

Species From the Heliiothinae Complex (Lepidoptera: Noctuidae) in Tucumán, Argentina, an Update of Geographical Distribution of *Helicoverpa armigera*

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Abstract

The Heliiothinae complex in Argentina encompasses *Helicoverpa gelotopoeon* (Dyar), *Helicoverpa zea* (Boddie), *Helicoverpa armigera* (Hübner), and *Chloridea virescens* (Fabricius). In Tucumán, the native species *H. gelotopoeon* is one of the most voracious soybean pests and also affects cotton and chickpea, even more in soybean-chickpea succession cropping systems. Differentiation of the Heliiothinae complex in the egg, larva, and pupa stages is difficult. Therefore, the observation of the adult wing pattern design and male genitalia is useful to differentiate species. The objective of this study was to identify the species of the Heliiothinae complex, determine population fluctuations of the Heliiothinae complex in soybean and chickpea crops using male moths collected in pheromone traps in Tucumán province, and update the geographical distribution of *H. armigera* in Argentina. The species found were *H. gelotopoeon*, *H. armigera*, *H. zea*, and *C. virescens*. Regardless of province, county, crop, and year, the predominant species was *H. gelotopoeon*. Considering the population dynamics of *H. gelotopoeon* and *H. armigera* in chickpea and soybean crops, *H. gelotopoeon* was the most abundant species in both crops, in all years sampled, and the differences registered were significant. On the other hand, according to the Sistema Nacional Argentino de Vigilancia y Monitoreo de Plagas (SINAVIMO) database and our collections, *H. armigera* was recorded in eight provinces and 20 counties of Argentina, and its larvae were found on soybean, chickpea, sunflower crops and spiny plumeless thistle (*Carduus acanthoides*). This is the first report of *H. armigera* in sunflower and spiny plumeless thistle in Argentina.

Key words: Fabaceae, invasive species, old world bollworm, population fluctuations

The Heliiothinae (Lepidoptera: Noctuidae) complex encompasses 381 described species, distributed in 28 genera (Pogue 2013). The most economically important species included in *Helicoverpa* Hardwick and *Chloridea* Duncan and (Westwood) are polyphagous. The females lay eggs, and the larval stage can survive and feed on a very wide range of host plant species. Many of these hosts are crops, including many field crops: cotton (*Gossypium hirsutum* L.; Malvales: Malvaceae), sorghum (*Sorghum* spp. Moench; Poales: Poaceae), sunflower (*Helianthus annuus* L.; Asterales: Asteraceae), chickpea (*Cicer arietinum* L.; Fabales: Fabaceae), alfalfa (*Medicago sativa* L.; Fabales: Fabaceae), lupinus (*Lupinus* spp. L.; Fabales: Fabaceae), soybean (*Glycine max* L.; Fabales: Fabaceae), tobacco

(*Nicotiana tabacum* L.; Solanales: Solanaceae), corn (*Zea mays* L.; Poales: Poaceae), wheat (*Triticum* spp. L.; Poales: Poaceae), horticultural crops such as tomatoes (*Solanum lycopersicum* L.; Solanales: Solanaceae), lettuce (*Lactuca sativa* L.; Asterales: Asteraceae), capsicum (*Capsicum annuum* L.; Solanales: Solanaceae), various bean crops, and flowers such as chrysanthemum (*Chrysanthemum* spp. L.; Asterales: Asteraceae), gladiolus (*Gladiolus* spp. L.; Asparagales: Iridaceae), and rose (*Rosa* spp. L.; Rosales: Rosaceae) (Cunningham and Zalucki 2014).

The native species of *Helicoverpa* in South America are divided into two groups: (a) *gelotopoeon*, including *Helicoverpa gelotopoeon* (Dyar), *Helicoverpa titicacae* Hardwick, *Helicoverpa*

bracteae Hardwick, and *Helicoverpa atacamae* Hardwick; and (b) *zea*, including only *H. zea* (Boddie). Species of *Chloridea* are represented by *Chloridea virescens* (Fabricius), *Chloridea tergemina* (Felder and Rogenhofer), and *Chloridea subflexa* (Guenée) (Mitter et al. 1993, Pastrana 2004, Pogue 2013).

