

Lack of Reproductive Isolation in *Helicoverpa gelotopoeon* (Lepidoptera: Noctuidae) Populations from Different Host Plant Species in Argentina

Authors: María Inés Herrero, Sofía Victoria Fogliata, Luciana Cecilia Dami, Augusto Casmuz, Gerardo Gastaminza, et. al.

Source: Florida Entomologist, 102(2) : 291-297

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.102.0201>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Lack of reproductive isolation in *Helicoverpa gelotopoeon* (Lepidoptera: Noctuidae) populations from different host plant species in Argentina

María Inés Herrero¹, Sofía Victoria Fogliata¹, Luciana Cecilia Dami¹, Augusto Casmuz², Gerardo Gastaminza², and María Gabriela Murúa^{1,*}

Abstract

Helicoverpa gelotopoeon (Dyar) (Lepidoptera: Noctuidae) is a polyphagous pest that belongs to the Heliiothinae complex, and has been described as feeding on numerous crops. Larvae damage mainly pods and seeds of soybean and other legumes. Adaptation to different host plants might reduce gene flow between phytophagous insect populations, which could lead to reproductive isolation. Therefore, studies on gene flow among host-associated populations are necessary to design management strategies. The objective of this study was to evaluate reproductive compatibility between 2 populations of *H. gelotopoeon* coming from different host plants. We collected *H. gelotopoeon* larvae in alfalfa and soybean crops, and studied biological and reproductive parameters, and mating compatibility. *Helicoverpa gelotopoeon* populations from soybean and alfalfa reared on artificial diet showed similar biological and reproductive characteristics. Intra- and inter-population crosses revealed that there are no signs of host-associated isolation, allowing gene exchange between these populations, which can be explained by the high mobility and generalist behavior of this species. This information will be useful to establish control measures for this pest.

Key Words: phytophagous insects; South American bollworm; host adaptation; reproductive compatibility

Resumen

Helicoverpa gelotopoeon (Dyar) (Lepidoptera: Noctuidae) es una plaga polífaga que pertenece al complejo de Heliiothinae y fue descrita alimentándose sobre numerosos cultivos. Las larvas dañan principalmente las vainas y los granos de soja y otras leguminosas. La adaptación a diferentes plantas hospederas podría reducir el flujo entre poblaciones de insectos fitófagos, lo cual podría conducir a un aislamiento reproductivo. Por lo tanto, estudios sobre el flujo entre poblaciones asociadas a plantas hospederas son necesarios para diseñar estrategias de manejo. El objetivo de este estudio fue evaluar la compatibilidad reproductiva entre 2 poblaciones de *H. gelotopoeon* provenientes de diferentes plantas hospederas. Nosotros recolectamos larvas de *H. gelotopoeon* en los cultivos de alfalfa y soja y estudiamos los parámetros biológicos, reproductivos y la compatibilidad reproductiva. Las poblaciones de *H. gelotopoeon* de soja y alfalfa criadas sobre dieta artificial mostraron características biológicas y reproductivas similares. Las cruces dentro y entre poblaciones revelaron que no hay signos de aislamientos asociados a la planta hospedera, favoreciendo el intercambio de genes entre estas poblaciones, lo cual puede ser explicado por la alta movilidad y el comportamiento generalista de esta especie. Esta información será útil para establecer medidas para esta plaga.

Palabras Claves: insectos fitófagos; oruga bolillera Sudamericana; adaptación planta hospedera; compatibilidad reproductiva

Helicoverpa gelotopoeon (Dyar) (Lepidoptera: Noctuidae), the South American bollworm, belongs to the Heliiothinae complex together with *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), and *Chloridea virescens* (F.) (Lepidoptera: Noctuidae) in northwestern Argentina (Murúa et al. 2014, 2016). This species occurs throughout Argentina, Chile, southern Brazil, Paraguay, and Uruguay (Pastrana 2004; Navarro

et al. 2009). It is a polyphagous pest described as feeding on 10 families of host plants in nature (Pastrana 2004). Larvae principally damage pods and seeds of soybean and other legumes. It also affects cotton (*Gossypium hirsutum* L.; Malvaceae), alfalfa (*Medicago sativa* L.; Fabaceae), sunflower (*Helianthus annuus* L.; Asteraceae), soybean (*Glycine max* L.; Fabaceae), chickpea (*Cicer arietinum* L.; Fabaceae), and corn (*Zea mays* L.; Poaceae).

¹Instituto de Tecnología Agroindustrial del Noroeste Argentino, Estación Experimental Agroindustrial Obispo Colombres, Consejo Nacional de Investigaciones Científicas y Técnicas (ITANOA-EEAOC-CONICET), Las Talitas 4001, Tucumán, Argentina; E-mail: maria_inesherrero@hotmail.com (M. I. H.), sofiavfogliata@gmail.com (S. V. F.), ludami@eeaoc.org.ar (L. C. D.), gmurua@eeaoc.org.ar (M. G. M.)

²Estación Experimental Agroindustrial Obispo Colombres (EEAOC), Las Talitas 4001, Tucumán, Argentina; E-mail: acasmuz@eeaoc.org.ar (A. C.), ggastaminza@eeaoc.org.ar (G. G.)

