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Short Communication

Timeline and geographical distribution of *Helicoverpa armigera* (Hübner) (Lepidoptera, Noctuidae: Heliothinae) in Brazil

Daniel R. Sosa-Gómez[a,*], Alexandre Specht[b], Silvana V. Paula-Moraes[b], Adilson Lopes- Lima[c], Silvia A.C. Yano[a], Adriana Micheli[a], Elisangela G.F. Morais[d], Paulo Gallo[a], Paulo R.V.S. Pereira[e], José R. Salvadori[f], Marcos Botton[g], Maurício M. Zenker[h], Wilson S. Azevedo-Filho[i]

[a] *Embrapa Soja, Londrina, PR, Brazil*
[b] *Embrapa Cerrados, Planaltina, DF, Brazil*
[c] *Embrapa Amapá, Macapá, AP, Brazil*
[d] *Embrapa Roraima, Boa Vista, RR, Brazil*
[e] *Embrapa Trigo, Passo Fundo, RS, Brazil*
[f] *Faculdade de Agronomia e Medicina Veterinária, Universidade de Passo Fundo, Passo Fundo, RS, Brazil*
[g] *Embrapa Uva e Vinho, Bento Gonçalves, RS, Brazil*
[h] *Departamento de Zoologia, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, SP, Brazil*
[i] *Laboratório de Biologia, Centro de Ciências Exatas, da Natureza e de Tecnologia, Universidade de Caxias do Sul, Bento Gonçalves, RS, Brazil*

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ABSTRACT

This study presents registers of *Helicoverpa armigera* (Hübner) occurrence to assess its spatial and temporal distribution in Brazil. We used data from collections, especially from the Southern Region, systematic collections in Rio Grande do Sul, occasional collections of caterpillars and adults in different regions of Brazil, as well as literature registers. We conclude that the introduction of *H. armigera* in Brazil probably occurred before October 2008. We also register that in August 2012 *H. armigera* was already present from the extreme southern part (Rio Grande do Sul) to the extreme northern part (Amapá) of Brazil.

In March 2013, the Old World bollworm, *Helicoverpa armigera* (Hübner), was reported in Brazil (Embrapa, 2013). On that occasion, specimens were collected in the states of Bahia, Mato Grosso, Paraná, and Goiás, and also in Distrito Federal, causing damage on corn, soybean, and cotton (Czepak et al., 2013; Specht et al., 2013b). The identification was based on morphological characters of male genitalia (Czepak et al., 2013; Specht et al., 2013b) and molecular analysis (Specht et al., 2013b). This species is genetically and morphologically closely related to *Helicoverpa zea* (Boddie), and the identification based on genitalia characters is time-consuming and requires a well-trained taxonomist. These two species are almost indistinguishable when characterization is based on external morphological characters (Hardwick, 1965; Pogue, 2004; Specht et al., 2013b). For these reasons, surveys were performed in different parts of the country to confirm the geographical distribution, using reliable diagnostic methods (inner male genitalia and/or molecular tools), and establish the approximate time of introduction of this pest in Brazil.

The geographical distribution was obtained after identifying field collected larvae, or rearing immatures until the adult phase, or collecting owlet moths with light traps. The occurrence of *H. armigera* before 2013 in Brazil was investigated through analysis of samples deposited in scientific collections from Embrapa Cerrados, Embrapa Soybean, the Museum of Science and Technology from Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), and the collection of the Universidade de Caxias do Sul, considering mainly the samples collected using light traps in Rio Grande do Sul since 1998 (Teston and Corseuil, 2004; Specht et al., 2005, 2013a; Zenker et al., 2010) and systematized monthly collections using light traps, from May 2011 to April 2013, in Pinto Bandeira, RS. The morphological and molecular identifications were performed as described by Pogue (2004) and Behere et al. (2008).

The PCR-RFLP molecular analysis, was performed with the amplification product corresponding to positions between 2443 and 2953 from the COI gene of *Helicoverpa* species (Accession number GU188273 from *H. armigera* mitogenome) and restriction

* Corresponding author.
E-mail: daniel.sosa-gomez@embrapa.br (D.R. Sosa-Gómez).

