

Reproductive isolation between two populations of *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae) from different host plant species and regions in Argentina

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Abstract

The sugarcane borer, *Diatraea saccharalis* (F.), has a widespread distribution throughout the Western Hemisphere and is a pest of many crop plants including sugarcane, corn, sorghum and rice. The use of *Bacillus thuringiensis* (Bt) corn has been the primary tool for managing this species in corn fields. Sugarcane borer control has been recently threatened by observations of susceptibility and/or resistance to certain varieties of Bt corn and the protein used in many newer varieties. This has led to increased interest in understanding sugarcane borer genetic diversity and gene flow within and among its populations and the consequent exchange of alleles between geographically distant populations. The objective of this study was to examine reproductive compatibility between host-associated geographic populations of *D. saccharalis* in Argentina and to determine whether this pest represents a complex of host-associated cryptic species rather than a wide ranging generalist species. Intra and inter-population crosses revealed that *D. saccharalis* populations from the north-western and Pampas regions presented evidence of prezygotic and postzygotic incompatibility. Such a result is likely to be the product of an interruption of gene flow produced by either geographic or host plant associated isolation, suggesting that Tucumán (northwestern) and Buenos Aires (Pampas) populations of *D. saccharalis* are a distinct genotype and possibly an incipient species.

Keywords: sugarcane borer, fitness, reproductive compatibility, cryptic species, insect resistance management

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Introduction

Specialized phytophagous insects are by far the most diverse and abundant group of animals on earth (May, 2000).

Within insects, and particularly within two of the major insect orders (Coleoptera and Lepidoptera), several taxa are currently undergoing rapid radiation. Herbivorous insect radiation has been found to track angiosperm radiation, and has been proposed to be driven by adaptation to novel host plants (Farrell, 1998). Agricultural development has resulted in wide scale introduction of novel host plants to native environments. In many cases, phytophagous insect ‘species’, formerly thought to be generalists, are composed of complexes of

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morphologically and genetically uniform cryptic species exhibiting marked biological differences associated with the ability to exploit particular plants (Bickford *et al.*, 2007). Cryptic species are often the result of local adaptation to plants differing in chemical content, phenology or other structural and physiological components (Bickford *et al.*, 2007). Recent host plant driven radiation is difficult to track based on morphological or molecular methods (Frey *et al.*, 2013) and can be inferred through examination of host plant-associated biological features of insect populations and through the evolution of reproductive isolation between demes exploiting different host plants (Frey *et al.*, 2013). Cryptic species identification is important not only to explain phytophagous insect radiation but also because it has profound implications in insect pest management (Rull *et al.*, 2012).

The sugarcane borer, *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae), is currently thought to be a dominant stalk borer species in corn that is widely distributed in the Western Hemisphere throughout much of South America, Central America, the Caribbean and the southern USA (Dyar & Heinrich, 1927; Box, 1931, 1951, 1956). The host plants of *D. saccharalis* are numerous and include sugarcane (*Saccharum* spp.), corn (*Zea mays* L.), sorghum (*Sorghum bicolor* L.) and rice (*Oryza sativa* L.) (Long & Hensley, 1972; Joyce *et al.*, 2014). Damage caused by *D. saccharalis* larvae feeding in cultivated host plants includes a reduction of plant biomass and increased susceptibility to plant pathogens by providing points entry (Capinera, 2001; Salvatore *et al.*, 2010; Alves Lopes *et al.*, 2014). In northwestern Argentina, *D. saccharalis* is the most damaging sugarcane pest and causes considerable losses in sugar and ethanol production (Willink 1982; Salvatore *et al.*, 2010). By contrast in central Argentina (Pampas region), it is the most important pest in corn and sorghum and also causes high annual losses (Aragón, 2002; Iannone, 2002; Serra & Trumper, 2006).

