

Biological characterization and mating compatibility of *Helicoverpa gelotopoeon* (D.) (Lepidoptera: Noctuidae) populations from different regions in Argentina

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Abstract

Helicoverpa gelotopoeon, the South American bollworm, is a polyphagous pest of the Heliiothinae complex that causes damage to soybean, cotton, and chickpea crops. Some species within this complex have developed resistance to genetically modified crops and insecticides, which has led to increased interest in their genetic diversity and population structure. The objective of this study was to characterize biological and reproductive parameters of two populations of *H. gelotopoeon* collected in two different provinces of Argentina. Intra- and inter-population crosses revealed that *H. gelotopoeon* populations from both regions of Argentina did not present evidence of pre-zygotic and post-zygotic incompatibility, suggesting that Tucumán and Córdoba populations of *H. gelotopoeon* belong to a single wide-ranging species. Our data support the occurrence of substantial gene flow between *H. gelotopoeon* populations, probably due to the widely documented, long-range migratory capacity of Heliiothinae species.

Keywords: South American bollworm, biology, reproductive compatibility, fitness, insect resistance management

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Introduction

The subfamily Heliiothinae (Lepidoptera: Noctuidae) encompasses 381 described species, many of which are important agricultural pests. The genera *Helicoverpa* Hardwick and *Chloridea* Duncan and Westwood (Pogue, 2013) contain several

economically important pest species, which are native to South America including *H. gelotopoeon* (Dyar), *H. zea* (Boddie), *C. virescens* (Fabricius), *C. subflexa* (Guenée), and *C. tergemina* (Felder & Rogenhofer) (Mitter *et al.*, 1993; Pastrana, 2004; Pogue, 2013). The South American invasion of *Helicoverpa armigera* Hübner added a new species into the Heliiothinae complex; this species is now found in Brazil (Czepak *et al.*, 2013; Specht *et al.*, 2013; Tay *et al.*, 2013), Paraguay (Senave, 2013), Argentina (Murúa *et al.*, 2014), Bolivia, and Uruguay (Kriticos *et al.*, 2015). In addition to *H. armigera*, three other Heliiothinae species are present in the North of Argentina: *H. gelotopoeon*, *H. zea*, and *C. virescens* (Murúa *et al.*, 2016).

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Helicoverpa gelotopoeon, the South American bollworm, is a polyphagous pest and has been reported in cotton (*Gossypium hirsutum* L.; Malvales: Malvaceae), alfalfa (*Medicago sativa* L.; Fabales: Fabaceae), sunflower (*Helianthus annuus* L.; Asterales: Asteraceae), soybean (*Glycine max* L.; Fabales: Fabaceae), chickpea (*Cicer arietinum* L.; Fabales: Fabaceae), and corn (*Zea mays* L.; Poales: Poaceae) (Pastrana, 2004; Navarro *et al.*, 2009). Larvae cause damage in the vegetative and reproductive plant growth stages. *Helicoverpa gelotopoeon* occurs in Argentina, Chile, southern Brazil, Paraguay, and Uruguay (Pastrana, 2004; Navarro *et al.*, 2009). In Tucumán and other provinces of Argentina, *H. gelotopoeon* is a major pest of soybean and can be difficult to control with insecticides (Navarro *et al.*, 2009; Scalora *et al.*, 2012). Consequently, it causes a significant economic impact, since Argentina is the third major soybean producer in the world, covering an area of 20.1 million hectares (Bolsa de Cereales, 2016). *Helicoverpa gelotopoeon* also affects cotton and chickpea, and damage is most severe when these crops are grown in succession (Pastrana, 2004; Fichetti *et al.*, 2009; Navarro *et al.*, 2009; Scalora *et al.*, 2012).

The bioecological characteristics of this Heliiothinae complex, in addition to the numerous occurrences of insecticide resistance within this subfamily (Forrester, 1990; Forrester *et al.*, 1993; Armes *et al.*, 1996), necessitate establishing alternatives to insecticide control for the 34 species of *Helicoverpa*. Since 1996, *Bacillus thuringiensis* (Bt) crops have been the primary tool for managing major pests, such as *H. armigera*, *H. zea*, and *C. virescens*, in cotton and corn fields worldwide (Tabashnik *et al.*, 2009; Blanco *et al.*, 2016). Currently, genetically modified soybeans are one method to control primary crop pests, but these also impact on secondary pests, such as *H. gelotopoeon* and *H. zea* (MacRae *et al.*, 2005; Wu *et al.*, 2005; Casmuz *et al.*, 2014; Cazado *et al.*, 2014; Monsanto, 2014).