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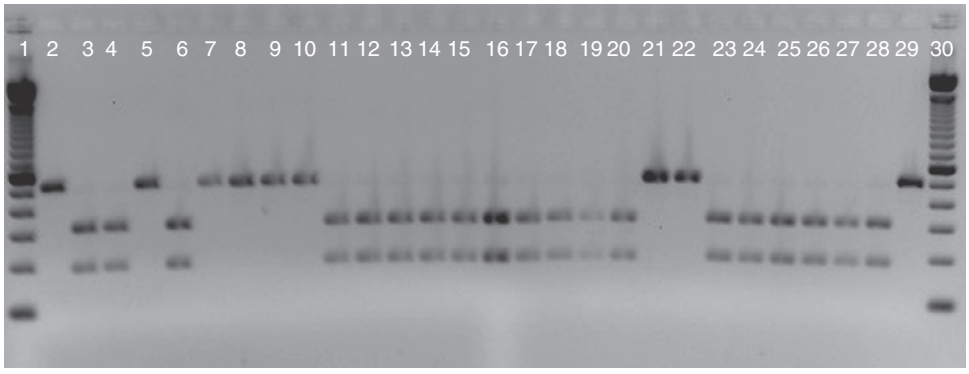

Fig. 1. PCR-RFLP agarose gel (1.5%) for COI amplification products (511 b) using *BstZ*17I endonuclease. Lane 1: 100 bp MW; lanes 2 and 5: undigested product of *Helicoverpa zea* (Londrina, PR); lanes 3 and 4: digestion product of *Helicoverpa armigera* (Londrina, PR); lane 6: digestion product of *H. armigera*; lanes 7–10: undigested product of *H. zea* (Planaltina, DF); lanes 11–15: digestion product of *H. armigera* (Luiz Eduardo Magalhães, BA); lanes 16–20: digestion product of *H. armigera* (Carambeí, PR); lanes 21 and 22: undigested product of *H. zea* (Arapoti, PR); lanes 23 and 24: digestion product of *H. armigera* (Sengés, PR); lanes 25–28: digestion product of *H. armigera* (Taquarituba, SP); lane 29: undigested product of *H. zea* (Taquarituba, SP); lane 30: 100 bp MW.

Table 1
Helicoverpa spp. in Brazil collections.

