# Comparison of Biology between Helicoverpa zea and Helicoverpa armigera (Lepidoptera: Noctuidae) Reared on Artificial Diets

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# Comparison of biology between *Helicoverpa zea* and *Helicoverpa armigera* (Lepidoptera: Noctuidae) reared on artificial diets

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### Abstract

The objective of this study was to compare growth and development of *Helicoverpa zea* Boddie and *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) larvae that were feeding on an artificial diet. Neonate larvae of *H. zea* and *H. armigera* were collected in maize fields cultivated at the Brazilian Agricultural Research Corporation, Embrapa Maize & Sorghum, in Sete Lagoas (Minas Gerais) and in farmers' cotton fields in Luís Eduardo Magalhães (Bahia), respectively. Bioassays were conducted in the laboratory using individual larvae in 50 mL plastic cups fed a white bean–based artificial diet and maintained at a temperature of 26 ± 2 °C and a relative humidity of 47 ± 10%. The following larval biological parameters were evaluated: number and duration of instars, survival of larval instars and pupae, larval biomass, larval head capsule size, and larval length. An adaptation index was computed for comparison of development of the 2 species. Significant differences were observed between the species for all variables except for the number of instars and pupal survival. Although *H. armigera* larvae developed faster and were smaller than *H. zea* larvae, the diet tested can be considered adequate for rearing both species in the laboratory.

Key Words: adaptation index; corn earworm; Old World bollworm; cotton earworm; bollworm; polyphagous caterpillar

### Resumen

El objetivo de este estudio fue comparar el crecimiento y desarrollo de larvas de *Helicoverpa zea* Boddie y *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) que fueron alimentadas con una dieta artificial. Se recolectaron larvas recién nacidas de *H. zea* y *H. armigera* en campos de maíz cultivadas en la Cooperación Brasileña de Investigación Agropecuaria, Maíz y Sorgo de Embrapa, en Sete Lagoas (Minas Gerais), y en los campos de algodón de los agricultores en Luís Eduardo Magalhães (Bahia), respectivamente. Se realizaron los bioensayos en el laboratorio utilizando larvas individuales en tasas plásticas de 50 mL alimentadas con una dieta artificial a base de frijól blanco y se mantuvieron a una temperatura de 26 ± 2 °C y una humedad relativa de 47 ± 10%. Se evaluaron los siguientes parámetros biológicos larvales: número y duración de los estadios, la sobrevivencia del estadio de larva y de pupa, la biomasa, el tamaño de la cápsula de la cabeza y la longitud de las larvas. Se calculó el índice de adaptación para la comparación del desarrollo de las 2 especies. Se observaron diferencias significativas entre las especies para todas las variables excepto para el número de estadios y la sobrevivencia de la pupa. Aunque las larvas de *H. armigera* se desarrollaron más rápido y fueron más pequeñas que las larvas de *H. zea*, la dieta probada puede considerarse adecuada para la cría de las dos especies en el laboratorio.

Palabras Clave: índice de adaptación; gusano elotero del maíz; gusano bellotero; gusano del algodón; gusano; oruga polífaga

The corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), is a pest of significant economic importance in agriculture throughout much of the world. In the United States, this species is reported to cause damage to cotton, soybean, sorghum, and tomato, and is a major pest of corn used for human consumption (ICAC Recorder 2011). In Brazil, *H. zea* mainly damages tomatoes and the tips of corn ears (Avila et al. 2013). In addition to direct losses caused by larval consumption of developing kernels, subsequent damage is also significant and includes lack of 2nd ear formation, ovule fertilization failure in late ears, and failure of ears to fill properly (Cruz et al. 2008). In addition, corn earworm damage creates holes through which fungal infection occurs, causing grain rot and mycotoxin production, which leads to qualitative grain loss due to devaluation of the product and a threat to the health of humans and livestock (Luiz & Magro 2007).

*Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), sometimes known as Old World bollworm, African bollworm, or cotton bollworm, is a major pest of many crops in Africa, Asia, Europe, and Oceania, but is new to the Western Hemisphere. Until 2013, *H. armigera* was considered an A1 quarantine species and was not been reported in the Americas. However, this species was identified morphologically and molecularly in Brazil in 2013, reportedly causing serious damage to crops such as cotton and soybean (Czepak et al. 2013; Specht et al. 2013). *Helicoverpa armigera* is highly dispersive and has the ability to develop on various crops, both conventional and genetically modified (Liu et al. 2004). The existing damage and potential threats posed by this pest in Brazil have caused various agribusiness sectors to mobilize to develop sustainable management strategies (Avila et al. 2013; Embrapa 2013), but extensive losses to Brazilian agriculture have been experienced (Brasil 2014; Oliveira et al. 2014).

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*Helicoverpa zea* and *H. armigera* are genetically and physiologically closely related species that have mating compatibility under laboratory conditions (Tay et al. 2013). Therefore, the presence of *H. armigera* in Brazilian crop fields during 2 seasons (2011/2012 and 2012/2013) was initially confused with *H. zea*, even though the observed aggressiveness and high degree of resistance to insecticides were not consistent with the expected responses of *H. zea*. The observation of *H. armigera* in South America and its subsequent spread into Central America and the Caribbean have increased the concern about the invasion risk to North America. Previously, the perceived dispersal threat was based on the goods transported into North America from various locations worldwide, including South and Central America and the Caribbean. Currently, there are 2 additional natural dispersal pathways: via the land bridge between North and South America and via island hopping across the Caribbean (Kriticos et al. 2015).

Information on growth rate, instar duration, and survival under certain environmental conditions are important for understanding the population dynamics and competition of these 2 species in the field. Such information can aid in predicting the ability of these species to successfully exploit agroecosystems (Cunningham & Zalucki 2014). Among its basic assumptions, integrated pest management requires an understanding of the biology of the pest complex in the target crop (Kogan 1998). In Brazil, there are few bioecological studies comparing *H. zea* and *H. armigera*. Because such information could be critical for the development of integrated management strategies, a study was initiated to compare selected biological characteristics of *H. zea* and *H. armigera* reared on artificial diet.

## Materials and Methods

This experiment was conducted at the Laboratory of Ecotoxicology and Insect Management of Embrapa Maize & Sorghum, Sete Lagoas (Minas Gerais, Brazil), under controlled conditions (26 ± 2 °C, 50 ± 10% RH, 12:12 h L:D photoperiod). The Greene modified standard artificial diet, considered to be the most suitable for development of *H. zea* (Garcia et al. 2006), was used for insect culture. The following biological parameters were assessed: number and duration of instars, larval and pupal survival, and larval biomass, head capsule size, and body length.

### INSECT SOURCES AND REARING

Larvae of *H. zea* (approx. 200 individuals) were collected from corn fields at the experimental station of Embrapa Maize & Sorghum. *Helicoverpa armigera* larvae (approx. 300 individuals) were collected from cotton fields in Luís Eduardo Magalhães (Bahia). F1 neonate *H. zea* larvae and F2 neonate *H. armigera* larvae were cultured individually in 50 mL screw-top cups made of transparent autoclavable polypropylene, each containing a 4.5 $cm^3$ portion of artificial diet (approx. 5 g). The larvae used in these studies were derived from the 2nd generation of laboratory-reared insects.

### NUMBER AND DURATION OF INSTARS

To establish the number of instars, the cups (*n* = 72 per species) were observed daily for the presence of exuviae. At each molt, the exuviae, which are dark brown to black in color, were removed from the cups by using a fine bristle brush. The duration of the 1st instar was considered to be the time between egg hatching and the 1st molt, and the duration of each subsequent instar was the period between 2 molts. Except where noted, for statistical analysis, each insect was considered to be 1 replicate.

### SURVIVAL

For survival assessment, the numbers of live and dead individuals were assessed daily throughout the development cycle. For this variable only, each group (*n* = 18 per species) of 4 individuals was considered to be 1 replicate, to guarantee an adequate number of replicates.

