difference ($p<0.05$) was only observed in the green bean diet (Table 2). The sex rate was not influenced by the different diets (Table 3).

**Adults:** The longevity of the adults there was no difference between the males and the females on the evaluated diets. Only the moths that developed on the natural diet showed a longevity that was significantly less ($p<0.05$) than on the other two diets, where the females lived 16.57 days on average and the males 17.25 days. On the diets based on beans and chickpeas the adults survived a week longer on average (Table 3).

**Eggs:** The pre-oviposition, oviposition and incubation of eggs periods did not present a statistical difference among

Table 2: Comparison of biological parameters of immature *Helicoverpa armigera* fed with natural diet and artificial diets

| | Period of the stage (days)* | | | | | |
|---|---|---|---|---|---|---|
| | | | Pupa | | Pupae weight (g)** | |
| Diets | Larval | Pre-pupa[ns] | Female | Male | Female (Mean±SE) | Male (Mean±SE) |
| Green Bean | 14.54[a] | 2.00 | 12.87[aA] | 15.00[aB] | 0.273±0.007[aA] | 0.252±0.007[aB] |
| Diet A (Bean) | 13.053[b] | 1.92 | 10.74[bA] | 11.83[bB] | 0.381±0.006[bA] | 0.370±0.007[bA] |
| Diet B (Chickpea) | 11.6765[c] | 1.82 | 10.98[bA] | 12.14[bB] | 0.372±0.005[bA] | 0.362±0.006[bA] |

*Non-normal data submitted to the Kruskal-Wallis non-parametric analysis, averages compared by the Dunn method ($p<0.05$), **Normal data submitted to one-way ANOVA and averages compared by the Tukey's test ($p<0.05$), [ns]H or F test non significant, averages followed by the same lower-case letter in the column and capital letter in the same row do not present statistical difference

Table 3: Viability (%) of the stages larva, pre-pupa, pupa, adult, viability (larva-adult), total cycle and sexual orientation of *Helicoverpa armigera* fed with natural diet and artificial diets

| | Diets | | |
|---|---|---|---|
| Stages | Green bean | Diet A (Bean) | Diet B (Chickpea) |
| **Viability (%)** | | | |
| Larva | 32.80[a] | 89.93[b] | 92.57[b] |
| Pre-pupa | 81.71[a] | 97.76[b] | 97.81[b] |
| Pupa | 73.13[a] | 92.37[b] | 92.54[b] |
| Viability larva-adult | 19.00[a] | 81.21[b] | 83.79[b] |
| Egg development period to adult emergence (days) | 31.93[a] | 28.83[b] | 27.76[b] |
| Sexual orientation | 0.45 | 0.50 | 0.45 |

Means within a row followed by the same letter are not significantly different

Table 4: Comparison of biological parameters of *Helicoverpa armigera* adults fed in the larval phase with natural diet and artificial diets

| | Longevity of adults (days) | | Duration (days)$^{ns}$ | | | No. of eggs | |
|---|---|---|---|---|---|---|---|
| | Female | Male | Pre-oviposition | Oviposition | Incubation of eggs | Total$^{ns}$ | Viables$^{ns}$ |
| Diets | (Mean±SE) | | | | | | |
| Green bean | 16.57±1.25$^{aA}$ | 17.25±1.15$^{aA}$ | - | - | 2.47±0.24 | 974.06±152.16 | 622.11±120.24 |
| Diet A (Bean) | 24.37±0.91$^{bA}$ | 24.71±0.62$^{bA}$ | 6.25±0.53 | 17.43±2.07 | 2.58±0.09 | 1076.65±136.36 | 986.00±142.41 |
| Diet B (Chickpea) | 25.17±0.90$^{bA}$ | 25.41±0.83$^{bA}$ | 5.71±0.84 | 17.50±1.02 | 2.70±0.07 | 1246.68±136.61 | 674.25±175.67 |

Normal data submitted to one-way ANOVA and averages compared by the Tukey's test ($p<0.05$), Averages followed by the same lower-case letter in the column and capital letter in the same row do not present statistical difference, $^{ns}$F-test not significant

Table 5: Fertility life table of *Helicoverpa armigera* from the parameters of moths reared on natural and artificial diets

| Parameter | Green bean | Diet A (Bean) | Diet B (Chickpea) |
|---|---|---|---|
| ($R_o$) Net reproductive rate (offspring) | 25.220 | 199.890 | 195.660 |
| (T) Mean generation time (day) | 45.590 | 44.980 | 42.760 |
| (r) Intrinsic rate of increase (day) | 0.031 | 0.051 | 0.054 |
| (λ) Finite rate of increase (days) | 1.030 | 1.050 | 1.060 |

themselves regarding the evaluated diets. The same occurred with the total number of eggs and the number of viable eggs (Table 4).

**Fertility life table:** The net reproduction rate $R_o$ indicated that the population can increase each generation: 25.22 on the green bean diet, 199.89 on the bean-based diet and 195.66 on the chickpea diet (Table 5). Regarding the finite rate of increase (λ) values of 1.06, 1.05 and 1.03 were observed, respectively, for the chickpea diet, the bean diet and the natural green bean diet. In other words, the population would increase daily by 6, 5 and 3% on the respective diets (Table 5).