Brazilian municipalities/states	Geographic coordinates (UPS)	Plant or trap collect	Species	Sampling date	Identification
Boa Vista, Roraima	02°53′45″ N; 60°39′42″ W	Soybean	*H. armigera*	05/08/2013	Morphological
Bonfim, Roraima	03°19′55″ N; 59°50′44″ W	Soybean	*H. armigera*	05/08/2013	Morphological
Bonfim, Roraima	03°19′55″ N; 59°50′44″ W	Corn	*H. armigera*, *H. zea*	05/08/2013	Morphological
Macapá, Amapá	00°14′48″ S; 51°17′54″ W	Soybean	*H. armigera*	08/08/2012	Morphological
Baixada Grande do Ribeiro, Piauí	07°51′01″ S; 45°12′49″ W	Cotton	*H. armigera*	12/05/2013	Morphological
Luís Eduardo Magalhães, Bahia	12°07′12″ S; 45°48′41″ W	Cotton,	*H. armigera*, *H. zea*	06/2012	Molecular, morphological
Luís Eduardo Magalhães, Bahia	12°07′12″ S; 45°48′41″ W	Soybean	*H. armigera*	06/2012	Molecular, morphological
Roda Velha, Bahia	12°42′9″ S; 45°49′30″ W	Cotton, Soybean	*H. armigera*	21/02/2013	Morphological
Correntina, Bahia	13°27′33″ S; 45°44′11″ W	Cotton	*H. armigera*, *H. zea*	23/12/2012	Morphological
Correntina, Bahia	13°27′33″ S; 45°44′11″ W	Soybean	*H. armigera*	23/12/2012	Morphological
Nova Mutum, Mato Grosso	13°53′59″ S; 55°47′53″ W	Soybean	*H. armigera*	23/02/2013	Morphological
Diamantino, Mato Grosso	14°21′29″ S; 56°23′12″ W	Soybean	*H. armigera*	21/01/2013	Morphological
Tangará da Serra, Mato Grosso	14°37′44″ S; 57°34′32″ W	Soybean	*H. armigera*	06/03/2013	Morphological
Campo Verde, Mato Grosso	15°28′19″ S; 55°08′40″ W	Soybean	*H. armigera*	08/02/2013	Morphological
Rondonópolis, Mato Grosso	16°42′43″ S; 54°39′43″ W	Cotton	*H. armigera*	11/04/2013	Morphological
Alto Taquari, Mato Grosso	17°50′08″ S; 53°27′46″ W	Soybean	*H. armigera*	14/01/2013	Morphological
Palmeira de Goiás, Goiás	16°39′29″ S; 49°56′13″ W	Soybean	*H. armigera*	23/02/2013	Morphological
Jataí, Goiás	17°56′25″ S; 51°47′46″ W	Soybean	*H. armigera*	23/02/2013	Morphological
Santa Juliana, Minas Gerais	19°21′29″ S; 47°28′36″ W	Cowpea	*H. armigera*	23/02/2013	Morphological
Afonso Cláudio, Espiritu Santo	20°02′55″ S; 41°06′18″ W	Tomato	*H. armigera*	02/03/2014	Morphological
Alegre, Espírito Santo	20°45′45″ S 41°32′28″ W	Tomato	*H. armigera*	02/03/2014	Morphological
Planaltina, Distrito Federal	15°35′05″ S; 47°53′14″ W	Corn, soybean	*H. zea*, *H. armigera*	03/10/2013	Molecular, morphological
Planaltina, Distrito Federal	15°35′05″ S; 47°53′14″ W	soybean	*H. armigera*	03/10/2013	Molecular, morphological
Brasília, Distrito Federal	15°44′06″ S; 47°53′14″ W	Tomato	*H. armigera*, *H. zea*	20/11/2013	Morphological
Costa Rica, Mato Grosso do Sul	18°34′49″ S; 53°09′08″ W	Soybean	*H. armigera*	21/12/2013	Morphological
Londrina, Paraná	23°11′44″ S; 51°10′34″ W	Soybean, light trap	*H. armigera*, *H. zea*	07/02/2013	Molecular, morphological
Rolândia, Paraná	23°17′47.0″ S 51°26′01.9″ W	Soybean	*H. armigera*	10/14/2008	Molecular
Taquarituba, São Paulo	23°42′49″ S; 49°11′40″ W	Soybean	*H. armigera*	03/27/2013	Molecular
Sengés, Paraná	24°07′22″ S; 49°28′54″ W	Soybean	*H. armigera*	03/27/2013	Molecular
Arapoti, Paraná	24°11′52″ S; 49°55′12″ W	Wheat	*H. zea*	03/27/2013	Molecular
Carambei, Paraná	24°59′36″ S; 50°08′27″ W	Soybean	*H. armigera*	03/27/2013	Molecular, morphological
Passo Fundo, Rio Grande do Sul	28°22′55″ S; 52°38′96″ W	Soybean	*H. armigera*, *H. zea*	20/12/2012	Morphological
Pinto Bandeira, Rio Grande do Sul	29°02′50″ S; 51°28′12″ W	Light trap	*H. armigera*	04/05/2011	Morphological
Espumoso, Rio Grande do Sul	28°43′11″ S; 52°47′16″ W	Soybean	*H. armigera*	21/12/2012	Morphological
Carazinho, Rio Grande do Sul	28°20′26″ S; 52°52′57″ W	Soybean	*H. armigera*, *H. gelotopoeon*	20/12/2012	Morphological

fragment length polymorphism (RFLP) analysis with the BstZ17I endonuclease (Fig. 1), as well as the morphological characters indicate that so far *H. armigera* occurs in the following states of Brazil: Roraima, Amapá, Piauí, Bahia, Mato Grosso, Mato Grosso do Sul, Distrito Federal, Goiás, Espírito Santo, São Paulo, Paraná, and Rio Grande do Sul (Table 1 and Fig. 1).

We identified one *H. armigera* specimen in a larval sample collected in Rolândia, Paraná, in October 14, 2008. This was the first time that this species was observed in samples analyzed for the present study. After this, the exam of adults from systematized monthly collections in Pinto Bandeira, RS (extreme southern Brazil) provided the detection of *H. armigera* on May 4, 2011. In the same year, *H. armigera* specimens were collected in August ($n = 6$) and September ($n = 1$). In 2012, adults of *H. armigera* were found in February ($n = 1$), August ($n = 4$), September ($n = 3$), October ($n = 1$), and December ($n = 1$). In 2013, adults of this species were collected in February ($n = 2$), March ($n = 2$), and April ($n = 4$).