*Corresponding author; Email: gmurua@eeaoc.org.ar; mgmurua@yahoo.com

According to Herrero et al. (2017), 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*. Currently, genetically modified plants expressing genes from the entomopathogenic bacterium *Bacillus thuringiensis* Berliner (Bacillaceae) (*Bt*) are available as an alternative strategy to control major lepidopteran pests. However, *H. 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).

Although several strategies have been proposed to delay resistance to *Bt* crops, the use of refuges has been recognized as an important component of resistance management for transgenic crops (Tabashnik 1994; Alstad & Andow 1995; Gould 1998). Refuges normally consist of host plants that do not produce *Bt* toxins to promote survival of susceptible individuals that would mate with the resistant ones from *Bt* plants. Nevertheless, for these individuals to be effectively considered as a source of susceptibility to resistance management, alternative hosts should contain a population able to randomly mate with individuals emerging from *Bt* crops (Martel et al. 2003; Tabashnik & Carrière 2008). Adaptation to different host plants might reduce gene flow between phytophagous insect populations, which could lead to reproductive isolation (Groot et al. 2011). Under these circumstances, a refuge would lose its function, since it wouldn't be possible for these populations to randomly mate with each other. Therefore, studies on genetic diversity and gene flow among populations of insect pests, and the possible exchange of alleles between host-associated and geographic distant populations are necessary to design resistance management strategies for this Heliiothinae complex.

In this context, a study made by Herrero et al. (2017) with *H. gelotopoeon* populations collected from chickpea in 2 regions of Argentina, showed similar biological and reproductive characteristics when reared on artificial diet in the laboratory. In addition, intra- and inter-population crosses revealed that *H. gelotopoeon* populations from neither region presented evidence of pre-zygotic or post-zygotic incompatibility, suggesting extensive gene flow between Tucumán (northwestern region) and Córdoba (Pampas region) populations of *H. gelotopoeon*. Other studies used molecular tools and documented unstructured genetic populations in other species of this genus collected on different hosts (Behere et al. 2007; Groot et al. 2011; Albernaz et al. 2012; Asokan et al. 2012). However, other authors found host-associated genetic differentiation in distinct species of the Heliiothinae complex (Subramanian & Mohankumar 2006; Behere et al. 2013). The objective of this study was to evaluate reproductive compatibility between 2 populations of *H. gelotopoeon* collected in soybean and alfalfa crops in northwestern Argentina.

Materials and Methods

INSECT COLLECTIONS AND IDENTIFICATION

Helicoverpa gelotopoeon larvae were collected from Dec 2015 to Jan 2016 in commercial soybean and alfalfa fields located in 2 counties of Tucumán province, Argentina. Collections were made on alfalfa in Vipos County (26.466666°S, 65.300000°W), and on soybean in San Agustín County (26.839166°S, 64.858888°W). At each sampling site, a minimum of 300 larvae (instars 2–5) were gathered and placed in-

dividually in glass tubes (12 cm high, 1.5 cm diam) with pieces of artificial diet. Collected larvae were taken to the laboratory and placed in breeding chambers under controlled conditions of 27 ± 2 °C, 70 to 75% relative humidity, 14:10 h (L:D) photoperiod until adult emergence. 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 Colombres, Tucumán, Argentina.

INSECT REARING

Approximately 250 adults (125 females, 125 males) were selected randomly from reared larvae collected in soybean and alfalfa to establish the experimental colony for each population. These adults were arranged in 5 cylindrical oviposition cages (40 cm high, 20 cm diam) lined with polyethylene bags with approximately 25 females and 25 males per population. 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 to 75% relative humidity, and 14:10 h (L:D) photoperiod. Both populations were reared according to the methodology described by Murúa et al. (2003) and Herrero et al. (2017). 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 d. Cages were checked daily for oviposition and adult mortality. Rearing of each population was maintained between Dec 2015 and May 2016.

Eggs deposited in the cages were collected and put into 750 mL plastic containers (Polituc®, Tucumán, Argentina). 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 (TodoDroga®, Córdoba, Argentina) (Murúa et al. 2003). Artificial diet was replaced every 2 to 3 d. As larvae pupated, pupae were sexed and placed in containers with moistened filter paper until adult emergence. Adults were used to establish a new generation. After establishing a colony for each population, individuals from the second to fourth generations were used for studies of fitness and reproductive compatibility.

BIOLOGY OF SOUTH AMERICAN BOLLWORM POPULATIONS FROM DIFFERENT HOST PLANTS

From each experimental colony of *H. gelotopoeon*, once emerged, 5 groups of neonate larvae were selected randomly (126 and 95 larvae from soybean and alfalfa, 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, 46 females and 46 males from the soybean population, and 33 females and 33 males from the alfalfa population were selected randomly to determine incubation period, longevity, and reproductive parameters. One 24-h-old virgin female and 1 virgin male from the same population were placed in cylindrical oviposition cages similar to those described above (25 cm high, 15 cm diam). These single pair matings represented the parental cross that was used for reproductive compatibility studies. Moths were maintained in this cage, and daily mortality and oviposition were recorded. Dead females were dissected to establish the presence of spermatophores in the

bursa copulatrix, which was considered evidence of copulation. Pre-oviposition, oviposition, and post-oviposition (number of d that a female survives after its last oviposition) period, 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 HOST PLANTS

A crossing experiment was performed to determine reproductive compatibility between soybean (S) and alfalfa (A) populations, according to the methodologies described by Herrero et al. (2017). To determine compatibility, a 24-h-old virgin female and virgin male were used. Four different types of crosses were performed: (i) parental crosses using parents from the same population, (ii) hybrid crosses using 1 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 cage was monitored for survival until pupation and then reared into adulthood. The parameters measured to determine compatibility were as follows: number of spermatophores, pre-oviposition, oviposition, and post-oviposition period duration, total fecundity, and total fertility.