In the *gelotopoeon* group, species can only be separated by morphological characters of the adults, since the biology and morphology of the immature stages remain unknown (Navarro et al. 2009). Recently, *H. armigera* (Hübner) has been detected in Brazil (Czepak et al. 2013, Specht et al. 2013, Tay et al. 2013), Paraguay (SENAVE 2013), Argentina (Murúa et al. 2014), Bolivia, Uruguay (Kriticos et al. 2015), Puerto Rico (NAPPO 2014), and the United States (NAPPO 2015). Thus, the presence of *H. armigera* in northwestern Argentina (NOA) has led to the inclusion of this species in the Heliiothinae complex, together with *H. gelotopoeon*, *H. zea*, and *C. virescens*.

Helicoverpa gelotopoeon is a polyphagous pest and has been reported in cotton, alfalfa, sunflower, soybean, chickpea, and corn, to name a few crops it attacks. Larvae cause damage in the vegetative and reproductive plant growth stages. This species occurs in Argentina, Chile, southern Brazil, Paraguay, and Uruguay (Pastrana 2004, Navarro et al. 2009). In Tucumán and other provinces of Argentina, *H. gelotopoeon* causes severe damage to soybean crops and can be difficult to control with insecticides (Navarro et al. 2009, Scalora et al. 2012). Consequently, it causes a significant economic impact, because Argentina is the third major soybean producer in the world, covering an area of 20.1 million hectares (Bolsa de Cereales 2015). This insect species also affects cotton and chickpea crops, causing more severe problems when they are grown in succession cropping systems (Pastrana 2004, Fichetti et al. 2009, Navarro et al. 2009, Scalora et al. 2012).

The differentiation of the Heliiothinae complex in the egg, larva, and pupa stages is very difficult, but adults can be distinguished by the pattern of wing veins and male genitalia using traditional taxonomic methods (Pogue 2004, Navarro et al. 2009). A further complication is distinguishing between *H. armigera* and the native *H. zea* (North and South America), since the latter is estimated to have diverged from the former only approximately 1.5–2 million years ago, and the same pheromone compounds, although in different concentrations, are found in both species (Pogue 2004, Witzgall et al. 2004, Behere et al. 2007). Thus, males of both species are attracted to sex pheromone lures released by females of both species in the field. Yet another complication is the fact that *H. armigera* and *H. zea* have been shown to hybridize in the laboratory and could well be doing the same in the field (Hardwick 1965, Laster and Hardee 1995, Laster and Sheng 1995). It is unclear whether *H. armigera* can hybridize with other endemic Heliiothinae species such as *H. gelotopoeon*. Therefore, genitalic dissection or molecular techniques are necessary to accurately identify adult males collected in pheromone traps (Pogue 2004, Behere et al. 2008, Specht et al. 2013, Tay et al. 2013, Leite et al. 2014, Arneodo et al. 2015, Kriticos et al. 2015). Behere et al. (2008) reported the use of two partial mitochondrial DNA genes [cytochrome oxidase subunit I (COI), cytochrome b (Cyt b)] as markers to differentiate *H. armigera*, *H. punctigera*, *H. assulta*, and *H. zea*. Arneodo et al. (2015) designed a rapid and simple molecular tool to distinguish *H. armigera* from *H. zea* and *H. gelotopoeon*. The method was validated using *Helicoverpa* specimens collected across Argentina, and their identity was further corroborated by COI sequence and phylogenetic analysis.

Helicoverpa armigera seems to be well adapted to the climate of agricultural regions of South America and, according to Kriticos

et al. (2015) it is spreading rapidly throughout Argentina, Bolivia, Brazil, Paraguay, and Uruguay, and most recently to Puerto Rico. However, no studies have been performed to investigate if *H. armigera* detected in these non-Brazilian locations were due to natural spread of Brazilian populations, or if they represented separate incursion events.

