ECOSYSTEMS

Fitness and mating compatibility of *Chrysodeixis includens* (W.) (Lepidoptera: Noctuidae) populations collected in different provinces and crops in Argentina

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Abstract: *Chrysodeixis includens*, is an important Plusiinae pest that feeds on soybean, beans, and cotton. This species has a genetic structure determined by molecular markers in populations collected in soybean crops in Brazil and has developed resistance to insecticides. This had led to an increased interest in the genetic diversity of its populations in Argentina. This study aimed to characterize the fitness parameters and mating compatibility of geographically distant *C. includens* populations and on different host plants in Argentina. Intra- and inter-population crosses revealed that *C. includens* populations from Tucumán and Santiago del Estero provinces did not present any evidence of prezygotic and postzygotic incompatibilities, suggesting that both populations collected from soybean plants belonged to a single wide-ranging species. Prezygotic and postzygotic incompatibilities were detected between populations collected from different crops (soybean and bean). These incompatibilities may be caused by the different nutrients that each plant species renders, or the specific pest control strategies which may affect *C. includens* behavior. Studies of the genetic structure of *C. includens* populations based on mating isolation on other crops, as well as of the impact of management strategies on the specie, will increase understanding of the reproductive compatibility revealed by the present study.

Key words: Bean, fitness, mating compatibility, northern Argentina, soybean, soybean looper.

INTRODUCTION

The soybean looper *Chrysodeixis includens* Walker 1857 (Lepidoptera: Noctuidae), is a Plusiine pest that feeds of numerous crops plants of great economic value (Specht et al. 2015). Its origin is unclear, but it is recognized as a migratory species restricted to the Western hemisphere, from The United States of America (USA) in the north to southern South America (Navarro et al. 2009, Barrionuevo et al. 2012). *Chrysodeixis includens* is a polyphagous species that attacks beans (*Phaseolus vulgaris* L.), cotton (*Gossypium*

hirsutum L.), tobacco (*Nicotiana tabacum L.*), flax (*Linum usitatissimum* L.), sunflower (*Helianthus annuus* L.), alfalfa (*Medicago sativa* L.), and horticultural crops, among others (Navarro et al. 2009, Barrionuevo et al. 2012, Specht et al. 2015), but it shows preference for legumes, such as soybean (*Glycine max* L.). The period of greatest leaf consumption of *C. includens* in soybean, takes place after growth stage R3, which is a critical phase that directly impact on yield (Carter-Wientjes et al. 2004, Casmuz et al. 2015). In The USA, *C. includens* is the most destructive defoliation pest that attacks the soybean crop

(Moonga & Davis 2016). In Brazil, it has also become an important phytosanitary problem in soybean and cotton production since early 2000's (Moscardi et al. 2012, De Lima Viana et al. 2014, Yano et al. 2015), and their populations have been considered to be of growing importance for bean , due to the damage caused in this crop (Baldin et al. 2014).

Chrysodeixis includens is an important pest in Argentina from 33° south latitude northwards being present in the northwest and the northeast regions, Córdoba and Buenos Aires provinces (Navarro et al. 2009, Barrionuevo & San Blas 2015). In the 2013/2014 growing season, in soybean field of San Agustín (Tucumán province, Argentina) this species caused a 20.2% defoliation and a 19.7% yield reduction (Casmuz et al. 2014). In the last season 2017/2018 farmers of Rosario de la Frontera county (Salta province, Argentina), recorded outbreaks of this pest in bean crops above the level of the economic damage and have had to resort to three agrochemical sprays to their control. Currently, insecticides and genetically modified soybean and cotton that express the insecticidal protein Cry1Ac of *Bacillus thuringiensis* are used in Argentina to control *C. includens*. In The USA, resistance to insecticides (organophosphates, carbamates and pyrethroids) and control failures with IGR (insect growth regulator) insecticides (methoxyfenozide) (Owen et al. 2013) have been reported for *C. includens* (Felland et al. 1990, Higley & Boethel 1994, Mascarenhas & Boethel 2000). Casmuz et al. (2014) also indicated that the mixture of pyrethroid insecticides with organophosphates had no control over *C. includens* in soybean in Tucumán (Argentina). Moreover, Mascarenhas et al. (1998) found that *C. includens* field-collected larvae from U.S. had a lower susceptibility to the *Bacillus thuringiensis* variety *kurstaki* insecticide as compared to a reference laboratory population.