DATA ANALYSIS

Fitness data (duration of egg, larval instars and pupal stage, and pupal mass (obtained 24 h after pupation) between both populations were compared by the 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 the Kruskal-Wallis (1952) test ($P < 0.05$).

For all of these 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. Statistical analyses were performed using IBM SPSS Statistics, version 22.0 (IBM 2013).

Results

BIOLOGICAL AND REPRODUCTIVE PARAMETERS

In total, 46 and 33 parental crosses were used to determine biological and reproductive parameters of soybean and alfalfa populations, respectively. The duration of each life stage and reproductive parameters are presented in Table 1.

Soybean Population

The incubation period ranged from 2 to 5 d (3.00 ± 0.06 [SE]). This population displayed 5 larval instars (the fifth instar was the longest), and completed this stage in 14.15 ± 0.14 d. Duration of the pupal stage was 11.35 ± 0.16 d, and longevity was 14.5 ± 0.99 and 19.39 ± 1.30 d for female and male, respectively. Sex ratio was 1.18:1 (F:M). The entire life span in this population lasted about 45.49 ± 0.92 d.

The number of spermatophores transferred per female was 1.11 ± 0.11 . The pre-oviposition, oviposition, and post-oviposition periods

duration were 4.33 ± 0.36 , 6.09 ± 0.52 , and 5.00 ± 0.69 d, respectively. Total fecundity was 330.51 ± 34 eggs per female, ranging from 0 to 770, and total fertility $77.57 \pm 4.98\%$ with a range of 0 to 100% hatching.

Alfalfa Population

The incubation period was 3.08 ± 0.07 and ranged from 2 to 4 d. As seen with soybean, this population displayed 5 larval instars, and completed this stage in 14.54 ± 0.15 d. Duration of pupal stage was 10.98 ± 0.19 d, and longevity 13.66 ± 1.16 and 15.65 ± 1.26 d for female and male, respectively. The sex ratio was 1:1.28 (F:M), and the entire life span duration was about 42.97 ± 0.88 , 2 d less than the soybean population.

The average number of spermatophores transferred per female was 1.39 ± 0.32 . Regarding the reproductive parameters, duration of the pre-oviposition, oviposition, and post-oviposition periods were 5.38 ± 0.47 , 5.19 ± 0.64 , and 4.19 ± 0.51 d, respectively. Total fecundity was 366.44 ± 0.76 eggs laid per female, ranging from 0 to 1,182 eggs. Total fecundity was $79.62 \pm 4.97\%$ with a range of 0 to 99.12 hatched eggs.

The biological parameters that presented significant differences between soybean and alfalfa populations were second instar larval development time ($W = 9,305$; $P = 0.003$), fourth instar larval development time ($W = 6,754$; $P = 0.02$) (these parameters were a longer duration for the alfalfa population), and life span ($W = 1,630$; $P = 0.032$), that was longer for the soybean population (Table 1). Reproductive parameters were not significantly different between populations.

REPRODUCTIVE COMPATIBILITY BETWEEN SOUTH AMERICAN BOLLWORM POPULATIONS

In total, 79 parental crosses, 42 hybrid crosses, 115 backcrosses, and 65 inter-hybrid matings were carried out between soybean and alfalfa populations (Table 2). Significant differences were found only in the post-oviposition period ($H = 13.35$; $P = 0.003$), while no significant differences were observed for those parameters that may indicate pre-zygotic and post-zygotic incompatibility between populations, such as presence and number of spermatophores, fecundity, or fertility (Table 2).

The lowest fecundity values resulted from inter-hybrid matings, but they were not low enough to conclude that significant differences existed in comparison to parental crosses. In addition, inter-hybrid matings showed the highest fertility when compared to the other types of crosses (Table 2). In general, all hybrid crosses, backcrosses, and inter-hybrid matings showed similar values to both parental crosses for all parameters (Tables 2, 3). Therefore, no indications of pre-zygotic or post-zygotic incompatibility was observed between these 2 populations.

Discussion

This study compared biological and reproductive traits, and mating compatibility between 2 *H. gelotopoeon* populations collected from different host plants in Tucumán, Argentina. This research complements previous contributions made by Herrero et al. (2017), who used *H. gelotopoeon* populations collected in 2 regions, but in the same crop in Argentina.

The results of the present study showed that the South American bollworm populations from soybean and alfalfa crops take approximately 45 and 43 d, respectively, to complete a single generation (from egg to adult) under laboratory conditions. A similar development time was observed by Herrero et al. (2017) for *H. gelotopoeon* and Ali et al. (2009) for *H. armigera*.