### LARVAL DEVELOPMENT

To measure the head capsule size and body biomass and length, 400 individuals of each species were observed. Daily, 10 individuals were removed to evaluate insect biomass, which was measured using a precision scale (Precision = 0.1 mg, Mettler Toledo AB204, Switzerland). Then, larvae were killed in microtubes containing 2 mL of 70% ethanol, and the maximum head capsule width was measured using a stereomicroscope at 25´ magnification (Carl Zeiss, Germany) with an ocular micrometer for the first 3 d of insect life and thereafter at 6´ magnification until the pre-pupa stage. Length of larvae was measured using graph paper.

### ADAPTATION INDEX (AI)

The data were used to calculate the adaptation index (AI) to compare the relative development of the 2 species fed an artificial diet under laboratory conditions. AI was calculated according to the method proposed by Boregas et al. (2013) using the formula AI = (LSV * PB) / (PLD), where LSV = larval survival in %, PB = pupal biomass in mg, and PLD = pupal and larval development period in days.

### STATISTICAL ANALYSES

Data of the number and duration of instars and larval development times were subjected to analysis of variance (ANOVA), and the means were compared using the Scott-Knott test. The survival percentages were transformed using $(x + 1)^{1/2}$ to meet the ANOVA normality assumptions. The variables biomass, head capsule size, and body length were subjected to ANOVA and to polynomial regression at a probability level of 5% ($P < 0.05$) using the software SISVAR® (Ferreira 2003). The regression model was used because the linearized model rule (Dyar 1890) is not robust enough to explain the head capsule growth of *H. zea*, according to Ambrosano et al. (1997).

## Results

Up to 7 instars were observed for both species of *Helicoverpa*. *Helicoverpa armigera* had 3 to 7 instars; 10% of all individuals progressed to the pupal stage after the 4th instar, 42% after the 5th instar, and 48% after the 6th or 7th instar. *Helicoverpa zea* had 5 to 7 instars; 28% of the individuals moved to the pupal stage after the 5th instar, 63% after the 6th instar, and 9% after the 7th instar.

Larval survival during the 1st and 2nd instars of *H. zea* was lower than that for *H. armigera* (Table 1). In general, higher insect mortality was observed in early instars compared with late instars. A significant difference between the species was observed in the duration of the larval period: the periods of the 2nd, 5th, and 6th instars for *H. zea* (3.9, 5.4, and 6.7 d, respectively) were longer than those for *H. armigera* (Table 1), and the total larval period of the former was longer than that of the latter (17.7 and 12.7 d, respectively).

The biomass accumulation curves also differed between the species; the quadratic equation for *H. zea* was $y = 3.933x^2 - 28.083x$ with $R^2 = 0.9542$ and that for *H. armigera* was $y = 6.2744x^2 - 32.08x$ with $R^2 = 0.9486$. Through the 6th day, there was no difference between the spe-

**Table 1.** Larval survival (%) and duration (d) by instar for *Helicoverpa zea* and *H. armigera* reared using the same artificial diet and laboratory conditions (26 ± 2 °C, 50 ± 10% RH, 12:12 h L:D photoperiod), Sete Lagoas, Minas Gerais, Brazil.