Helicoverpa armigera, *H. zea*, and *C. virescens* have developed resistance to insecticides and Bt Cry proteins (Forrester *et al.*, 1993; Gould *et al.*, 1995; Armes *et al.*, 1996; Hardee *et al.*, 2001; Li *et al.*, 2007; Mahon *et al.*, 2007; Pietrantonio *et al.*, 2007; Gao *et al.*, 2009; Liu *et al.*, 2010; Bird & Downes, 2014; Brévault *et al.*, 2015; Reisig & Reay-Jones, 2015; Tay *et al.*, 2015); this has led to increased interest in understanding Heliiothinae genetic diversity and gene flow among its populations and the possible exchange of alleles between geographically distant populations. Some insect populations identified as the same species may yet display different biological and genetic traits and show reproductive isolation, preventing complete gene flow among populations. Therefore, identifying cryptic species is important to not only explain phytophagous insect evolution but also help understand implications for insect pest management and the emergence of insecticide and Bt resistance (Pérez Contreras, 1999; Rull *et al.*, 2012). In general, unstructured genetic populations have been reported for *H. armigera*, *H. zea*, and *C. virescens* species, which were based on several molecular markers, including mtDNA, allozymes, and microsatellites (Korman *et al.*, 1993; Roehrdanz *et al.*, 1994; Nibouche *et al.*, 1998; Zhou *et al.*, 2000; Han & Caprio, 2002, 2004; Behere *et al.*, 2007; Endersby *et al.*, 2007; Groot *et al.*, 2011; Perera & Blanco, 2011; Asokan *et al.*, 2012; Leite *et al.*, 2014; Arneodo *et al.*, 2015). However, other studies (Nibouche *et al.*, 1998; Khiaban *et al.*, 2010; Domingues *et al.*, 2012; Yenagi *et al.*, 2012), have found genetic population structure in different species of this complex.

Genetically distinct populations of insects can vary in their susceptibility to control tactics (Joyce *et al.*, 2014); therefore,

understanding the biology and genetic population structure of *H. gelotopoeon* is essential for the development of sustainable management strategies. The objective of this study was to characterize biological and reproductive parameters of two *H. gelotopoeon* populations collected in Tucumán and Córdoba provinces of Argentina. Our research provided detailed information about South American bollworm biology, unknown until now, and will be useful to define the management strategies for this species in Argentina.

Materials and methods

Insect collections

Helicoverpa gelotopoeon larvae were collected from September to October 2014 in commercial chickpea fields located in two Argentine provinces. In Tucumán province (Northwestern region), collections were made in San Agustín county (26°50'21"S, 64°51'32"W), and in Córdoba province (Pampas region), collections were made in Marcos Juárez county (32°43'14.25"S, 62°07'00.30"W). Each sampling location was treated as a population. At each sampling site, a minimum of 300 larvae (instars 2–5) were collected and placed individually in glass tubes (12 cm high and 1.5 cm diameter) with pieces of artificial diet. Collected larvae were returned to the laboratory and placed in breeding chambers under controlled conditions (27 ± 2°C, 70–75% relative humidity, 14 : 10 light : dark photoperiod) until adult emergence. Then, all adults that emerged in the laboratory were examined using male genitalia to confirm the species according to Velasco de Stacul *et al.* (1969). Sampled insects from each of these populations were deposited as voucher specimens in the insect collection of the Sección Zoología Agrícola, Estación Experimental Agroindustrial Obispo Colombes, Tucumán, Argentina.

Insect rearing

Approximately 250 adults (125 females and 125 males) were randomly selected from reared larvae to establish an experimental colony for each population. Adults were arranged in five cylindrical oviposition cages (40 cm high and 20 cm diameter) lined with polyethylene bags with approximately 25 females and 25 males per cage. For aeration, both ends of the cage were covered with a nylon cloth. Each population was maintained in the same chamber under identically controlled conditions at 27 ± 2°C, 70–75% relative humidity and a photoperiod of 14 : 10 light:dark. The food for adults was provided via a cotton plug saturated with a mixture of honey and water (1 : 1 volume : volume) which was replaced every day. Cages were checked daily for oviposition and adult mortality. Rearing of each population was maintained between September 2014 and February 2015.