Additionally, some insect populations identified as the same species may yet display different biological and genetic traits and show reproductive isolation (cryptic species), preventing complete gene flow among populations as consequence of the presence of prezygotic and postzygotic barriers (Drès & Mallet 2002, Bickford et al. 2007). Therefore, identifying cryptic species is important not only to explain the evolution of phytophagous insects, but also to understand the effect of susceptibility of pest populations to different control tactics (Pérez Contreras 1999, Rull et al. 2012, Joyce et al. 2014). It has led to an increased interest in fully understanding *C. includens* genetic diversity and gene flow between geographically distant populations of *C. includens*, and between populations collecting in different crops. Palma et al. (2015) carried out the first molecular characterization of *C. includens* populations in soybean crops from Brazil with molecular markers of the Inter Simple Sequence Repeat (ISSR) type, determining the existence of genetic structuring in populations which was not related to geographical distances. Also, structured genetic populations have been reported for other Plusiinae species, the cabbage looper *Trichoplusia ni* Hübner 1800-1803 (Lepidoptera: Noctuidae) (Zhu et al. 1997, Evenden et al. 2002, Franklin et al. 2010). These studies revealed variation in their pheromone composition and the presence of haplotypes.

The objective of this study was to characterize biological, reproductive parameters, and mating compatibility of *C. includens* populations collected in different host plant and geographical distance in Argentina, to determine whether this pest comprises a complex of cryptic species, or simply consist of a single wide-ranging species.

Thus, *C. includens* specimens were collected from soybean crops in Tucumán and Santiago del Estero provinces, to establish the influence

of geographic distance and from bean crop in Salta province, to establish the influence of host plant association on reproductive isolation. This research provides information about reproductive compatibility of soybean loopers, which has remained undocumented until now. This information will be useful for understanding reproductive compatibility among *C. includens* populations, which is necessary for designing sustainable strategies to control this species in Argentina.

MATERIALS AND METHODS

Insect sampling

Soybean looper larvae were collected during March and April 2016 in the northern Argentina. Larvae were collected from soybean crops in Tucumán (7 de Abril, 26° 20' 27.99" S, 64° 30' 47.46" W) and Santiago del Estero provinces (Sacháyoj, 26° 41' 23.63'' S, 61° 49' 41.23'' W). In Salta province, larvae were collected from bean crops (Rosario de la Frontera, 25° 56' 53.91" S, 65° 6' 57.63" W). The three sampling points are located within the Chacoan Province Region, according to the Biogeographical regionalization of the Neotropical region proposed by Morrone (2014).

At each sampling site, a minimum of 300 larvae (instars 3-6) were collected using a vertical cloth as described by Drees & Rice (1985). Each larva was placed in a plastic tube (12 cm tall and with a 1.5 cm diameter) containing one soybean or bean leaf. The larvae were taken to the laboratory and kept in breeding chambers under controlled conditions (at 27 ± 2 ºC, 70-75% relative humidity and 14:10 h light:dark photoperiod). All larvae were examined following procedures described by Barrionuevo & San Blas (2015) to confirm the species. After the emergence of adults a voucher specimens from each population were deposited in the insect collection of Sección Zoología Agrícola, at Estación Experimental Agroindustrial Obispo Colombres (EEAOC) (Tucumán, Argentina).