| Species | 1st instar | 2nd instar | 3rd instar | 4th instar | 5th instar | 6th instar | 7th instar | Total larval period |
|---|---|---|---|---|---|---|---|---|
| % survival (mean ± SE) | | | | | | | | |
| *H. zea* | 81.9 ± 3.4 b | 72.2 ± 8.3 b | 72.2 ± 10.9 a | 70.8 ± 10.7 a | 63.9 ± 10.4 a | 16.7 ± 9.0 a | 13.9 ± 7.6 a | 13.9 ± 7.6 a |
| *H. armigera* | 97.2 ± 1.9 a | 95.8 ± 2.3 a | 83.3 ± 7.6 a | 76.4 ± 8.7 a | 65.3 ± 10.1 a | 16.7 ± 9.0 a | 14.4 ± 7.9 a | 14.4 ± 7.9 a |
| df | 34 | 34 | 34 | 34 | 34 | 34 | 34 | 34 |
| *F* | 14.942 | 14.972 | 1.031 | 0.320 | 0.006 | 0.000 | 0.001 | 0.053 |
| *P* | 0.0005 | 0.0005 | 0.3171 | 0.5755 | 0.9397 | 0.9975 | 0.9769 | 0.8192 |
| Duration in days (mean ± SE) | | | | | | | | |
| *H. zea* | 2.9 ± 0.2 a | 3.9 ± 0.1 a | 2.7 ± 0.3 a | 3.4 ± 0.4 a | 5.4 ± 0.7 a | 6.7 ± 1.7 a | 6.0 ± 2.0 a | 17.7 ± 0.6 a |
| *H. armigera* | 3.3 ± 0.1 a | 1.2 ± 0.1 b | 2.6 ± 0.3 a | 3.3 ± 0.3 a | 3.4 ± 0.4 b | 3.0 ± 0.6 a | 3.0 ± 0.6 a | 12.7 ± 0.3 b |
| df | 128(1) | 117(1) | 112(1) | 101(1) | 21(1) | 18(1) | 4(1) | 87(1) |
| *F* | 3.600 | 282.430 | 0.074 | 0.066 | 0.133 | 3.216 | 3.240 | 46.614 |
| *P* | 0.0600 | 0.0001 | 0.7861 | 0.7984 | 0.0000 | 0.0908 | 0.1697 | 0.0000 |

For each variable, means followed by the same letter in each column did not differ significantly by the Scott-Knott test ($P > 0.05$).

cies; however, beginning on the 7th day, biomass accumulation in *H. armigera* was more rapid than in *H. zea*. Because *H. zea* spent a longer period of time in the larval period, its final biomass was greater than that of *H. armigera* (Fig. 1). Both species lost weight during the pre-pupal period; relative to the last larval period in each species, *H. zea* lost 16.0% of its biomass and *H. armigera* 24.9%. The higher biomass accumulation by *H. zea* resulted in higher pupal biomass (402 mg for *H. zea* and 358 mg for *H. armigera*) (Table 2).

Differences between the species were observed with regard to increases in body length and head capsule size during larval development, with *H. zea* represented by the quadratic equation $y = 0.0099x^2 + 0.0693x$, with $R^2 = 0.9875$, and *H. armigera* represented by $y = 0.0208x^2 + 0.0344x$, with $R^2 = 0.9724$ (Fig. 2). Similar to the biomass accumulation variable, the head capsules of the 2 species presented similar patterns through the 6th day, during which the head capsule size increased, particularly in *H. armigera.* However, as with the other parameters, the final head capsule size was greater in *H. zea* than in *H. armigera*.

The increase in larval body length followed the same pattern as the biomass and head capsule size. In both species, growth could be fitted to quadratic equations: $y = 0.0966x^2 + 0.6944x$ with $R^2 = 0.9866$ for *H. zea* and $y = 0.2507x^2 + 0.4177x$ with $R^2 = 0.9773$ for *H. armigera* (Fig. 3). The body length in the pre-pupal stage of *H. zea* remained constant, 32.6 mm, unlike that for *H. armigera*, in which the body length was 35.2 mm on the 1st day of the pre-pupal stage and 30.36 mm at the end.

**Table 2**. Pupal survival, pupal biomass, and adaptation index (AI) of *Helicoverpa zea* and *H. armigera* reared using the same artificial diet and laboratory conditions (26 ± 2 °C, 50 ± 10% RH, 12:12 h L:D photoperiod), Sete Lagoas, Minas Gerais, Brazil.

| Species | Survival (%) (mean ± SE) | Biomass (mg) (mean ± SE) | AI |
|---|---|---|---|
| *H. zea* | 41.7 ± 9.9 a | 402.6 ± 0.8 a | 1,757.6 |
| *H. armigera* | 47.2 ± 8.3 a | 358.1 ± 0.2 b | 1,922.8 |
| df | 34(1) | 88(1) | |
| *F* | 0.185 | 13.693 | |
| *P* | 0.6700 | 0.0004 | |

For each variable, means followed by the same letter in a column do not differ significantly by the Scott-Knott test ($P > 0.05$).