Eggs deposited in the cages were collected and put into plastic containers of 1000 ml. Once emerged, neonate larvae were placed individually in glass tubes with artificial larval diet that included chickpea flour (Grandiet®, Buenos Aires, Argentina), wheat germ (Grandiet®, Buenos Aires, Argentina), brewer's yeast (Calsa®, Tucumán, Argentina), vitamin C (Anedra®, Buenos Aires, Argentina), sorbic acid (Anedra®, Buenos Aires, Argentina), sodium benzoate (TodoDroga®, Córdoba, Argentina), vitamin supplement amino acids (Ruminal®, Buenos Aires, Argentina), and methylparaben (Todo Droga®, Córdoba, Argentina) (Murúa *et al.*, 2003). Artificial diet was replaced every 2–3 days. As

larvae pupated, pupae were sexed and placed in containers with moistened filter paper until adult emergence. Adults were used to initiate a new generation. After establishing a colony for each population, individuals from the second to the fourth generation were used for studies of fitness and reproductive compatibility.

Biology of South American bollworm populations from different provinces

From each experimental colony of *H. gelotopoeon*, five groups of neonate larvae were randomly selected (total 120 and 90 larvae of Tucumán and Córdoba province, respectively) to analyze the following parameters: duration of larval instars and pupal stage, pupal mass (obtained 24 h after pupation), and adult sex ratio.

From the adults obtained, a set of approximately 35 females and 35 males of Tucumán population, and 22 females and 22 males of Córdoba population were randomly selected to determine incubation period, longevity, and reproductive parameters. One virgin female and one virgin male (24 h old) from the same population were placed in cylindrical oviposition cages similar to those described above (25 cm high and 15 cm diameter) (Tucumán population, $N = 35$; Córdoba population, $N = 22$). These single pair matings represented the parental cross that was used for reproductive compatibility studies. Moths were maintained in this cage, with mortality and oviposition recorded daily. Dead females were dissected to establish the number of spermatophores present in their reproductive tract to determine whether or not mating had occurred. Pre-oviposition, oviposition, and post-oviposition period (number of days that a female survives after its last oviposition); total fecundity (number of eggs deposited by a female during her entire life period); incubation period; total fertility (percentage of eggs hatching); and adult longevity were recorded.

Reproductive compatibility between South American bollworm populations from different provinces

A crossing experiment was performed to determine reproductive compatibility between Tucumán and Córdoba populations, according to the methodologies described by Pashley & Martin (1987); Pashley *et al.* (1990); Lopez-Edwards *et al.* (1999); Murúa *et al.* (2008) and Fogliata *et al.* (2016). To determine compatibility, we used one virgin female and one virgin male of 24 h old. Four different types of crosses were performed: (i) parental crosses using parents from the same population, (ii) hybrid crosses using one parent of each population, (iii) backcrosses with the female progenitor as the recurrent parent and backcrosses with the male progenitor as the recurrent parent, and (iv) inter-hybrid mating crosses between F1 hybrids from different populations.

A subset of larval progeny (F1) from each fertile cross was monitored for survival until pupation and then reared to adulthood. The parameters measured to determine compatibility were: number of spermatophores; pre-oviposition, oviposition, and post-oviposition period duration; total fecundity; and total fertility.

Data analysis

Fitness data between both populations were compared by Wilcoxon rank-sum test (Lehmann, 1975) ($P < 0.05$). For the

reproductive compatibility data, due to the high number of combinations, the performance of all parental crosses was compared with the results of other types of crosses (hybrid crosses, backcrosses, and inter-hybrid matings) using Kruskal–Wallis (1952) test ($P < 0.05$).

For all studies, pre-oviposition, oviposition, and post-oviposition periods were compared for those females that laid eggs. Total fecundity was compared for all females including those that laid no eggs. For total fertility, females that laid eggs but had no spermatophores were not included. All data were analyzed using InfoStat (2006).

Results

Biological and reproductive parameters

All adults from Tucumán and Córdoba sampled were identified as *H. gelotopoeon* based on the morphology of male genitalia (Velasco de Stacul, 1969).

In total, 35 and 22 parental crosses were used to determine biological and reproductive parameters of Tucumán and Córdoba populations, respectively. The duration of each life stage and reproductive parameters are presented in table 1. The biological parameters that showed significant differences between Tucumán and Córdoba populations were: incubation period ($W = 3969$; $P = 0.0005$), third instar larval development time ($W = 7055$; $P = 0.0014$) and female longevity ($W = 294.50$; $P = 0.0438$), which were all longer in duration for the Tucumán population and pupal mass ($W = 1982$, $P = 0.0066$) that was larger for the Córdoba population (table 1). Differences in reproductive parameters only occurred in pre-oviposition period ($W = 451$; $P = 0.0155$), which was longer for the Tucumán population.