Insect rearing

Approximately 180 adults (90 females and 90 males) were randomly selected among the larvae collected, and reared to establish an experimental colony for each population. Approximately 30 female and 30 males were placed in cylindrical oviposition cages (40 cm tall and with a 20 cm diameter), lined with polyethylene bags. For aeration, both ends of the cage were covered with a nylon cloth. The cages were placed on plastic trays lined with moistened polyurethane foam. Each population was maintained in the same chamber under identically controlled conditions (at 27 ± 2 ºC, 70 - 75% relative humidity and a 14:10 h light:dark photoperiod). Food was provided via a cotton plug saturated with a mixture of honey and water (1:1), which was replaced every day. Cages were checked daily for oviposition and adult mortality. Eggs were collected daily from the cages and placed in 1000 ml plastic containers lined with moistened filter paper. Once they had emerged, neonate larvae were placed individually in glass tubes and fed on an artificial larval diet that included bean 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 (Todo Droga®, 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 cup containers with filter paper, until adults emerged. Adults were used to initiate a new generation. After

establishing a colony for each population, F1 generation of field-collected strains was used for the fitness study, and F2 generation was used to begin the parental crosses in the mating compatibility study. The following generations for the mating compatibility studies (F3-F5) were originated from the parental crosses.

Fitness of soybean looper populations

From each experimental *C. includens* colony, 200 eggs were randomly selected to analyze the following parameters: adult sex ratio, duration of egg, instars larval and pupal stages, and pupal mass (obtained 24 h after pupation). Adult sex ratio was determined in pupal stage according to the Angulo & Weigert's methodology (1985).

Among the adults obtained, couples (females and males) were randomly selected to determine reproductive parameters. One virgin female and one virgin male (24 hours old) were selected from each population and placed in cylindrical oviposition cages, similar to those described above (40 cm tall and with a 20 cm diameter).

Dead females were dissected to establish the presence and number of spermatophores present in their reproductive tract, to determine whether mating had occurred. Duration of pre-oviposition, oviposition, and post-oviposition periods, fecundity (number of eggs deposited by a female during her entire life period), fertility (percentage of hatched eggs) and adult longevity were recorded.

Reproductive compatibility between soybean looper populations

Two crossing experiments were performed to determine reproductive compatibility between *C. includens* populations: 1) between populations distant geographically and collected from soybean crop (Tucumán and Santiago del Estero provinces); and 2) between populations collected from different host plants (soybean and bean crops from Tucumán and Salta provinces, respectively).

The crossings were performed following the methodologies described by Herrero et al. (2017). To determine compatibility, one virgin female and one virgin male (both being 24 hours old) were used. Four different types of crosses were performed: i) parental crosses using parents from the same population (in this case, single pair mating from the fitness studies were used); ii) hybrid crosses using one parent from different populations; iii) backcrosses, some with the female progenitor as the recurrent parent, and others with the male progenitor as the recurrent parent; and iv) interhybrid 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 as follows: presence and number of spermatophores per female, preoviposition, oviposition and postoviposition period duration, total fecundity, and total fertility.

Data analysis

Fitness data from different populations were compared by Kruskal–Wallis (1952) test ($P > 0.05$), because they did not show normal distribution. For the reproductive compatibility studies, due to the high number of combinations, the performance of all parental crosses was compared with that deriving from other types of crosses (hybrid crosses, backcrosses, and interhybrid mating), using Kruskal–Wallis (1952) test ($P > 0.05$). For all of these studies, the data of duration of preoviposition, oviposition and postoviposition periods were compared in the case of females that had laid eggs, whereas fecundity values were compared considering all females, including those that had not laid

eggs. For fertility, females that had laid eggs but had no spermatophores were not included. For the number of spermatophores, females that had none in their tract were included as a value representing copula failure in all cross types. All data were analyzed using InfoStat 2016 software (Di Rienzo et al. 2008).

RESULTS

All adults from the three sampled populations were identified as *C. includens* based on the morphology of their larvae (Barrionuevo & San Blas 2015).