Since 1999, use of *Bacillus thuringiensis* (Bt) corn has been the primary tool for managing this species in corn fields. Sugarcane borer control has been recently threatened by observations of susceptibility and/or resistance to certain varieties of Bt corn and the protein used in many newer varieties (Huang *et al.*, 2006, 2007a, b; ASA, 2014). This has led to increased interest in understanding sugarcane borer genetic diversity and gene flow within and among its populations and the consequent exchange of alleles between geographically distant populations (Joyce *et al.*, 2014). Although *D. saccharalis* apparently has a broad geographic distribution in addition to a wide host plant range, it is treated as a single species. The occurrence of distinct genetic structure of sugarcane borer populations has been confirmed in several regions of North and South America. Most of these studies used molecular tools. Lange *et al.* (2004) tested six populations of *D. saccharalis* and concluded that this species clustered into two groups: México and South America, and the Caribbean and southern USA. The differences could reflect two dispersal events (presumably human-assisted), one to the north and east and one to the south from an original evolution on grasses such as a wild ancestor of maize in southern México. Joyce *et al.* (2014) used two molecular markers (amplified fragment length polymorphism (AFLP) and cytochrome oxidase subunit I (COI) sequences) to examine variation in the population structure of *D. saccharalis* in the southern USA. They found a genetically distinct cluster of *D. saccharalis* from Florida that was different than those collected in Louisiana and Texas. They also found the existence of a *D. saccharalis* cryptic species. Previous studies with

D. saccharalis populations from Brazil and Colombia revealed variation in their pheromone composition and covariation in haplotypes (Cortés *et al.*, 2010). The average ratios of the two main sex pheromone components for Colombia were significantly different from those exhibited in Brazil populations. Colombia populations showed the highest genetic divergence from the most common haplotype.

The objective of this study was to examine reproductive compatibility between host-associated geographic populations of *D. saccharalis* in Argentina and to determine whether this pest represents a complex of host-associated cryptic species rather than a wide ranging generalist species. We addressed this question by collecting *D. saccharalis* in the northwestern and Pampas regions and studying biological, reproductive parameters and mating compatibility. This information will be useful to define the management strategies and control of *D. saccharalis* populations in Argentina.

Materials and methods

Insect collections

Sugarcane borer larvae were collected in February 2014 from commercial fields in two Argentine geographical regions. In the northwestern region, collections were made on sugarcane in La Cocha county (S:27°50'4.6", W:65°30'58.7") (Tucumán province) and in the Pampas region, collections were on conventional corn (non Bt) in Pergamino county (S:33°51'51.6", W:60°39'32.3") (Buenos Aires province). At each sampling site, a minimum of 200 larvae (instars 3–6) were gathered and placed individually in glass tubes (12 cm high and 1.5 cm diameter) with pieces of stalks of corresponding host plants. Collected larvae were taken back to the laboratory and placed in breeding chambers under controlled conditions (25 ± 2°C, 70–75% RH, 14L:10D photoperiod) until adult emergence. Then, all adults that emerged were examined using taxonomic morphological markers to confirm the species identity. 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, 350 adults (175 females and 175 males) were randomly selected from larvae collected in sugarcane and corn to establish the experimental colony for each population. These adults were arranged in 10 oviposition cages with approximately 20 females and 20 males per population. Each population was maintained in the same chamber under identically controlled conditions at 25 ± 2°C, 70–75% RH and a photoperiod of 14L:10D. Rearing of each population was carried out between February and May of 2014.

Cylindrical oviposition cages made out of plastic mesh (200 cm high and 150 cm diameter) were lined with polyethylene bags as an oviposition substrate. 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. Adult food was provided via a cotton plug saturated with a mixture of honey and water (1:1 vol:vol), which was replaced daily. Cages were checked daily for oviposition and adult mortality.

Eggs were collected daily with a moistened brush and deposited in Petri dishes lined with moistened filter paper.

Once emerged, neonate larvae were placed individually in glass tubes with 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), vitamin supplement amino acids (Ruminal®, Buenos Aires, Argentina) and methylparaben (Todo Droga®, Córdoba, Argentina) (Murúa *et al.*, 2003). Diet was replaced every 2–3 days. As larvae pupated, pupae were sexed and placed in cup containers with moistened filter paper until adults emerged. After establishing a colony for each population, individuals from the 2nd to the 5th generation were used for studies of fitness and inter-populations reproductive compatibility.

Fitness of sugarcane borer populations

For each experimental population colony, ten groups of neonate larvae were randomly selected (total 80 larvae of each population) to analyze the following parameters: duration of larval instars and pupal stage, pupal mass (obtained 24 h after pupation) and adult sex ratio.