Reproductive compatibility between South American bollworm populations

In total, 57 parental crosses, 35 hybrid crosses, 93 backcrosses, and 56 inter-hybrid matings were performed between Tucumán and Córdoba populations (table 2). No significant differences were observed in reproductive parameters between both populations. In general, all hybrid crosses, backcrosses, and inter-hybrid matings showed more similar values than both parental crosses for all parameters (tables 2 and 3).

Discussion

This study compared biological and reproductive demographic traits and mating compatibility between two *H. gelotopoeon* populations collected from chickpea in two regions of Argentina. *Helicoverpa gelotopoeon* populations from Tucumán and Córdoba showed similar biological and reproductive characteristics when reared on artificial diet in the laboratory. However, differences in incubation period, third instar larval development time, female longevity, pupal mass, and pre-oviposition period were found. These parameters were higher for the Tucumán population, but the pupal mass was higher for Córdoba population (table 1).

Results of our study showed that the South American bollworm populations from Tucumán and Córdoba provinces complete a single generation (from egg to adult) in approximately 40 and 38 days under laboratory conditions. A different development time was reported by Navarro *et al.* (2009) for this species, but a similar observation was reported by Naseri

Table 1. Duration in days (mean \pm SE) of egg, larval (L1–L5), and pupal stages; pupal mass (mg); female, male, and total longevity (days); sex ratio (F : M); and life span of *Helicoverpa gelotopoeon* populations collected in Tucumán and Córdoba provinces in Argentina and reared at 25 \pm 2 °C, 70–75% RH, and 14L:10D.

Life cycle stages	Tucumán population	<i>n</i>	Range	Córdoba population	<i>n</i>	Range
Egg	4.07 \pm 0.13a	9507	2–7	3.41 \pm 0.13b	9689	2–6
L1	2.54 \pm 0.06a	116	2–4	2.7 \pm 0.07a	87	2–4
L2	2.24 \pm 0.08a	116	1–4	2.04 \pm 0.06a	87	1–4
L3	1.94 \pm 0.04a	111	1–3	1.73 \pm 0.09b	83	1–4
L4	2.14 \pm 0.07a	111	1–6	1.99 \pm 0.08a	82	1–4
L5	5.81 \pm 0.14a	108	2–11	5.87 \pm 0.14a	62	3–9
Overall larval stage	14.75 \pm 0.18a	107	13–24	14.24 \pm 0.18a	62	12–19
Pupa	12.79 \pm 0.34a	90	9–20	11.86 \pm 0.33a	49	8–17
Pupal mass	224 \pm 5.6a	61	149–310	253 \pm 9.3b	34	131–434
Female longevity	12.68 \pm 0.8a	35	4–21	9.75 \pm 1.07b	22	4–17
Male longevity	13 \pm 1.06a	35	2–23	11.18 \pm 0.98a	22	4–19
Sex ratio ♀:♂	1: 1.04	90		1: 1.3	49	
Spermatophores per female	1.29 \pm 0.12a	35	1–4	1.27 \pm 0.12a	22	1–3
Pre-oviposition period	4.91 \pm 0.31a	34	3–10	3.81 \pm 0.42b	21	1–8
Oviposition period	5.44 \pm 0.68a	34	1–17	4.86 \pm 0.63a	21	1–11
Post-oviposition period	1.74 \pm 0.39a	34	0–8	2.19 \pm 0.47a	21	0–7
Total fecundity	340.74 \pm 51.49a	35	0–1326	517.68 \pm 84.11a	22	0–1397
Total fertility	79.73 \pm 1.55a	33	44–92	84.35 \pm 1.81a	19	63–98

Values followed by same letters within a row are not significantly different according to Wilcoxon test ($P > 0.05$).

Table 2. Number (mean \pm SE) of spermatophores per female; duration of pre-oviposition, oviposition, and post-oviposition periods; total fecundity (number of eggs per female); and total fertility (percentage of egg hatch) of *Helicoverpa gelotopoeon* parental crosses, hybrid crosses, backcrosses, and inter-hybrid matings using populations collected in Tucumán and Córdoba provinces in Argentina and reared at 25 \pm 2°C, 70–75% RH, and 14L:10D.