Table 1. Duration in days (mean \pm SE) of egg, larval (L1–L5), and pupal stages, pupal mass (mg), female and male longevity (d), sex ratio (F:M) and life span of *Helicoverpa gelotopoeon* populations collected in soybean and alfalfa crops in Tucumán, Argentina, and reared at 27 ± 2 °C, 70 to 75% RH, and 14:10 h (L:D) photoperiod.

Life cycle stages	Soybean population	n	Range	Alfalfa population	n	Range
Egg	3.00 \pm 0.06 a	229	2–5	3.08 \pm 0.07 a	894	2–4
L1	2.98 \pm 0.07 a	126	2–6	2.86 \pm 0.06 a	95	2–4
L2	2.02 \pm 0.05 a	106	1–4	2.26 \pm 0.07 b	86	1–4
L3	2.07 \pm 0.04 a	98	1–4	2.06 \pm 0.06 a	79	1–4
L4	2.32 \pm 0.07 a	90	1–4	2.77 \pm 0.13 b	73	1–9
L5	4.92 \pm 0.09 a	85	3–6	4.91 \pm 0.09 a	66	3–6
Overall larval stage	14.15 \pm 0.14 a	85	11–18	14.54 \pm 0.15 a	66	12–17
Pupa	11.35 \pm 0.16 a	59	8–15	10.98 \pm 0.19 a	56	8–14
Pupal mass	238.98 \pm 3.73 a	59	165–307	231.76 \pm 5.09 a	56	114–294
Female longevity	14.50 \pm 0.99 a	46	6–26	13.66 \pm 1.16 a	33	6–33
Male longevity	19.39 \pm 1.30 a	46	5–31	15.65 \pm 1.26 a	33	5–24
Life span	45.49 \pm 0.92 a	47	32–59	42.97 \pm 0.88 b	42	35–61
Sex ratio ♀:♂	1.18:1 a	59		1:1.28 a	56	
Spermatophores per female	1.11 \pm 0.11 a	46	0–3	1.39 \pm 0.32 a	33	0–5
Pre-oviposition period	4.33 \pm 0.36 a	33	1–9	5.38 \pm 0.47 a	26	1–10
Oviposition period	6.09 \pm 0.52 a	33	1–13	5.19 \pm 0.64 a	26	1–14
Post-oviposition period	5.00 \pm 0.69 a	33	1–19	4.19 \pm 0.51 a	26	0–10
Total fecundity	330.51 \pm 34 a	37	0–770	366.44 \pm 0.76 a	27	0–1,182
Total fertility	77.57 \pm 4.98 a	33	0–100	79.62 \pm 4.97 a	26	0–99.12

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

The duration of egg, larval, pupal, and adult stages, and sex ratio and number of larval instars were similar to those observed previously for *H. gelotopoeon* (Iannone & Leiva 1993; Navarro et al. 2009; Herrero et al. 2017) and *H. armigera* (Ali et al. 2009; Sharma et al. 2011; Jha et al. 2012; Amer & El-Sayed 2014; Reigada et al. 2016).

The number of spermatophores found in dissected females was generally 1 for both populations. Similar values were obtained by Herrero et al. (2017) for *H. gelotopoeon*, and by Coombs et al. (1993) for *H. armigera* and *Helicoverpa punctigera* (Lepidoptera: Noctuidae). Fecundity and fertility values were generally similar to those reported for *H. gelotopoeon* (Urretabizkaya et al. 2010; Herrero et al. 2017) and *H. armigera* (Nasreen & Mustafa 2000; Sharma et al. 2011).

Population parameters characterization is important because they may be affected by differences in host plant use. According to Matsubayashi et al. (2010), different plant species might differ in their chemistry and morphological structure; thus, selection might act in contrasting directions between populations of insects feeding on different host plant species, favoring opposing trait values on different hosts.

Helicoverpa gelotopoeon populations from soybean and alfalfa reared on artificial diet showed similar biological and reproductive traits. However, differences in second and fourth instar larval development time and life span were found. The duration of these parameters was higher for the alfalfa population, but the life span was longer for the soybean population (Table 1).

Silva et al. (2018) similarly characterized the immature stages of *H. armigera* populations from different regions and host plants (citrus, cotton, and corn) in Brazil. In this study, the mean development times of the larvae and pre-pupae from the 3 populations were similar, and mean life span (egg to adult) was not significantly different. They observed only small variations for some parameters, but they considered that some variation is commonly found in studies of Lepidoptera, and that the observed values represented normal variation.

Host-associated differentiation is widely recognized as a step towards speciation in phytophagous insects, because adaptations to different host plants often lead to reproductive isolation. Various pre-

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 soybean and alfalfa crops in Tucumán, Argentina, and reared at 27 ± 2 °C, 70 to 75% RH, and 14:10 h (L:D) photoperiod. (N) = sample size.