It is important to mention that *H. armigera* has developed resistance to insecticides and Cry proteins (Forrester et al. 1993, Armes et al. 1996, Li et al. 2007, Mahon et al. 2007, Gao et al. 2009, Liu et al. 2010, Bird and Downes 2014, Tay et al. 2015). Therefore, it is necessary to know how the Heliiothinae complex is distributed in legumes of economic importance in NOA, considering the difficulty of identifying these species.

The objective of this study was to determine population fluctuations of the Heliiothinae complex in soybean and chickpea crops using male moths collected in pheromone traps. The distribution of *H. gelotopoeon* in Tucumán province and an update of the geographical distribution of *H. armigera* in Argentina are also provided herein.

Materials and Methods

Identification of the Heliiothinae Complex

Larvae were collected in chickpea and soybean fields in Tucumán and Santiago del Estero provinces. In chickpea, collections took place on September 12, 2013 in Viclos (27°10'38"S, 64°52'42"W). In soybean, larvae were collected on January 31 and February 26, 2014 in La Cocha (27°45'55"S, 65°30'6"W), on February 7, 2014 in Jiménez (26°52'51"S, 64°42'3"W), and on February 15, 2015 in San Agustín (26°50'21"S, 64°51'32"W). Samples were collected during vegetative and reproductive stages in both crops. One hectare was monitored in each locality. In each hectare, 10–15 plants of soybean or chickpea were sampled at each of ten locations. Larvae were collected using a sheet method as described by Drees and Rice (1985).

The collected larvae were taken to the laboratory and reared on artificial diet, which included dry bean flour, wheat germ (Grandiet, Buenos Aires, Argentina), brewer's yeast (Calsa, Tucumán, Argentina), vitamin C, sorbic acid (Anedra, Buenos Aires, Argentina), vitamin supplement, amino acids (Ruminal, Buenos Aires, Argentina), and methylparaben (Todo Droga, Córdoba, Argentina) (Murúa et al. 2003), in growth chambers under controlled conditions [27 ± 2°C, 70–75% relative humidity, 14:10 (L:D) h cycles] until adult emergence. This diet has been used to rear other polyphagous lepidopteran species such as *Spodoptera frugiperda* (J. E. Smith), *Diatraea saccharalis* (Fabricius), *Rachiplusia nu* (Guenée), and *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae) (Murúa et al. 2003, 2008; Prieto et al. 2008, Barrionuevo et al. 2012). Only male moths were examined using genitalic observation to confirm the species according to Velasco de Estacul et al. (1969) and Pogue (2004). The females collected were not considered for this study, which might have affected the accuracy of data regarding species composition in the Heliiothinae complex, mainly in species with unequal sex ratios. Some insects of each of these populations were deposited as voucher specimens in the collection of Sección Zoología Agrícola, at Estación Experimental Agroindustrial Obispo Colombes, Tucumán province, Argentina.

Population Fluctuations

Male moths were sampled in chickpea and soybean crops from 2013 to 2015 in Tucumán province. Traps were placed in chickpea

Table 1. Larvae of the Heliiothinae complex (*Helicoverpa* spp. and *Chloridea virescens*) collected in Viclos, La Cocha, San Agustín and Jiménez counties in chickpea and soybean crops used to identify the adults of each species

Province	County	Crop	Date	Larvae collected (no.)	Males obtained and identified ^a (no.)	<i>H. gelotopoeon</i>	<i>H. armigera</i>	<i>H. zea</i>	<i>C. virescens</i>
Tucumán	Viclos	Chickpea	September 12 2013	250	96	94	1	0	1
	La Cocha	Soybean	January 31 2014	200	94	89	4	1	0
			February 26 2014	100	54	53	1	0	0
		San Agustín	Soybean	January 15 2015	180	95	95	0	0
Santiago del Estero	Jiménez	Soybean	February 15 2014	150	77	71	2	3	1

^aOnly male moths obtained from larvae collected were examined using genitalia to confirm the species. Females obtained were not considered for this study.

crops from August to October 2013 in Viclos, and from July to December 2014 in San Agustín. In soybean, traps were placed from January to June 2014 and from January to May 2015 in San Agustín. Yellow traps (Unitrap, ChemTica Internacional S.A., Heredia, Costa Rica) were used and installed according to manufacturer's recommendations. The lures were changed every 20 or 30 d and the insects were collected every 7 or 10 d. Traps were baited with *H. armigera* and *H. gelotopoeon* pheromones (ChemTica Internacional S.A., Heredia, Costa Rica). Growth stages of chickpea and soybean crops were determined based on the description by Soltani et al. (2006) and Fehr et al. (1971), respectively.