	Spermatophores per female	Pre-oviposition period (days)	Oviposition period (days)	Post-oviposition period (days)	Fecundity	Fertility
Parental crosses	1.28 \pm 0.09a (57)	4.49 \pm 0.26a (55)	5.22 \pm 0.49a (55)	1.91 \pm 0.3a (55)	409.04 \pm 46.31a (55)	81.45 \pm 1.22a (52)
Hybrid crosses	0.86 \pm 0.11a (35)	4.36 \pm 0.26a (28)	5.18 \pm 0.62a (28)	1.71 \pm 0.38a (28)	366.81 \pm 60.58a (26)	75.96 \pm 2.73a (23)
Backcrosses	1.19 \pm 0.11a (93)	4.51 \pm 0.26a (75)	4.83 \pm 0.36a (75)	2.41 \pm 0.27a (75)	375.26 \pm 37.52a (70)	77.68 \pm 1.43a (58)
Inter-hybrid matings	1.27 \pm 0.15a (56)	5.03 \pm 0.38a (36)	6.06 \pm 0.54a (36)	2.44 \pm 0.37a (36)	297.88 \pm 40.81a (43)	77.95 \pm 1.67a (33)

Values followed by same letters within a column are not significantly different according to Kruskal–Wallis test ($P > 0.05$).

Table 3. Number (mean \pm SE) of mated female, total fecundity (number of eggs per female), and total fertility (percentage egg hatch) of each *Helicoverpa gelotopoeon* crosses and backcrosses using populations collected in Tucumán (T) and Córdoba (C) provinces in Argentina and reared at 25 \pm 2°C, 70–75% RH, and 14L:10D.

Type of crosses	♀	♂	N° paired	Mated female	Fecundity	Fertility
Parental crosses	T	T	35	35	340.74 \pm 51.49 (35)	79.73 \pm 1.55 (33)
Parental crosses	C	C	22	22	517.68 \pm 84.11 (22)	89.35 \pm 1.81 (19)
Hybrid crosses	T	C	17	13	389.23 \pm 85.03 (13)	71.53 \pm 3.82 (12)
Hybrid crosses	C	T	18	13	344.38 \pm 89.30 (13)	80.80 \pm 3.50 (11)
Backcrosses	C	F ₁ (C♀ \times T♂)	16	12	379.08 \pm 101.32 (12)	82.07 \pm 2.08 (10)
Backcrosses	C	F ₁ (T♀ \times C♂)	14	10	280 \pm 99.19 (10)	76.61 \pm 5.38 (9)
Backcrosses	T	F ₁ (C♀ \times T♂)	12	12	473.58 \pm 78.91 (12)	77.72 \pm 3.63 (11)
Backcrosses	T	F ₁ (T♀ \times C♂)	13	8	183.38 \pm 87.50 (8)	79.64 \pm 4.40 (5)
Backcrosses	F ₁ (C♀ \times T♂)	C	8	5	563.40 \pm 140.97 (5)	68.70 \pm 6.95 (4)
Backcrosses	F ₁ (C♀ \times T♂)	T	10	8	507.75 \pm 137.12 (8)	72.93 \pm 4.74 (7)
Backcrosses	F ₁ (T♀ \times C♂)	C	10	8	268.75 \pm 89.81 (8)	80.50 \pm 1.52 (7)
Backcrosses	F ₁ (T♀ \times C♂)	T	10	7	390.29 \pm 108.70 (7)	78.73 \pm 1.21 (5)
Inter-hybrid matings	F ₁ (T♀ \times C♂)	F ₁ (C♀ \times T♂)	16	12	260.67 \pm 72.55 (12)	78.49 \pm 3.37 (9)
Inter-hybrid matings	F ₁ (T♀ \times C♂)	F ₁ (T♀ \times C♂)	14	10	176.30 \pm 49.03 (10)	75.14 \pm 5.16 (7)
Inter-hybrid matings	F ₁ (C♀ \times T♂)	F ₁ (C♀ \times T♂)	15	14	406.50 \pm 79.76 (14)	79.10 \pm 2.05 (12)
Inter-hybrid matings	F ₁ (C♀ \times T♂)	F ₁ (T♀ \times C♂)	11	7	318.14 \pm 120.19 (7)	78.12 \pm 4.30 (5)

et al. (2009) for *H. armigera*. The incubation period for both populations was similar to that reported by Navarro *et al.* (2009), but it differed compared with the report made by Urretabizkaya *et al.* (2010). Five larval instars of both populations were found and this result was similar to that reported by