Fitness of *C. includens* populations

In total, 135, 137 and 124 individuals from Tucumán, Salta and Santiago del Estero populations, respectively, were used in this study. The life stage and reproductive parameters are presented in Table I. Significant differences were detected in the duration of the L1, L2, L3, L5 and L6 instars larval, duration of overall larval stage and pupal stages. The duration of L1, L2 and L6 instars larval from Salta population collected in bean crop (4.04, 3.05 and 5.55 days, respectively) were significantly longer than those from Tucumán (2.86, 2.37 and 4.06 days, respectively) and Santiago del Estero (2.19, 2.35 and 4.31 days, respectively) populations collected in soybean crop (L1: H= 251.61, *P*= <0.0001; L2: H= 82.99, *P*= <0.0001 and L6: H= 92.89, *P*= <0.0001). However, the duration of L3 instar larval from Salta population (1.32 days) was significantly low than others populations of Tucumán and Santiago del Estero (1.94 and 2.04 days, respectively) (H= 85.70, *P*= <0.0001). The L5 instar larval from Tucumán and Salta populations (3.14 and 2.84 days, respectively) were significantly longer than from Santiago del Estero population (2.67 days) (H= 7.55, *P*= 0.0134). The duration of overall

larval stage from Salta population collected in bean crop (18.38 days), was significantly longer than others population collected from soybean crop (14.7 days for both populations) (H= 208.22, *P*= <0.0001). Duration of the pupal stage from Santiago del Estero and Salta populations (8.6 days for both) was significantly longer than from Tucumán populations (7.78 days) (H= 59.90, *P*= <0.0001). The duration of the preoviposition period from Santiago del Estero population (5.13 days) was significantly longer than from Salta and Tucumán populations, in decreasing order (4.58 and 3.25 days, respectively) (H= 9.39, *P*= 0.0074). No significant differences were detected between the remaining fitness and reproductive parameters (Table I).

Reproductive compatibility between *C. includens* populations distant geographically and collected from the same crop

In total, 43 parental crosses, 43 hybrid crosses, 153 backcrosses and 63 interhybrid matings were made between Tucumán and Santiago del Estero populations (Table II). Significant differences were found in the duration of preoviposition (H= 9.07, *P*= 0.0225), and postoviposition periods (H= 7.91, *P*= 0.0407), fecundity (H= 20.58, *P*= 0.0001) and fertility rates (H= 18.83, *P*= 0.0003) (Tables II and IV). The results obtained in the four types of crosses, show a successful matings between Tucumán and Santiago del Estero populations.

Reproductive compatibility between *C. includens* populations collected from different crops

In total, 37 parental crosses and 60 hybrid crosses were made between Tucumán and Salta populations collected in soybean and bean crops, respectively (Table III). Significant differences were found in preoviposition period duration (H= 5.84, *P*= 0.0141), fecundity (H= 8.27, *P*= 0.0033) and fertility rates (H= 12.22, *P*= 0.0003),

Table I. Biological and reproductive parameters of *Chrysodeixis includens* populations, collected in Tucumán, Santiago del Estero and Salta provinces in Argentina.