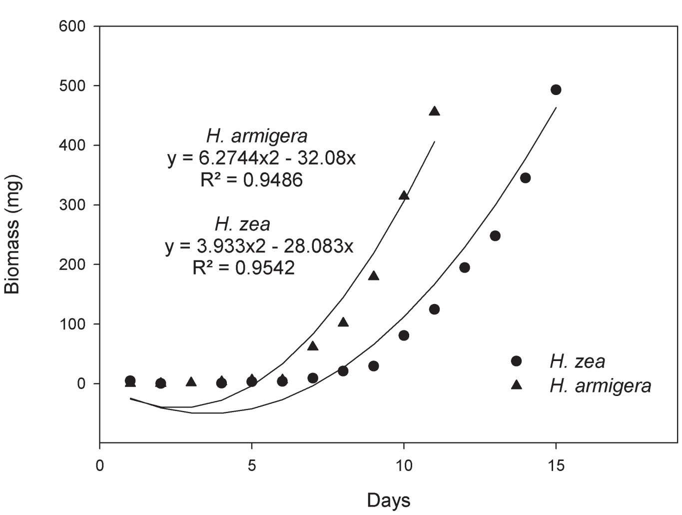


**Fig. 1.** Cumulative daily mass of *Helicoverpa zea* and *H. armigera* reared using the same artificial diet and laboratory conditions (26 ± 2 °C, 50 ± 10% RH, 12:12 h L:D photoperiod), Sete Lagoas, Minas Gerais, Brazil.

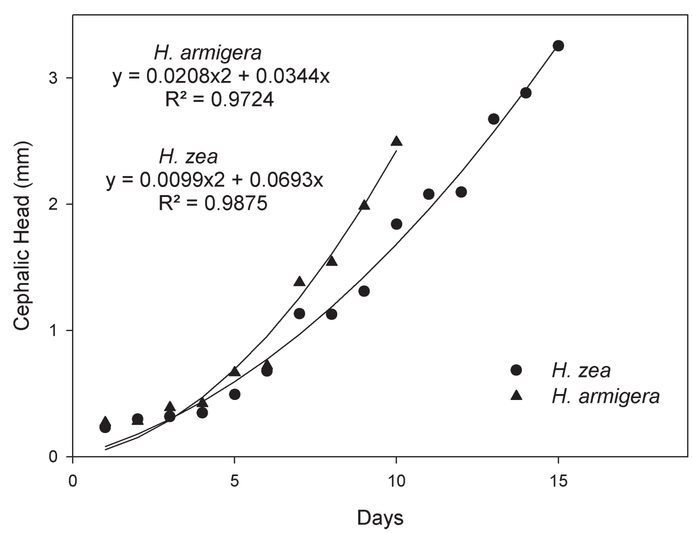


**Fig. 2.** Cumulative daily size of head capsule of *Helicoverpa zea* and *H. armigera* reared using the same artificial diet and laboratory conditions (26 ± 2 °C, 50 ± 10% RH, 12:12 h L:D photoperiod), Sete Lagoas, Minas Gerais, Brazil.