Iannone & Leiva (1993) and Sharma *et al.* (2011) for *H. gelotopoeon* and *H. armigera*, respectively. Larval development time was similar to that reported by Iannone & Leiva (1993); Urretabizkaya *et al.* (2010); and Navarro *et al.* (2009) for *H. gelotopoeon*. Pupal stage duration was shorter than

reported by other studies (Iannone & Leiva, 1993; Urretabizkaya *et al.*, 2010), but the values were consistent with those obtained by Naseri *et al.* (2009) and Mironidis (2014) for *H. armigera*. No previous studies have examined *H. gelotopoeon* pupal mass, but in general it was smaller than those observed for *H. armigera* (Arghand *et al.*, 2014) and *H. zea* (Giolo *et al.*, 2006). Adults from the *H. gelotopoeon* populations had a similar survival time as those reported by Simmons & Lynch (1990); Liu *et al.* (2004); Naseri *et al.* (2009); and Pérez & Suris (2012) for *Helicoverpa* and *Chloridea* species. The sex ratio found for the *H. gelotopoeon* population from Tucumán and Córdoba (1 female:1 male) was similar to that reported by Álvarez Hernández *et al.* (2010) for *C. virescens* reared on leaves and pods of chickpea. In this study, we found that females emerged before males, suggesting that *H. gelotopoeon* is a protogynous species, a characteristic also observed by Giolo *et al.* (2006) and Colvin *et al.* (1994) with *H. zea* and *H. armigera*, respectively. According to Rhainds *et al.* (1999), protogyny may be an evolved mechanism to reduce inbreeding, given that early emerged females are less likely to mate with their brothers.

The number of spermatophores found in dissected females was generally one for both populations. Similar observations were reported by Callahan (1958) and Navarro (1987) for *H. zea*. The periods of pre-oviposition, oviposition, and post-oviposition were similar with those found by other reports for *H. armigera* (Sharma *et al.*, 2011). Navarro *et al.* (2009) reported higher fecundity of *H. gelotopoeon* than that recorded in our study. However, our fecundity results were similar to that found by Urretabizkaya *et al.* (2010) for the same species. No previous studies have examined *H. gelotopoeon* fertility. Nevertheless, our results showed values lower than those reported for *C. virescens* and *H. zea* (Navarro, 1987; Méndez Barceló, 2003), but higher than those found for *H. armigera* (Laster & Sheng, 1995; Ali *et al.*, 2009) (table 1).

Intra- and inter-population crosses revealed that *H. gelotopoeon* populations from the northwestern and Pampas regions in Argentina did not present evidence of pre-zygotic or post-zygotic incompatibility, suggesting that Tucumán and Córdoba populations of *H. gelotopoeon* belong to a single wide-ranging species (tables 2 and 3).

As previously mentioned, unstructured genetic populations have been reported for different species of the Heliiothinae complex. Most of these studies used molecular tools (Korman *et al.*, 1993; Roehrdanz *et al.*, 1994; Nibouche *et al.*, 1998; Zhou *et al.*, 2000; Han & Caprio, 2002, 2004; Behere *et al.*, 2007; Endersby *et al.*, 2007; Groot *et al.*, 2011; Perera & Blanco, 2011; Asokan *et al.*, 2012; Leite *et al.*, 2014; Arneodo *et al.*, 2015). Only Colvin *et al.* (1994) compared reproductive compatibility between different African, Indian, Chinese, and Australian populations of *H. armigera*, suggesting that *H. armigera* exists as a single species over its geographical range.

This study is the first to report a lack of reproductive isolation between *H. gelotopoeon* populations from Argentina based on reproductive parameters and mating compatibility. Our results clearly indicate that there are no signs of geographical isolation, since populations mated successfully in both directions. This supports the presumption that substantial gene flow occurs between *H. gelotopoeon* populations, probably due to the long-range migratory capacity of Heliiothinae species, which has been widely documented (Hartstack *et al.*, 1982; Farrow & Daly, 1987; Gregg *et al.*, 1995; Westbrook, 2008; Westbrook & Lopez, 2010).

This study provides useful information about South American bollworm biology to define the management strategies and control of this species in Argentina. However, additional research on the population genetics of this species in other crops will further increase our understanding of its unstructured genetic populations.

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