	Spermatophores per female	Pre-oviposition period (d)	Oviposition period (d)	Post-oviposition period (d)	Fecundity	Fertility (%)
Parental crosses	1.23 \pm 0.11 a (79)	4.79 \pm 0.29 a (59)	5.69 \pm 0.40 a (59)	4.64 \pm 0.44 b (59)	345.67 \pm 30.03 a (64)	78.47 \pm 3.51 a (59)
Hybrid crosses	1.07 \pm 0.13 a (42)	3.77 \pm 0.40 a (26)	5.73 \pm 0.51 a (26)	3.15 \pm 0.55 a (26)	376.41 \pm 53.38 a (31)	69.72 \pm 6.98 a (26)
Backcrosses	0.97 \pm 0.07 a (115)	4.60 \pm 0.30 a (72)	5.01 \pm 0.27 a (72)	3.13 \pm 0.27 a (72)	298.00 \pm 2.33 a (84)	76.87 \pm 3.16 a (58)
Inter-hybrid matings	1.18 \pm 0.10 a (65)	4.51 \pm 0.30 a (43)	4.95 \pm 0.43 a (43)	2.74 \pm 0.35 a (43)	250.26 \pm 34.26 a (53)	80.34 \pm 3.67 a (43)

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 soybean (S) and alfalfa (A) crops in Tucumán, Argentina, and reared at 27 ± 2 °C, 70 to 75% RH, and 14:10 h (L:D) photoperiod. (N) = sample size.

Type of crosses	♀	♂	No. paired	Mated female	Fecundity	Fertility
Parental crosses	S	S	46	36	339.69 \pm 34.21 (36)	77.57 \pm 4.98 (33)
Parental crosses	A	A	33	27	366.44 \pm 54.09 (27)	79.61 \pm 4.97 (26)
Hybrid crosses	S	A	16	13	317.38 \pm 87.27 (13)	58.6 \pm 13.23 (11)
Hybrid crosses	A	S	26	18	419.05 \pm 67.63 (18)	77.86 \pm 6.91 (15)
Backcrosses	A	F1 (A ♀ \times S ♂)	12	8	261.00 \pm 72.30 (8)	86.82 \pm 3.85 (6)
Backcrosses	A	F1 (S ♀ \times A ♂)	10	7	285.43 \pm 65.17 (7)	67.83 \pm 13.88 (6)
Backcrosses	S	F1 (A ♀ \times S ♂)	11	9	352.11 \pm 70.14 (9)	61.61 \pm 15.47 (9)
Backcrosses	S	F1 (S ♀ \times A ♂)	12	8	238.12 \pm 71.25 (8)	81.84 \pm 5.40 (6)
Backcrosses	F1 (A ♀ \times S ♂)	A	12	10	259.20 \pm 64.94 (10)	74.21 \pm 14.47 (8)
Backcrosses	F1 (A ♀ \times S ♂)	S	10	5	428.60 \pm 51.25 (5)	85.41 \pm 3.36 (5)
Backcrosses	F1 (S ♀ \times A ♂)	A	22	14	316.50 \pm 60.03 (14)	70.28 \pm 5.53 (14)
Backcrosses	F1 (S ♀ \times A ♂)	S	26	23	290.95 \pm 46.73 (23)	84.24 \pm 5.18 (18)
Inter-hybrid matings	F1 (S ♀ \times A ♂)	F1 (A ♀ \times S ♂)	14	11	344.36 \pm 99.73 (11)	82.86 \pm 5.25 (9)
Inter-hybrid matings	F1 (S ♀ \times A ♂)	F1 (S ♀ \times A ♂)	15	9	169.88 \pm 60.93 (9)	75.07 \pm 15.80 (6)
Inter-hybrid matings	F1 (A ♀ \times S ♂)	F1 (A ♀ \times S ♂)	21	20	236.50 \pm 45.36 (20)	81.30 \pm 4.66 (19)
Inter-hybrid matings	F1 (A ♀ \times S ♂)	F1 (S ♀ \times A ♂)	15	13	247.46 \pm 77.30 (13)	79.31 \pm 10.04 (9)

mating and post-mating barriers may reduce gene flow between host-associated populations (Danon et al. 2017).

Considering species from the genus *Helicoverpa*, Subramanian and Mohankumar (2006) made a study using microsatellites to provide insight into the genetic variability of *H. armigera* populations from 6 different host plants. They found strong genetic variability among *H. armigera* populations, indicating high similarity among populations collected from vegetable crops, whereas the population collected from the cotton crop was found to be more variable. They suggested that the genetic variation among these populations collected from different host plants may be due to host characteristics. This has importance for integrated pest management programs, because isolated populations can have different responses to control measures. In this regard, Shrivankumar and Jagdishwar Reddy (2004) found differences in susceptibility to different insecticides among *H. armigera* populations collected from 3 hosts: chickpea, tomato, and grapes, suggesting that these differences may be due to plant factors.

However, the occurrence of unstructured genetic populations has been reported for different species of the Heliiothinae complex (*H. armigera*, *C. virescens*, and *Chloridea* [*Heliiothis*] *subflexa* [Guenée]; Lepidoptera: Noctuidae). Most of these studies used molecular tools (Behere et al. 2007; Groot et al. 2011; Albernaz et al. 2012; Asokan et al. 2012). Only Vijaykumar et al. (2007) tested the extent of compatibility and fecundity of populations of *H. armigera* occurring in 8 different host plants. They found high cross compatibility among these populations, suggesting that these crops (especially chickpea and pigeonpea) could play an important role as natural refuges. Studies conducted by Green et al. (2003) and Ravi et al. (2005) also found that pigeonpea and chickpea may act as important sources of refuge for *Bt* cotton plantings.