The exam of adults obtained from immatures of *H. armigera* collected in soybean fields in the extreme northern Brazil (Amapá) confirmed the occurrence of this species since August 8, 2012. Most of Heliothinae larvae collected in soybean fields were *H. armigera*, a very small proportion of specimens belong to *Chloridea virescens*

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(0.2%). Evaluations of collections performed from 1998 (Teston et al., 2006) until 2008 (Zenker et al., 2010; Specht et al., 2013a) in the Southern Region of Brazil did not reveal the occurrence of *H. armigera*. We did not find *H. zea* specimens from immature insects collected in soybean. However, this former species is a soybean pest in United State of America (Swenson et al., 2013). All the *Helicoverpa* samples collected from corn were obtained from corn ear, revealing host preference of Brazilian populations of *H. zea* for this host plant.

The analysis of insects previously collected and deposited in museums by several authors (Teston and Corseuil, 2004; Specht et al., 2005, 2013a; Zenker et al., 2010) suggested that at least in Rio Grande do Sul the introduction of *H. armigera* occurred after December 2008 and before May 2011. The earliest *H. armigera* samples mentioned in the published literature are from May 22, 2012 (Leite et al., 2014) in this study we found one larvae of *H. armigera* in soybean fields from Rolândia, Paraná in October of 2008. Based on the evidence provided by these data and considering a scenario of introduction of *H. armigera* in the country before 2008, it is reasonable to infer that this pest had enough time to build up populations detected as outbreaks in the 2012/2013 growing season (Embrapa, 2013). In August 2011, six specimens were captured in one sampling date, indicating that populations of this insect were already established in this area. Interestingly, in two consecutive years of monthly sampling a progressive increase of adults captured mainly at the end of the winter (August and September) and at the end of the summer (April) was observed.

The fact that *H. armigera* was detected from northern to southern Brazil, at least during the 2012/2013 growing season, when substantial damage was reported by several researchers (Czepak et al., 2013; Specht et al., 2013b; Tay et al., 2013), reveals that the identification of this species in the country was carried out a long time after its introduction, constraining management control decisions to minimize the impact of this pest in several crops. Soon after being reported in Brazil, *H. armigera* was also found in Paraguay and Argentina (Senave, 2013; Murúa et al., 2004), Puerto Rico (North American Plant Protection Organization, 2014) and later in USA (http://www.freshfromflorida.com/Divisions-Offices/Plant-Industry/Plant-Industry-Publications/Pest-Alerts/Pest-Alert-The-Old-World-Bollworm).

The late detection of *H. armigera* after its introduction could be attributed to the high similarity between this species and *H. zea*, as well as to the scarce number of studies for the identification of pest species, such as insect identification guides and other related publications (Hoffmann-Campo et al., 2012; Sosa-Gómez et al., 2014). Moreover, the specific identification of insect species is carried out, almost exclusively, based on external morphology of the adult specimens, which requires rearing immature individuals. This technique is time consuming and, many times, natural enemies kill the immature individuals. Therefore, only some studies of the immature stages of insects (Angulo et al., 2008) and species identification using molecular markers (Gomez-Rolim et al., 2013), which requires more investments, especially in equipment and specialized technicians, have been performed. However, most of these studies focus on determined taxa, usually genera, and most species continue not having a complete morphological or molecular characterization. These deficiencies are evidenced in many countries, especially in South America, due to a biological megadiversity and lack of taxonomists (Marques and Lamas, 2006). Furthermore, the morphological similarities between *H. armigera* and *H. zea* turn the situation even more critical.

In conclusion, the detection of *H. armigera* as a devastating component in high-value commercial crops, such as cotton and soybean (Czepak et al., 2013; Specht et al., 2013b), shows the vulnerability of Brazil and other countries in South America regarding quarantine pests. Additionally, efforts to elucidate invasion paths and origin are essential to design strategies for preventing future pest introduction and adopting science-based policies (Estoup and Guillemaud, 2010; Guillemaud et al., 2011).

Conflicts of interest

The authors declare no conflicts of interest.

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