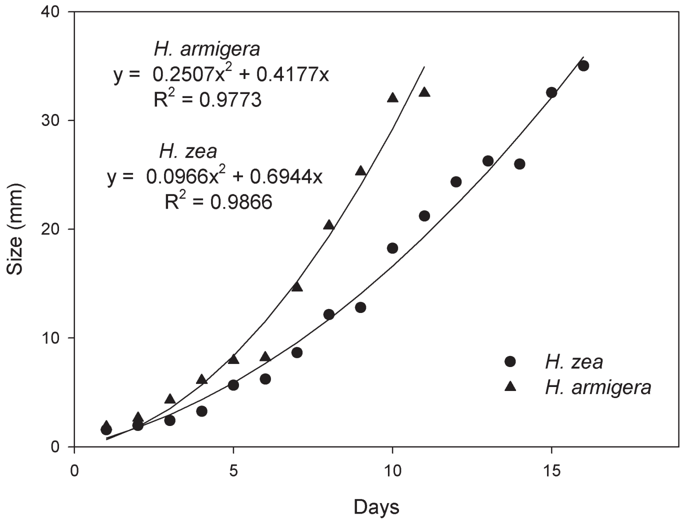


**Fig. 3.** Cumulative daily larval body length of *Helicoverpa zea* and *H. armigera* reared using the same artificial diet and laboratory conditions (26 ± 2 °C, 50 ± 10% RH, 12:12 h L:D photoperiod), Sete Lagoas, Minas Gerais, Brazil.

The adaptation index (AI) to the diet was higher for *H. armigera* than for *H. zea*. This difference was mainly due to higher survival and shorter duration of the insect development period in *H. armigera* (Table 2).

## Discussion

The number of instars may change with the larval food source. Kumar et al. (2009) observed 5 instars of *H. armigera*, whereas Jha et al. (2014) observed 6 instars on an artificial diet, and Liu et al. (2004) reported up to 7 instars depending on the host. Jha et al. (2014) also reported variation in the number of instars for *H. armigera* fed natural diets, with 6 for larvae reared on corn and 7 for those reared on asparagus. The number of instars that we obtained for *H. armigera* differs from the reports of Avila et al. (2013) and Czepak et al. (2013), who described the existence of 5 to 6 instars for larval development of this species. The number of instars tends to vary for most lepidopteran species, and this variation may be a function of factors including the environment as well as food (Parra 1999).

In this study, *H. armigera* survival rates were relatively high during the initial stages of development, which has also been observed in the field (Avila et al. 2013). Moreover, the herein determined biomass values are consistent with those found for *H. armigera* by Liu et al. (2010) using an artificial diet (350 mg) and (in part) by Storer et al. (2001) for *H. zea* (between 450 and 500 mg). The head capsule values are similar to those observed for *H. zea* by Zuniga et al. (2011), who found differences in values for *H. zea* populations from different regions of Chile, with the last instar ranging from 3.02 to 3.33 mm; in our study, we observed a mean head capsule width of 3.27 mm for the last instar.

The development period values of *H. zea* larvae are comparable to those found in another study using an artificial diet (Giolo et al. 2006). *Helicoverpa armigera* total larval development times were less than those found by Liu et al. (2004) for this species using different hosts. Feeding an artificial diet can provide adequate nutritional parameters and may accelerate the development of the insect. The larval growth parameters were differentiated only after the 6th day, whereas differences in survival and development period between the species were observed beginning at the 1st and 2nd instars, respectively. These results may explain the greater ability of *H. armigera* to cause economic damage on various hosts in the production system in Brazil (Avila et al. 2013; Embrapa 2013).

Introduced exotic insects cause extensive annual losses to Brazilian agriculture (Oliveira et al. 2013), and since its introduction in Brazil, *H. armigera* has caused substantial economic losses to several crops (Avila et al. 2013; Brasil 2014; Oliveira et al. 2014). The higher adaptation index obtained in the present study may explain the higher potential for damage by *H. armigera* compared with *H. zea*. Theoretically, because it is an introduced species, this pest may present competitive advantages over congeners—such as *H. zea,* which was already established in Brazil—including a smaller number of natural enemies. Furthermore, these results indicate that *H. armigera* has a shorter life cycle (approx. 28% faster) than *H. zea*, which suggests a greater number of generations per year and faster population growth. More generations of *H. armigera* may also suggest a greater likelihood of developing insecticide resistance, mainly in response to the inappropriate use of insecticides.

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