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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 Luis 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 cultivados 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 Luis Eduardo Magalhães (Bahia), respectivamente. Se realizaron los bioensayos en el laboratorio utilizando larvas individuales en tarros de plástico de 50 mL alimentadas con una dieta artificial a base de frijol 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
**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 (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 cm² 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 AB8204, Switzerland). Then, larvae were reared on artificial diet 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)½ 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. armigera* were lower 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 \( y = 6.274x^2 - 32.08x \) with \( R^2 = 0.9486 \). Through the 6th day, there was no difference between the spe-
cies; however, beginning on the 7th day, biomass accumulation in \textit{H. armigera} was more rapid than in \textit{H. zea}. Because \textit{H. zea} spent a longer period of time in the larval period, its final biomass was greater than that of \textit{H. armigera} (Fig. 1). Both species lost weight during the pre-pupal period; relative to the last larval period in each species, \textit{H. zea} lost 16.0\% of its biomass and \textit{H. armigera} 24.9\%. The higher biomass accumulation by \textit{H. zea} resulted in higher pupal biomass (402 mg for \textit{H. zea} and 358 mg for \textit{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 \textit{H. zea} represented by the quadratic equation $y = 0.0099x^2 + 0.0693x$ with $R^2 = 0.9875$, and \textit{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 \textit{H. armigera}. However, as with the other parameters, the final head capsule size was greater in \textit{H. zea} than in \textit{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 \textit{H. zea} and $y = 0.2507x^2 + 0.4177x$ with $R^2 = 0.9773$ for \textit{H. armigera} (Fig. 3). The body length in the pre-pupal stage of \textit{H. zea} remained constant, 32.6 mm, unlike that for \textit{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 1. Larval survival (%) and duration (d) by instar for \textit{Helicoverpa zea} and \textit{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.

Table 2. Pupal survival, pupal biomass, and adaptation index (AI) of \textit{Helicoverpa zea} and \textit{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*. The development period values of *H. armigera* differ from those observed for *H. armigera* - *H. zea* populations from different regions of Chile, by Liu et al. (2010) 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).

The development period values of *H. zea* larvae are comparable to those found in another study using an artificial diet (Giole 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 observed 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 congeneric—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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