The data obtained were tested for normality using the Shapiro-Wilk test. The data regarding *H. gelotopoeon* and *H. armigera* adults obtained from each trap that did not show normal distribution or homogeneity of variance were subjected to a square root transformation [$\sqrt{(X+0.5)}$]. The transformed data were analyzed using a *t*-test to detect differences between the adults of both species collected on both crops from different years. All the analyses were conducted using the software InfoStat (2006).

Geographical Distribution of *H. armigera*

Pheromone and light traps were used to update the distribution of this noctuid pest in Tucumán province. As described previously, males were collected using pheromone traps in San Agustín (Cruz Alta). A light trap (A todo campo, Buenos Aires, Argentina) was installed in Las Talitas (26°47'S, 65°11'W) from September to November 2014 and only male moths were selected for further identification. Moreover, larvae of this species were caught directly from sunflower, and spiny plumeless thistle (*Carduus acanthoides* L.; Asterales: Asteraceae).

To report the geographical distribution of *H. armigera* in other regions of Argentina, the database of Sistema Nacional Argentino de Vigilancia y Monitoreo de Plagas (SINAVIMO) of the Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA) was utilized.

Results

Identification of Heliiothinae complex

A total of 880 larvae were collected from 2013 to 2015 on chickpea and soybean crops and 416 male moths were obtained and identified. Species identification used adult males only, because using male genitalic characters are more reliable than using females (Perera et al. 2015). Female obtained were 394 and remainder of larvae died by unknown reasons. The species found were determined as *H. gelotopoeon*, *H. armigera*, *H. zea*, and *C. virescens*. Regardless of province, county, crop, and year, the predominant species was *H. gelotopoeon* (Table 1).

Population Fluctuations

Population fluctuations of *H. gelotopoeon* and *H. armigera* males in chickpea and soybean crops are shown in Fig. 1. Considering the total number of moths obtained (*H. gelotopoeon*, $n = 440$; *H. armigera*, $n = 53$), *H. gelotopoeon* was the most abundant species in both crops, in all years sampled, and the difference in number of moths between the two crops was statistically significant ($t = -6.15$; $df = 89$; $P < 0.0001$). In chickpea (Fig. 1a and b), the first species that appeared was the native, *H. gelotopoeon*, and it was present, in general, during the entire crop cycle in 2013 and 2014. The largest collections of *H. gelotopoeon* and *H. armigera* were from October to November and from September to October in 2013, respectively, and adults were only collected in some weeks from September to November in 2014.

In soybeans (Fig. 1c and d), the largest collection of *H. gelotopoeon* was recorded in February in both years and, in general, this pest was present during all months in 2015. Male *H. armigera* individuals were only recorded in 2014 from mid-February to April. In 2014, male *H. gelotopoeon* specimens were observed before *H. armigera*.

Geographical Distribution of *H. armigera*

Considering our collections, *H. armigera* was found in two counties (Las Talitas and San Agustín) of Tucumán province and one county (Jiménez) of Santiago del Estero province, and these were new records for both provinces. Larvae of this species were recorded on soybean, chickpea, sunflower, and spiny plumeless thistle. This is the first report of *H. armigera* in sunflower and spiny plumeless thistle in Argentina (Table 2 and Fig. 2).

Taking into account SINAVIMO database, the reports of Arneodo et al. (2015), and our own collections, *H. armigera* was recorded in eight provinces and 20 counties in Argentina (Table 2 and Fig. 2).