In the present study, different crosses made between *H. gelotopoeon* populations from soybean and alfalfa crops in Tucumán, Argentina, did not present signs of pre-zygotic or post-zygotic incompatibility, suggesting that soybean and alfalfa populations of *H. gelotopoeon* can freely interbreed. This observation is consistent with that observed by Herrero et al. (2017) for the same species, but collected in different regions of Argentina.

These findings have direct implications for management, considering that populations from *H. gelotopoeon* coming from different crops and regions are capable of freely interbreeding without significant re-

duction in their reproductive capacity. On one hand, using alternative hosts as refuges for this pest may be considered as an option to provide sufficient susceptible individuals to interbreed with any resistant insects emerging from the *Bt* soybean. Also, the existence of gene flow between these populations indicates that if resistance to *Bt* or insecticides were to evolve in 1 of *H. gelotopoeon* populations, quick action would be necessary to deter the rapid spread of resistance genes over large geographical areas.

This study is the second record of a lack of reproductive isolation between 2 *H. gelotopoeon* populations, using reproductive parameters and mating compatibility in Argentina (Herrero et al. 2017). These results clearly indicate that there are no signs of host-associated isolation between these 2 populations. Therefore, considering previous studies of Herrero et al. (2017), populations of *H. gelotopoeon* from different regions and host plants can mate successfully. This lack of isolation allows gene exchange between these populations, which can be explained by the high mobility and generalist behavior of this species (Navarro et al. 2009). However, future studies should include more populations to be able to confirm if *H. gelotopoeon* can be considered a wide-ranging generalist species.

Acknowledgments

We thank Lucas Fadda at Estación Experimental Agroindustrial Obispo Colombres for excellent technical support and assistance in the collection of material. We also thank Andrew Michel (Department of Entomology, The Ohio State University, Columbus, Ohio) for critical review of the manuscript and valuable comments, as well as Eduardo Willink (Estación Experimental Agroindustrial Obispo Colombres) for constructive comments on an earlier draft of the manuscript. This study was supported by Estación Experimental Agroindustrial Obispo Colombres, the Agencia Nacional de Promoción Científica de Argentina, through the Fondo Nacional de Ciencia y Tecnología, Ministerio de Ciencia, Tecnología e Innovación Productiva (grants PICT/2015 No. 3109), Proyectos de Desarrollo Tecnológico y Social (PDTS No. 302), Consejo Nacional de Investigaciones Científicas y Técnicas, and Consejo de Investigaciones de la Universidad Nacional de Tucumán (PIUNT No. G638/1). This study is part of the first author's doctoral thesis.