Groups of approximately 15 females and 30 males for each population were randomly selected to determine egg duration, longevity and reproductive parameters. To promote female mating success, 1 virgin female and 2 virgin males (24 h old) from the same population were placed in cylindrical oviposition cages similar to those described above (30 cm high and 15 cm diameter). These cages were labeled as Triple cages (Tucumán population, $N = 12$; Buenos Aires population $N = 15$). This was the parental cross that was used for reproductive compatibility studies. Moths were maintained in this cage, and daily mortality and oviposition were recorded until the 3 moths died. Dead females were dissected to establish the number of spermatophores present in their reproductive tract to determine whether or not mating had occurred. Preoviposition, oviposition and postoviposition period duration (days that the female survives after carrying out last oviposition), total fecundity (number of eggs deposited by a female during her entire life period), egg duration, total fertility (percentage of eggs hatching) and adult longevity were recorded.

Reproductive compatibility between sugarcane borer populations

A crossing experiment was performed to determine reproductive compatibility between Tucumán (T, northwestern region) and Buenos Aires (B, Pampas region) populations, according to the methodologies described by Pashley & Martin (1987); Pashley *et al.* (1990); Lopez-Edwards *et al.* (1999) and Murúa *et al.* (2008).

To determine compatibility the Triple cage method described above was used. 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 (backcrosses) and (iv) interhybrid mating crosses between F_1 hybrids from different populations. A subset of larval progeny (F_1) from each fertile triple cage crossing was monitored for survival to pupation and then reared to adulthood. The parameters measured to determine compatibility were as follows: number of spermatophores, preoviposition, oviposition and postoviposition period duration, total fecundity and total fertility.

Data analysis

Set data were tested for normality using the Shapiro–Wilk test. Only data on spermatophores per female, preoviposition, oviposition and postoviposition period duration, total fecundity and adult longevity that did not show normal distribution or homogeneity of variance were transformed to square root ($x + 0.5$). Data expressed as percentages and total fertility, were converted to arcsine ($x/100^{0.5}$).

For the fitness studies, data were analyzed using a *t*-test ($P < 0.05$) to detect differences between sugarcane borer populations. For the reproductive compatibility studies, due to the high number of combinations, the performance of all parental crosses was compared with the results of other type of crosses (hybrid crosses, backcrosses and interhybrids mating) using analysis of variance. Multiple comparisons among the means for the different type of crosses were performed with a Tukey's test.

For all of these studies, preoviposition, oviposition and postoviposition duration 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 the data were analyzed using InfoStat (2006).

Results

Morphological identification of D. saccharalis populations

All adults from the northwestern and Pampas regions sampled (Tucumán and Buenos Aires provinces, respectively) were identified as *D. saccharalis* based on the morphology of male genitalia (Dyar & Heinrich, 1927).

Biological and reproductive parameters

In total, 12 and 15 parental crosses were used to determine biological and reproductive parameters of T and B populations, respectively. The duration of each life stage and reproductive parameters are presented in table 1.

The biological parameter that presented significant differences between populations was the longer duration of the larval stage for the Tucumán population ($t = 2.3$; $df = 145$; $P < 0.0001$ (table 1). Duration of L1 ($t = -6.13$; $df = 128$; $P < 0.0001$) was higher for the Buenos Aires population than for the Tucumán population, while duration of L5 ($t = 3.26$; $df = 148$; $P < 0.0001$) was longer for the Tucumán population (table 1). Reproductive parameters were not significantly different between populations.

Reproductive compatibility between sugarcane borer populations

In total, 27 parental crosses, 85 hybrid crosses, 202 backcrosses and 91 interhybrids mating were carried out between the T and B populations (table 2). Significant differences were found for all parameters evaluated. In general, all hybrid crosses, backcrosses and interhybrids mating showed lower values than both parental crosses for all parameters (table 2).

According to the analysis of hybrid crosses, backcrosses and interhybrids mating, evidence of prezygotic and postzygotic incompatibility were detected (table 3). Hybrid crosses showed that 239 of 378 females (63%) did not carry spermatophores in their reproductive tract, a common and practical approach to quantify mating success (Perfectti, 2002; Rhainds,

Table 1. Biological and reproductive parameters of *Diatraea saccharalis* populations collected in Tucumán and Buenos Aires provinces in Argentina and reared at 25 ± 2°C, 70–75% RH and 14L:10D.