Life cycle stages	Tucumán population (soybean)	N	Range	Santiago del Estero population (soybean)	N	Range	Salta population (bean)	N	Range
Egg	4,19 ± 0,05a	135	3-6	4,09 ± 0,06a	124	3-5	4,12 ± 0,05a	137	3-5
L1	2,86 ± 0,05b	135	2-5	2,19 ± 0,04a	124	2-4	4,04 ± 0,05c	135	3-8
L2	2,37 ± 0,06a	131	1-5	2,35 ± 0,05a	122	2-4	3,05 ± 0,05b	131	2-5
L3	1,94 ± 0,05b	126	1-4	2,04 ± 0,05b	122	1-9	1,32 ± 0,06a	128	1-4
L4	2,13 ± 0,06a	126	1-5	2,13 ± 0,05a	119	1-4	2,23 ± 0,06a	128	1-5
L5	3,14 ± 0,15b	126	1-10	2,67 ± 0,15a	119	1-15	2,84 ± 0,13b	127	1-14
L6	4,06 ± 0,11a	71	2-7	4,31 ± 0,11a	90	3-9	5,55 ± 0,09b	114	3-13
Overall larval stage	14,75 ± 0,13a	126	11-22	14,72 ± 0,13a	118	12-22	18,38 ± 0,15b	124	16-28
Pupa	7,78 ± 0,08a	122	6-10	8,65 ± 0,07b	114	7-10	8,61 ± 0,08b	105	7-11
Pupal mass	0,25 ± 0,003a	122	0,14 – 0,32	0,25 ± 0,0028a	114	0,11 -0,30	0,26 ± 0,0023a	105	0,12 -0,30
Female longevity	9,06 ± 0,65a	18	4-15	8,24 ± 0,46a	25	5-12	7.89 ± 0,59a	19	3-19
Male longevity	9,00 ± 0,72a	18	5-14	7,84 ± 0,50a	25	4-12	7.63 ± 0,58a	19	3-18
Sex ratio ♀:♂	0,9:1a	122		0,8:1a	114		0,8:1a	105	
Spermatophores per female	1,78 ± 0,33a	18	0-5	1,32 ± 0,19a	25	0-4	1,05 ± 0,16a	19	0-3
Preoviposition period	3,25 ± 0,39a	12	1-6	5,13 ± 0,35b	16	2-8	4,58 ± 0,45ab	12	3-8
Oviposition period	4,50 ± 0,72a	12	1-8	2,81 ± 0,53a	16	1-8	3,83 ± 0,71a	12	1-8
Postoviposition period	1,58 ± 0,65a	12	0-7	1,00 ± 0,27a	16	0-3	1,50 ± 0,42a	12	0-4
Fecundity	416,69 ± 110,27a	16	0-1337	274,05 ± 68,18a	21	0-1015	306,19 ± 83,37a	16	0-1145
Fertility	48,10 ± 9,10a	12	0-86,37	54,74 ± 7,69a	16	0-100	55,36 ± 10,33a	12	0-100

Duration in days (mean ± SE) of egg, larval (L1-L6), and pupal stages, pupal mass (g), female and male longevity (days), sex ratio (F:M), number of spermatophores per female, duration of preoviposition, oviposition and postoviposition periods (days), fecundity (number of eggs per female), and fertility (%).
Values followed by same letters within a row are not significantly different according to Kruskal–Wallis test (P>0.05).

and number of spermatophores per female (H= 17.83, *P*= <0.0001). The low fecundity (an average of 103.4 eggs per female) and fertility (6.4%) rates recorded for the hybrid crosses did not allow making backcrosses and interhybrids crosses between Tucumán and Salta populations. Spermatophores per female, fecundity and fertility parameters showed higher values in parental crosses than in hybrid crosses. Such values pointed to the existence of postzygotic incompatibility. In this study, hybrid crosses resulted in 27 of 60 females (45%) not carrying spermatophores in their reproductive tracts, which is a common and practical approach to quantify mating success (Table III and V). This analysis also revealed prezygotic incompatibility (Rhainds 2010).

DISCUSSION

This study compared biological, reproductive parameters and mating compatibility between *C. includens* populations from different geographically distance and from different hosts in Argentina. This study is the first report of mating compatibility among *C. includens* populations in Argentina.

The results of this study showed that under laboratory conditions, soybean looper populations from Salta collected in bean crop complete a single generation (from egg to adult) in approximately 35 days, longer than Santiago del Estero and Tucumán (31 and 32 days, respectively), collected in soybean crop. Similar results were reported by Combe & Pérez (1978). A life cycle duration of 43 days was recorded by Barrionuevo et al. (2012) and 38-42 days by Moonga & Davis (2016).

Egg stage duration was similar to those reported in previous studies (Canerday & Arant 1967, Combe & Peréz 1978, Navarro et al. 2009, Barrionuevo et al. 2012).