Discussion

In Tucumán province, Argentina, *H. gelotopoeon*, the native species, was the most abundant and predominant Heliiothinae in chickpea and soybean crops. Our results showed that *H. gelotopoeon* and *H. armigera* had a seasonal distribution in both crops during all years studied in Tucumán province, being more prevalent in September and October on chickpea and during February and March on soybean.

The influence of crops on the abundance of *H. armigera* was reported by Maelzer and Zalucki (1999). The authors found that alfalfa and corn always had positive effects, whereas sorghum presented a negative effect on the light-trap catch in the second and third generations.

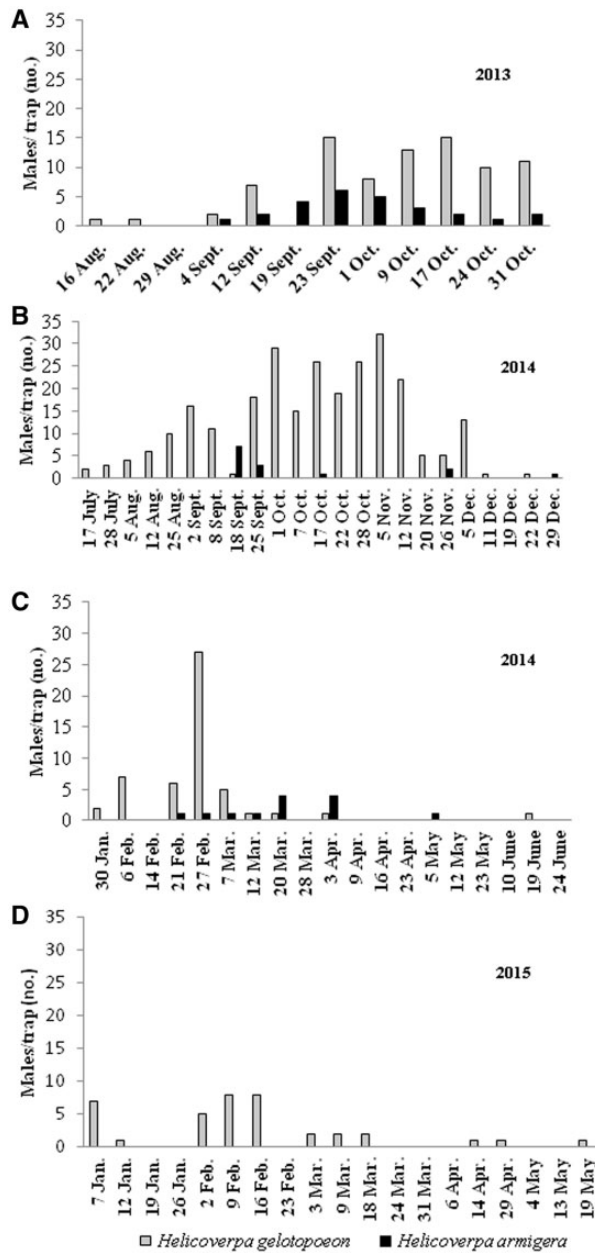


Fig. 1. Population fluctuations of *H. gelotopoeon* and *H. armigera* adults, in chickpea and soybean crops, from 2013 to 2015, in Tucumán province, Argentina. Chickpea crop: (A) Viclos county; (B) San Agustín county. Soybean crop: (C, D) San Agustín county.

Considering crop phenology, Ahmed and Khaliq (2012) observed that the appearance of *H. armigera* always coincided with the initiation of flowering of chickpea, irrespective of the fact of how early or late the crop started flowering. In our study, an increase in population of both species was detected from September to October, coinciding with the beginning of the podding stage (R3) of chickpea. In soybean, *H. armigera* was not abundant and the first adults were detected at R2, by the end of February, whereas *H. gelotopoeon* adults were found from V3, in the beginning of January, in both years.