References Cited

- Albernaz KC, Silva-Brandão KL, Fresia P, Cónsoli FL, Omoto C. 2012. Genetic variability and demographic history of *Heliothis virescens* (Lepidoptera: Noctuidae) populations from Brazil inferred by mtDNA sequences. *Bulletin of Entomological Research* 102: 333–343.
- Ali A, Choudhury RA, Ahmad Z, Rahman F, Khan FR, Ahmad SK. 2009. Some biological characteristics of *Helicoverpa armigera* on chickpea. *Tunisian Journal of Plant Protection* 4: 99–106.
- Alstad DN, Andow DA. 1995. Managing the evolution of insect resistance to transgenic plants. *Science* 268: 1894–1896.
- Amer AEA, El-Sayed AAA. 2014. Effect of different host plants and artificial diet on *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) development and growth index. *Journal of Entomology* 11: 1–7.
- Armes NJ, Jadhav DR, De Souza KR. 1996. A survey of insecticide resistance in *Helicoverpa armigera* in the Indian subcontinent. *Bulletin of Entomological Research* 86: 499–514.
- Asokan R, Rebijith KB, Krishna Kumar NK, Manamohan M. 2012. Genetic diversity of tomato fruit borer, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) inferred from mitochondrial cytochrome oxidase-I (mtCO-I). *Pest Management in Horticultural Ecosystems* 18: 29–34.
- Behere GT, Tay WT, Russel DA, Heckel DG, Appleton BR, Kranthi KR, Batterham P. 2007. Mitochondrial DNA analysis of field populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae) and of its relationship to *H. zea*. *BMC Evolutionary Biology* 7: 117. doi: 10.1186/1471-2148-7-117.
- Behere GT, Tay WT, Russell DA, Kranthi KR, Batterham P. 2013. Population genetic structure of the cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in India as inferred from EPIC-PCR DNA markers. *PLOS ONE*. doi: 10.1371/journal.pone.0053448 PMID: 23326431
- Bird LJ, Downes SJ. 2014. Toxicity and cross-resistance of insecticides to Cry2Ab-resistant and Cry2Ab-susceptible *Helicoverpa armigera* and *Helicoverpa punctigera* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 107: 1923–1930.
- Brévault T, Tabashnik BE, Carrière Y. 2015. A seed mixture increases dominance of resistance to *Bt* cotton in *Helicoverpa zea*. *Scientific Reports* 5: 9807. doi: 10.1038/srep09807.
- Coombs M, Del Socorro AP, Fitt GP, Gregg PC. 1993. The reproductive maturity and mating status of *Helicoverpa armigera*, *H. punctigera* and *Mythimna convecta* (Lepidoptera: Noctuidae) collected in tower-mounted light traps in Northern New South Wales, Australia. *Bulletin of Entomological Research* 83: 529–534.
- Danon G, Ben-Shlomo R, Keidar N, Dorchin N. 2017. Geographic and behavioural isolation promote the differentiation of parapatric host-associated forms in bud-galling midges (Diptera: Cecidomyiidae). *Biological Journal of the Linnean Society* 121: 163–173.
- Forrester NW. 1990. Designing, implementing and servicing an insecticide resistance management strategy. *Pesticide Science* 28: 167–179.
- Forrester NW, Cahill M, Bird LJ, Layland JK. 1993. Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Australia. *Bulletin of Entomological Research* 1: 1–132.
- Gao Y, Wu K, Gould F, Shen Z. 2009. Cry2Ab tolerance response of *Helicoverpa armigera* (Lepidoptera: Noctuidae) populations from Cry1Ac cotton planting region. *Journal of Economic Entomology* 102: 1217–1223.
- Gould F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Annual Review of Entomology* 43: 701–726.
- Gould F, Anderson A, Reynolds A, Bumgarner L, Moar W. 1995. Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. *Journal of Economic Entomology* 88: 1545–1559.
- Green WM, De Billot MC, Joffe T, Van Staden L, Bennet-Nel A, Du Toit CLN, Van Der Westhuizen L. 2003. Indigenous plants and weeds on the Makthini flats as refuge hosts to maintain bollworm population susceptibility to transgenic cotton (Bollgard). *African Entomology* 11: 21–29.
- Groot AT, Classen A, Inglis O, Blanco CA, López J, Vargas AT, Schal C, Heckel G, Schöfl G. 2011. Genetic differentiation across North America in the generalist moth *Helicoverpa virescens* and the specialist *H. subflexa*. *Molecular Ecology* 20: 2676–2692.
- Hardee DD, Adams LC, Solomon WL, Sumerford DV. 2001. Tolerance to Cry1Ac in populations of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae): three-year summary. *Journal of Agricultural and Urban Entomology* 18: 187–197.
- Herrero MI, Fogliata SV, Vera AM, Casmuz AS, Sosa Gómez D, Castagnaro AP, Gastaminza G, Murúa MG. 2017. Biological characterization and mating compatibility of *Helicoverpa gelatopoeon* (D.) (Lepidoptera: Noctuidae) populations from different regions in Argentina. *Bulletin of Entomological Research* 1–9.
- Iannone N, Leiva P. 1993. Bioecología, umbrales de acción y control de la isoca bolillera *Heliothis gelatopoeon* Dyar en soja. *Carpeta de producción vegetal*. Tomo 3, Serie soja, Información 114. EEA Pergamino, Buenos Aires, Argentina.
- IBM. 2013. IBM SPSS Statistics for Windows, Version 22.0. IBM Corp., Armonk, New York, USA.
- Jha RK, Chi H, Tang LC. 2012. Life table of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) with a discussion on Jackknife vs. Bootstrap techniques and variations on the Euler-Lotka equation. *Formosan Entomologist* 32: 355–375.
- Kruskal WH, Wallis WA. 1952. Use of ranks on one-criterion variance analysis. *Journal of the American Statistical Association* 47: 583–621.
- Lehmann EL. 1975. *Nonparametrics: Statistical Methods Based on Ranks*. Holden-Day, Inc., San Francisco, California, USA.
- Li GP, Wu KM, Gould F, Wang JK, Miao J, Gao XW, Guo YY. 2007. Increasing tolerance to Cry1Ac cotton from cotton bollworm, *Helicoverpa armigera*, was confirmed in *Bt* cotton farming area of China. *Ecological Entomology* 32: 366–375.
- Liu F, Xu Z, Zhu YC, Huang F, Wang Y, Li H, Gao C, Zhou W, Shen J. 2010. Evidence of field-evolved resistance to Cry1Ac-expressing *Bt* cotton in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in northern China. *Pest Management Science* 66: 155–161.
- Mahon RJ, Olsen KM, Garsia KS, Young SR. 2007. Resistance to *Bacillus thuringiensis* toxin Cry2Ab in a strain of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Australia. *Journal of Economic Entomology* 100: 894–902.
- Martel C, Réjasse A, Rousset F, Bethenod MT, Bourguet D. 2003. Host-plant-associated genetic differentiation in Northern French populations of the European corn borer. *Heredity* 90: 141–149.
- Matsubayashi KW, Ohshima K, Nosil P. 2010. Ecological speciation in phytophagous insects. *Entomologia Experimentalis et Applicata* 134: 1–27.
- Murúa MG, Virla E, Defagó V. 2003. Evaluación de cuatro dietas artificiales para la cría de *Spodoptera frugiperda* (Lepidoptera: Noctuidae) destinada a mantener poblaciones experimentales de himenópteros parasitoides. *Boletín de Sanidad Vegetal Plagas* 29: 43–51.
- Murúa MG, Cazado LE, Casmuz AS, Herrero MI, Villagrán ME, Vera A, Sosa-Gómez DR, Gastaminza G. 2016. Species from the *Heliothinae* complex (Lepidoptera: Noctuidae) in Tucumán, Argentina, an update of geographical distribution of *Helicoverpa armigera*. *Journal of Insect Science* 16: 1–7.
- Murúa MG, Scalora FS, Navarro FR, Cazado LE, Casmuz A, Villagrán ME, Lobos E, Gastaminza G. 2014. First record of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in Argentina. *Florida Entomologist* 97: 854–856.
- Nasreen A, Mustafa G. 2000. Biology of *Helicoverpa armigera* (Hübner) reared in laboratory on natural diet. *Pakistan Journal of Biological Sciences* 10: 1668–1669.
- Navarro FR, Saini ED, Leiva PD. 2009. Clave pictórica de polillas de interés agrícola, agrupadas por relación de semejanza. Primera edición. Instituto Nacional de Tecnología Agropecuaria, INTA. Estación Experimental Agropecuaria Pergamino y IMyZA – CNIA Castelar, Facultad de Cs Naturales e Instituto “Miguel Lillo,” Universidad Nacional de Tucumán, Buenos Aires, Argentina.
- Pastrana JA. 2004. Los lepidópteros Argentinos, sus plantas hospedadoras y otros sustratos alimenticios. Sociedad Entomológica Argentina Ediciones, Buenos Aires, Argentina.
- Pietrantonio PV, June TA, Parker R, Mott D, Siders K, Troxclair N, Vargas-Camplis J, Westbrook JK, Vassiliou VA. 2007. Detection and evolution of resistance to the pyrethroid cypermethrin in *Helicoverpa zea* (Lepidoptera: Noctuidae) populations in Texas. *Environmental Entomology* 36: 1174–1188.
- Ravi KC, Mohan KS, Manjunath TM, Head G, Patil BV, Angeline Greba DP, Premalatha K, Peter J, Rao NGV. 2005. Relative abundance of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on different host crops in India and the role of these crops as natural refuge for *Bacillus thuringiensis* cotton. *Environmental Entomology* 34: 59–69.
- Reigada C, Guimarães KF, Parra JRP. 2016. Relative fitness of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on seven host plants: a perspective for IPM in Brazil. *Journal of Insect Science* 16: 1–5.
- Reisig DD, Reay-Jones FPF. 2015. Inhibition of *Helicoverpa zea* (Lepidoptera: Noctuidae) growth by transgenic corn expressing *Bt* toxins and development of resistance to Cry1Ab. *Environmental Entomology* 44: 1275–1285.
- Sharma KC, Bhardwaj SC, Sharma G. 2011. Systematic studies, life history and infestation by *Helicoverpa armigera* on tomato in semi-arid region of Rajasthan. *Biological Forum: An International Journal* 3: 52–56.
- Silva IF, Lopes Baldin EL, Specht A, Sosa-Gómez DR, Roque-Specht VF, Morando R, Paula-Moraes SV. 2018. Biological and molecular characterization of the post-invasion immature stages of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Florida Entomologist* 101: 25–32.
- Shravankumar R, Jagdishwar Reddy D. 2004. Insecticide resistance to *Helicoverpa armigera* (Hübner) on three host crops. *Indian Journal of Plant Protection* 32: 136–138.