Chrysodeixis includens generally completes larval stage in six instars (Moscardi et al. 2012). However, 56%, 76% and 88% of the insects from Tucumán, Santiago del Estero and Salta, respectively, presented only six instars. The population from Salta had the highest percentage of individuals that molted for six instars. Shour & Sparks (1981) observed a range from five to

Table II. Mean ± SE (N) of parental crosses, hybrid crosses, backcrosses and interhybrid mating of Tucumán and Santiago del Estero *Chrysodeixis includens* populations.

	Parental crosses*	Hybrid crosses	Backcrosses	Interhybrid mating
Spermatophores per female	1,51 ± 0,18a (43)	1,16 ± 0,19a (43)	1,29 ± 0,07a (153)	1,54 ± 0,16a (63)
Preoviposition period	4,32 ± 0,31a (28)	4,80 ± 0,45ab (20)	5,02 ± 0,13b (99)	4,57 ± 0,15a (47)
Oviposition period	3,54 ± 0,45a (28)	4,25 ± 0,73a (20)	2,94 ± 0,18a (99)	3,79 ± 0,29a (47)
Postoviposition period	1,25 ± 0,32a (28)	2,35 ± 0,52ab (20)	2,13 ± 0,18b (99)	1,64 ± 0,21ab (47)
Fecundity	335,73 ± 61,61b (37)	189,61 ± 46,05a (33)	201,52 ± 15,95ab (134)	338,72 ± 26,23b (50)
Fertility	51,89 ± 5,80 ab (28)	37,16 ± 8,57a (20)	59,62 ± 2,98 b (97)	72,40 ± 3,81c (47)

* Is the average of the values of Tucumán and Santiago del Estero populations.
Values followed by same letters within a row are not significantly different according to Kruskal Wallis test (*P* > 0.05).

seven instars larval, with a predominance of six instars when the larvae were fed on an artificial diet. The differences recorded in larval stage duration and number of instars may be due to the components of the artificial diet, and the rearing conditions (Barrionuevo et al. 2012). The duration of the overall larval stage from Tucumán and Santiago del Estero populations was in agreement with Moonga & Davis (2016) and Canerday & Arant (1967) who used soybean leaves and artificial diet to breed the larvae, respectively. The duration of the overall larval stage from Salta population was similar to that reported by Combe & Pérez (1978) and Boldt et al. (1975). Besides, all the populations had shorter larval stage duration than reported by Barrionuevo et al. (2012) on artificial diet.

The pupal stage duration in Tucumán population, was similar to those recorded by Moonga & Davis (2016), Navarro et al. (2009), Moscardi et al. (2012) and Canerday & Arant (1967). Pupal stage from Salta and Santiago del Estero populations was similar to that reported

by Barrionuevo et al. (2012). The pupal mass was similar to the one reported in other studies (Canerday & Arant 1967, Jensen et al. 1974, Boldt et al. 1975). By contrast, Kidd & Orr (2001) and Moonga & Davis (2016) reported a lower pupal mass using soybean, cotton, cowpea (*Vigna unguiculata* L.) and sweet potato (*Ipomoea batatas* L.) leaves as larval diet.

Adult longevity for three populations was lower than reported by Combe & Peréz (1978) and Canerday & Arant (1967), but similar to those recorded by other authors (Jensen et al. 1974, Barrionuevo et al. 2012). In this study, it was found that females emerged before males, suggesting that *C. includens* is a protogyny species. No previous studies have reported this female behavior. The sex ratio found was of 0.8/0.9 F – 1 M. Barrionuevo et al. (2012) and Combe & Pérez (1978) reported a sex ratio with more females than males.

Number of spermatophores per female was generally 1 for the three populations, with a

Table III. Mean ± SE (N) of parental crosses, hybrid crosses, backcrosses and interhybrid mating of Tucumán and Salta *Chrysodeixis includens* population.