The results in this study indicate that *H. armigera* is widely distributed and the species has been adapted to elevations ranging from 20 to 1,200 masl in Argentina. Zhou et al. (2000), Rochester et al. (2002), and Feng et al. (2004) reported that *H. armigera* adults are highly mobile. They can move many hundreds of kilometers

between regions and extensively between fields within regions. *Helicoverpa armigera* seems to be well adapted to the climate of agricultural regions of South America. According to Kriticos et al. (2015), this insect species has spread rapidly throughout Argentina, Bolivia, Brazil, Paraguay, Uruguay, and most recently to Puerto Rico. However, no studies have been conducted to investigate whether *H. armigera* detected in these non-Brazilian locations were due to natural spread of Brazilian populations or they represented separate incursion events. In addition, *H. armigera* has been detected in Brazil in October 2008 (Sosa-Gomez et al. 2016), meaning that the presence of this species in other countries could be due to late detection more than to fast spreading. Also, the detection in Brazil does not mean that this country has been the entrance of this insect species to Latin American countries. *Helicoverpa armigera* could have invaded South America through other countries where its identification might have been neglected.

So far, in Argentina, larvae of *H. armigera* have been found on chickpea (Arneodo et al. 2015, SINAVIMO 2015), soybean, cotton (SINAVIMO 2015), sunflower and spiny plumeless thistle. Studies performed in Brazil indicate a widespread distribution of *H. armigera* throughout Midwest and Northeast Brazil in a variety of crops, particularly dicotyledons, beans (*Phaseolus vulgaris* L. Fabales: Fabaceae), soybean, and cotton, and to a lesser extent on monocotyledons, e.g., millet (*Pennisetum glaucum* L. Poales: Poaceae), sorghum, and corn (Leite et al. 2014).

Considering the importance of *H. gelotopoeon* in the region, few studies on the bioecology, crop damage, chemical control, natural enemies, host plants, oviposition preference or monitoring resistance to insecticides and *Bt* cultivars, have been performed. Our study is the first to provide information about its seasonal distribution.

Despite the low frequency of *H. armigera*, the results from the current study show that this pest is widely distributed in Argentina. Because of that, it is important to intensify the monitoring and correctly identify the different species of the Heliiothinae complex using taxonomic methods or molecular techniques.

The sustainable management of this species poses a significant challenge for Argentinean entomology in the coming years, especially considering its polyphagous feeding habit, great dispersal ability, and numerous reports of resistance to insecticides and *Bt* crops (Asokan et al. 2012, Li et al. 2007, Gao et al. 2009, Liu et al. 2010, Nair et al. 2013, Bird and Downes 2014).

It is necessary to address different studies, as mentioned, in order to contribute to future studies regarding management for *H. gelotopoeon* and *H. armigera* in Argentina.