- Subramanian S, Mohankumar S. 2006. Genetic variability of the bollworm, *Helicoverpa armigera*, occurring on different host plants. *Journal of Insect Science* 26: 1–8.
- Tabashnik BE. 1994. Evolution of resistance to *Bacillus thuringiensis*. *Annual Review of Entomology* 39: 47–79.
- Tabashnik BE, Carrière Y. 2008. Evolution of insect resistance to transgenic plants, pp. 267–279 *In* Tilmon KJ [ed.], *Specialization, Speciation, and Radiation: The Evolutionary Biology of Herbivorous Insects*. University of California Press, Berkeley, California, USA.
- Tay WT, Mahon RJ, Heckel DG, Walsh TK, Downes S, James WJ, Lee SF, Reineke A, Williams AK, Gordon KHJ. 2015. Insect resistance to *Bacillus thuringiensis* toxin Cry2Ab is conferred by mutations in an ABC transporter subfamily A protein. *PLoS Genetics* 11: e1005534. doi: 10.1371/journal.pgen.1005534.
- Urretabizkaya N, Vasicek A, Saini E. 2010. Insectos perjudiciales de importancia agronómica. I. Lepidópteros. Instituto Nacional de Tecnología Agropecuaria (INTA). Universidad Nacional de Lomas de Zamora y Universidad Nacional de La Plata, Buenos Aires, Argentina.
- Velasco de Stacul M, Barral JM, Orfila RN. 1969. Taxonomía, especificidad y caracteres biológicos diferenciados del complejo de especies denominadas “oruga del capullo” del algodón, “oruga de la espiga” del maíz, “oruga del brote” del tabaco y “bolillera” del lino. *Revista de Investigaciones Agropecuarias, INTA, Bs. As., Rep. Argentina. Serie 5. Patología Vegetal* 6: 19–68.
- Vijaykumar FB, Krishnareddy KB, Kuruvinashetti MS, Patil BV. 2007. Mating compatibility among *Helicoverpa armigera* (Lepidoptera: Noctuidae) occurring on selected host plants and *Bt* cotton survivors. *Journal of Economic Entomology* 100: 903–908.