	Parental crosses*	Hybrid crosses	Backcrosses**	Interhybrid mating**
Spermatophores per female	1,41 ± 0,19a (37)	0,5 ± 0,08b (60)	-	-
Preoviposition period	4,04 ± 0,33a (24)	5,37 ± 0,40b (19)	-	-
Oviposition period	4,28 ± 0,49a (24)	3,00 ± 0,62a (19)	-	-
Postoviposition period	1,48 ± 0,37a (24)	2,58 ± 0,54a (19)	-	-
Fecundity	361,44 ± 68,72a (32)	103,41 ± 41,54b (27)	-	-
Fertility	51,73 ± 6,78a (24)	6,41 ± 4,14b (14)	-	-

* Is the average of the values of the Tucumán and Salta populations.
**The low values of spermatophores per female, fecundity and fertility prevented carrying out the blackcrosses and Hybrid.
Values followed by same letters within a row are not significantly different according to Kruskal Wallis test with comparisons of pairs (*P* > 0.05).

range from 1 to 5. This observation was similar to reported by Jensen et al. (1974).

The duration of preoviposition period from Tucumán and Salta populations was similar to those recorded by Canerday & Arant (1967) and Combe & Pérez (1978). The duration of this period for Santiago del Estero population was higher than those reported (Canerday & Arant 1967, Combe & Pérez 1978). The oviposition period was similar to that reported by Barrionuevo et al. (2012). No previous studies have examined *C. includens* postoviposition period.

Fecundity was similar to those reported by Barrionuevo et al. (2012) and Jensen et al. (1974); but it was lower than those reported by other authors (Combe & Pérez 1978, Kidd & Orr 2001). Total fertility for all populations, was higher than reported by Moonga & Davis (2016) and lower than reported by Barrionuevo et al. (2012).

The results about mating compatibility between *C. includens* populations from Tucumán and Santiago del Estero) collected in soybean crop, did not show signs of geographical isolation. The presence of spermatophores in bursa copulatrix of females, and fecundity and fertility values, recorded in the different types of crosses, demonstrated successfully mating in both directions. This may account for the long-range migratory capacity of this species, whose presence has been widely documented in the American continent (Pogue 2005, Navarro et al. 2009). Palma et al. (2015) carried out the first molecular characterization of *C. includens* populations in soybean crops in Brazil and determined the existence a low but significant genetic differentiation among populations not related to geographical distance. The successful mating between *C. includens* populations from soybean crops presented in this study support the results reported by Palma et al (2015). The present study can contribute to the understanding of gene flow among *C. includens*

individuals from different provinces, a knowledge that is necessary to plan management programs aimed at offsetting resistance to Bt crops and insecticides (Grimi et al. 2018). Transgenic cotton and soybean crops that express Cry1Ac insecticidal proteins have been commercialized in Argentina since 1998 and 2012, respectively, and *Chrysodeixis includens* is one of the target pests of these technologies (Massoni et al. 2015). In 2017, Bt soybean was produced on 3.08 million hectares and Bt cotton on 250.000 hectares in Argentina. Currently the area cultivated with Bt crops is increasing rapidly and Argentina ranks third in planted biotech crops, with a total of 23.9 million hectares (ISAAA 2018). Furthermore, the soybeans that express a single insecticidal protein Cry1Ac, did not fully meet the high-dose concept for *C. includens*, which is one of the most important parameters in IRM programs (Bernardi et al. 2012).

Mating compatibility between Tucumán and Salta populations collected in soybean and bean crops, respectively, showed lower values of fecundity (103.4 eggs per female) and fertility (6.4%) parameters, spermatophores per female (0.5) and percentage mated female (41.9 and 48.3 %) in hybrid crosses than in parental crosses (Table IV). Also, these low values put off the possibility of making subsequent backcrosses and interhybrid mating of this study (Table V). These results suggested the presence of a barrier of gene flow between *C. includens* populations from soybean and bean which may be based on the different nutrients each plant species (Pashley 1988, Murúa et al. 2008) or pest control strategies used in the fields that would affect the behavior of this species (Palma et al. 2015). Besides, in Argentina, bean production is located in the northwest region, with the Salta province covering 44% of total national bean production, which makes it the largest producer in the country (Ministerio de Agroindustria de

Table IV. Detailed results of parental crosses, hybrid crosses, backcrosses and interhybrid mating of Tucumán (S) and Santiago del Estero (E) *Chrysodeixis includens* populations.