## DISCUSSION

The artificial diets provided the lowest development cycle (egg-adult) for *H. armigera* (Table 3). On the order hand to complete the cycle on the natural diet the species took four more days. The development indeed tends to be slower in the individuals raised with vegetable than in those fed with artificial diets[27]. Furthermore, the most appropriate foods provide lower duration of the development stages of insects[28]. The use of artificial diets considered adequate for mass rearing when the survival of the larval stage is greater than 75%[14]. Therefore, the artificial diets in this study are appropriate for rearing in laboratory conditions, since the larval viability values were 89.93 and 92.57% for beans and chickpeas, respectively. However, between the bean and chickpea diets, despite not observing a significant difference, 3% more survivors occurred with the chickpea diet. Taking into account a mass raising, this becomes a relevant fact, considering the number of larvae produced daily. Barbosa *et al.*[29] using an artificial diet based on white beans obtained larval viability of 14.4%, lower than the percentage obtained in this study which was higher than 89% on an artificial diet and 32% on a natural diet. The authors found an accentuated mortality in the last larval stages, which caused a low final viability, however they do not discuss the cause for this fact.

The pre-pupa period was similar in the diets evaluated, with two days in the green bean, 1.92 days in the bean diet and 1.82 days in the chickpea diet. Ali *et al.*[30] evaluating biological characteristics of *H. armigera* species in chickpeas found a similar result (2.15 days) to those obtained in this study. As in the larval stage, the viability among the individuals raised on the artificial diets was higher than that of the individuals kept on the natural green bean diet (Table 3). Evaluation of the pre-pupa stage, despite being important data, is not considered by many authors, probably due to the difficulty of observing this development stage.

The pupa period presented a significant difference ($p<0.05$) in the green bean diet when compared to the other two diets. In the natural diet this period lasted around two days more for females and three days more for males when compared to the artificial diets. Among the artificial diets there was no observed difference. Reigada *et al.*[31] evaluating only natural food observed that the host only influenced the pupa period of the females and did not affect the males. Jha *et al.*[32] when evaluating the development of the species did not observe a difference among the pupa period of *H. armigera* larvae that fed off of different natural hosts. The authors suggested that the immature stage feeding would have little influence on the pupa period. However, in this study the duration of the pupa period for the treatments was different, even between the sexes. Both females and males were influenced by the food offered.

Asynchrony was observed in the emergence of the adults, with the females emerging before the males (Table 2). Likely the females are skilled in searching for the best habitat for oviposition, while the males need to locate these females[27]. This explains the importance of the females emerging first, since they go to find the appropriate host while they mature sexually and prepare to liberate the sexual pheromone to attract the partners, which emerge two days later on average. Regarding the number of viable pupae the artificial chickpea diet provided a higher percentage than the other diets and again the natural green bean diet resulted in the lowest number of individuals (Table 2). Similar data were observed by Amer and El-Sayed[33] when comparing an artificial diet based on beans to natural diets. The weight of the pupae was 30% less on the natural diet when compared to the weight of the pupae on the artificial diets. The females weighed more than the males, however, a significant difference was only observed in the green bean diet. Higher *H. armigera* pupa weight is associated with more appropriate food ingested, which normally leads to a better reproductive performance in the adults[32,34].

The study showed that in artificial diets the increase was approximately 8 times regarding the natural diet, which indicated the inadequacy of maintaining this species on green beans in laboratory rearing. The time between generations was less for the chickpea diet, approximately 43 days. This means that on this diet *H. armigera* could have 8.5 generations/year, while on the other diets it will have 8 generations/year in the studied environmental conditions. This fact shows that using the chickpea diet insects can be obtained in a smaller space of time favoring the production schedule for the rearing of the pest in the laboratory.

A positive result for the intrinsic growth rate was also observed in the diet based on chickpeas and consists of the number of new individuals that each female of the population adds in a determined time in specific physical conditions in an unlimited environment[35]. Therefore, the value of the intrinsic rate of growth ($r_m$) will not be the same for different climates and food sources[36]. This value is the main fact that is obtained upon making a fertility life table[37], the higher the value of $r_m$ the more successful the species[36].

Finally, to maintain *H. armigera* in the laboratory, in a satisfactory way, it is necessary to offer food that is appropriate for the nutritional demands of each species. Therefore, the artificial bean and chickpea diets showed to be appropriate for the ideal biological parameters, fundamental for maintaining this species in the laboratory. From the results obtained in this study the chickpea diet was chosen to maintain the *H. armigera* in the laboratory and this has provided, until now, 18 uninterrupted generations without the introduction of individuals from the field.

## CONCLUSION

The present study indicated that the artificial diets were more adequate for *H. armigera* in laboratory conditions. The shortest larval duration was recorded on the chickpea-based diet, resulting in a shorter generation time. From the results obtained in this study the rearing of *H. armigera* on chickpea-based diet has been successfully maintained.

## SIGNIFICANCE STATEMENT

The importance of this study is due to the fact that it is the first to assess artificial diets for rearing of *H. armigera* in laboratory conditions in Brazil using life table. Advances in techniques for rearing insects on artificial diets are fundamental to solving issues of basic and applied entomology. This research presented that the artificial bean and chickpea diets are appropriate to rearing *H. armigera* in laboratory in Brazil.

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