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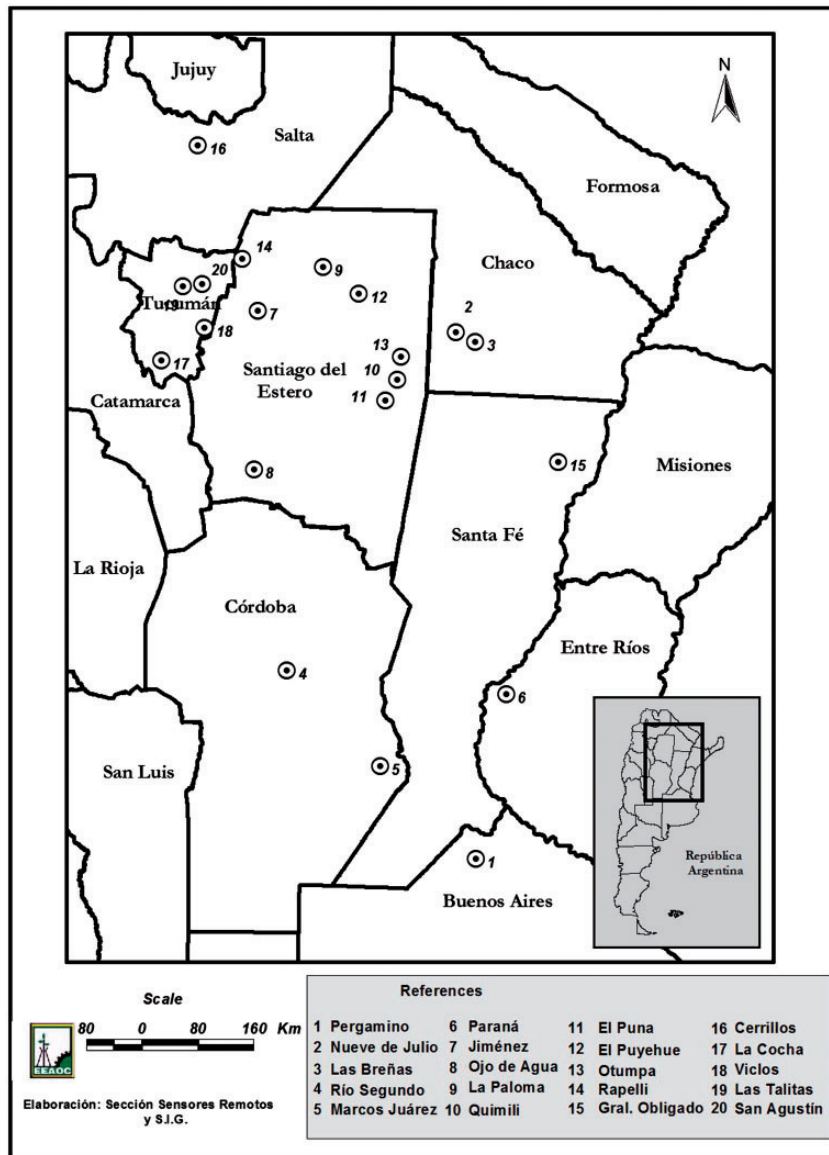


Fig. 2. Geographical distribution of *H. armigera* in different provinces of Argentina.

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Table 2. Geographical distribution of *H. armigera* in Argentina

Province	Sampling site	Elevation (masl)	Stage collected	Collection/host plant	Month/year
Buenos Aires	1 Pergamino ^a	56	Adults	Pheromone trap/soybean crops	July 2014
Chaco	2 Nueve de Julio ^b	50	Adults	Cotton and soybean	July 2014
	3 Las Breñas ^b	105	Adults	Pheromone trap	October 2014 to January 2015
Córdoba	4 Río Segundo ^a	335	Adults	Pheromone traps	June 2014
	5 Marcos Juárez ^a	112	Adults	Light trap near cereals and oleaginous crops	November 2014
Entre Ríos	6 Paraná ^a	77	Adults	Pheromone traps near to soybean and corn crops	July 2014
Santiago del Estero	7 Jiménez ^a	1,030	Larvae	Soybean	January 2014
	8 Ojo de Agua ^a	600	Adults	Pheromone traps on soybean crops	February 2015
	9 La Paloma ^a	606	Adults	Pheromone traps on soybean crops	February 2015
	10 Quimili ^a	137	Adults	Pheromone traps	June 2015
	11 El Puna ^a	130	Adults	Pheromone traps	June 2015
	12 El Puyehue ^a	191	Adults	Pheromone traps	June 2015
	13 Otumpa ^a	155	Adults	Pheromone traps	June 2015
	14 Rapelli ^b	395	Larvae	Chickpea	October 2014 to January 2015
Santa Fé	15 General Obligado ^a	21	Adults	Cotton	May 2014
Salta	16 Cerrillos ^a	1,217	Adults	Chickpea	November 2014
Tucumán	17 La Cocha	397	Adults ^c	Chickpea	November 2013
			Larvae	Soybean	February 2014
	18 Viclos	345	Adults ^c and larvae	Chickpea	September 2013
			Larvae	Spiny plumeless thistle	September 2014
	19 Las Talitas	591	Adults	Light trap near to sugarcane crops and lemon tree fields	October 2014
	20 San Agustín	1,190	Adults	Pheromone traps on soybean crops	January–June 2014
			Larvae	Sunflower	January 2015

^aDatabase of SINAVIMO.

^bData reported by Arneodo et al. (2015).

^cData used to report the first time of *H. armigera* collection in Argentina (Murúa et al. 2014).

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