Type of crosses	♀	♂	N° paired	Mated female	Mated %	Fecundity mean ± SE (N)	Fertility % ± SE (N)
Parental crosses	S	S	18	16	88,9	416,69 ± 110,27 (16)	48,10 ± 9,10 (12)
Parental crosses	E	E	25	21	84.0	274,05 ± 68,18 (21)	54,74 ± 7,69 (16)
Hybrid crosses	S	E	21	15	71.4	134,80 ± 55,07 (15)	39,42 ± 14,32 (7)
Hybrid crosses	E	S	22	18	81.8	235,28 ± 70,48 (18)	35,94 ± 11,1 (13)
Backcrosses	S	F_1 (S♀ x E♂)	14	13	92.9	201,08 ± 47,90 (13)	41,27 ± 10,74 (10)
Backcrosses	E	F_1 (S♀ x E♂)	18	17	94.4	286,71 ± 40,85 (17)	72,66 ± 6,66 (14)
Backcrosses	S	F_1 (E♀ x S♂)	19	18	94.7	244,11 ± 44,25 (18)	62,49 ± 7,95 (15)
Backcrosses	E	F_1 (E♀ x S♂)	27	25	92.6	205,00 ± 47,52 (25)	54,97 ± 7,35 (16)
Backcrosses	F_1 (S♀ x E♂)	S	13	13	100	164,31 ± 38,04 (13)	58,73 ± 12,57 (10)
Backcrosses	F_1 (S♀ x E♂)	E	15	13	86.7	147,77 ± 55,31 (13)	49,36 ± 8,40 (8)
Backcrosses	F_1 (E♀ x S♂)	S	20	15	75,0	216,67 ± 41,84 (15)	64,81 ± 8,14 (11)
Backcrosses	F_1 (E♀ x S♂)	E	27	20	74,1	134,5 ± 32,17 (20)	64,70 ± 5,67 (13)
Interhybrids mating	F_1 (S♀ x E♂)	F_1 (S♀ x E♂)	24	20	83.3	246,40 ± 27,50 (20)	53,86 ± 7,18 (19)
Interhybrids mating	F_1 (S♀ x E♂)	F_1 (E♀ x S♂)	7	6	85.7	488,17 ± 48,69 (6)	78,79 ± 2,77 (6)
Interhybrids mating	F_1 (E♀ x S♂)	F_1 (S♀ x E♂)	8	5	62.5	423,80 ± 82,65 (5)	83,26 ± 6,85 (5)
Interhybrids mating	F_1 (E♀ x S♂)	F_1 (E♀ x S♂)	24	19	79.2	366,32 ± 49,43 (19)	87,68 ± 2,20 (17)

Argentina 2016). This concentration of bean plantations may be promoting physiological adaptations of this pest to the crop. However, additional research based on reproductive compatibility studies of *C. includens* populations from other crops, and the effect of management strategies will further deepen our understanding of the mating isolation found in the present study, and clarify the possible existence of host races.

Table V. Detailed results of parental crosses, hybrid crosses, backcrosses and interhybrid mating of Tucumán (S) and Salta (B) *Chrysodeixis includens* populations.

Type of crosses	♀	♂	N° paired	Mated female	Mated %	Fecundity mean ± SE (N)	Fertility % ± SE (N)
Parental crosses	S	S	18	16	88,9	416,69 ± 110,27 (16)	48,10 ± 9,1 (12)
Parental crosses	B	B	19	16	84,2	306,19 ± 83,37 (16)	55,36 ± 10,33 (12)
Hybrid crosses	S	B	31	13	41.9	147,69 ± 69,52 (13)	11,08 ± 6,97 (8)
Hybrid crosses	B	S	29	14	48.3	62,29 ± 47,35 (14)	0,18 ± 0,18 (6)

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