Life cycle stages	Tucumán population (T)	<i>n</i>	Range	Buenos Aires population (B)	<i>n</i>	Range
Egg	8.15 ± 0.56a	2521	5–16	6.85 ± 0.38a	3366	4–12
L1	3.73 ± 0.2a	80	1–12	6.3 ± 0.4b	80	2–24
L2	5.47 ± 0.24a	76	2–11	5 ± 0.31a	74	1–17
L3	5.24 ± 0.3a	76	1–14	5.82 ± 0.27a	71	2–13
L4	4.82 ± 0.23a	76	2–12	5.01 ± 0.17a	71	2–12
L5	5.91 ± 0.41b	74	2–19	4.36 ± 0.15a	69	3–9
L6	6.11 ± 0.57	35	2–17	0	0	0
Overall larval stage	27.9 ± 0.95b	80	1–45	24.42 ± 0.68a	79	6–34
Pupa	5.47 ± 0.23a	70	3–10	5.21 ± 0.2a	68	2–11
Pupal mass	80 ± 2.5a	70	30–120	80 ± 2.7a	68	40–140
Female longevity	7.08 ± 0.4a	12	2–10	5.20 ± 0.4a	15	2–7
Male longevity ¹	6.13 ± 0.55a	12	2–10	5.0 ± 0.3a	15	2–8
Sex ratio ♀:♂	1: 1.03	70		1: 1.44	68	
Spermatophores per female	1.1 ± 0.08a	12	1–2	1 ± 0a	15	1–1
Preoviposition period	1.1 ± 0.58a	12	0–2	1.07 ± 0.07a	15	1–2
Oviposition period	2.58 ± 0.15a	12	2–3	2.27 ± 0.12a	15	2–3
Postoviposition period	3.75 ± 0.45a	12	0–6	2.4 ± 0.34a	15	1–5
Total fecundity	323 ± 30.82a	12	40–376	266.8 ± 20.64a	15	127–394
Total fertility	90.4 ± 1.1a	12	80–94	86.42 ± 3.13a	15	51–97

Duration in days (mean ± SE) of egg, larval (L1–L6) and pupal stages, pupal mass (mg), female and male longevity (days), sex ratio (F:M), number of spermatophores per female, duration of preoviposition, oviposition and postoviposition periods (days), total fecundity and total fertility (%).

Values followed by the same letters within a row are not significantly different according to Student's *t*-test ($P > 0.05$).

¹Male longevity was estimated from an average of two males.

Table 2. Number (mean ± SE) of spermatophores per female, duration of preoviposition, oviposition and postoviposition periods, total fecundity (number of eggs per female), and total fertility (percentage of egg hatch) of *Diatraea saccharalis* parental crosses, hybrid crosses, backcrosses and interhybrid matings using populations collected in Tucumán and Buenos Aires provinces in Argentina and reared at 25 ± 2°C, 70–75% RH and 14L:10D.

	Spermatophores per female	Preoviposition period (d)	Oviposition period (d)	Postoviposition period (d)	Fecundity	Fertility
Parental crosses ¹	1 ± 0.05b (27)	2.3 ± 0.08c (27)	2.41 ± 0.12c (27)	3 ± 0.23b (27)	251.59 ± 10.27c (27)	88.2 ± 3.98b (27)
Hybrid crosses	0.47 ± 0.05a (85)	1.83 ± 0.07b (36)	1.67 ± 0.1b (36)	1.75 ± 0.2a (36)	87.4 ± 8.44b (40)	86.06 ± 3.44b (36)
Backcrosses	0.37 ± 0.03a (202)	1.48 ± 0.09b (25)	1.16 ± 0.12ab (25)	1.44 ± 0.24a (25)	14.26 ± 6.29a (72)	72.67 ± 11.93ab (3)
Interhybrid mating	0.3 ± 0.05a (91)	1 ± 0.25a (3)	1 ± 0.35a (3)	3.33 ± 0.7b (3)	1.96 ± 10.27a (27)	65 ± 4.13a (25)

Values followed by same letters within a column are not significantly different according to Tukey's test ($P < 0.05$).

¹Parental crosses included data of both population (A × A and B × B).

2010). Considering successful crosses (139), the average of mated females ranged from 0.21 to 0.68, and carried one or two spermatophores.

Interhybrids mating using F_1 (B♀ × T♂) and F_1 (T♀ × B♂) individuals, resulted in unsuccessful matings (table 3). In total 75 of 139 mated females did not lay eggs. In the rest of the females (64), average fecundity was 32.9 ± 4.61 eggs/female and the fertility ranged from 10 to 100% (table 3).

Discussion

This study compared biological and reproductive demographic traits and mating compatibility between two *Diatraea saccharalis* populations collected in corn and sugarcane in two regions of Argentina.

The sugarcane population from Tucumán and corn population from Buenos Aires reared on artificial diet, showed

similar biological and reproductive characteristics. However, the Tucumán population displayed longer larval stage duration than that of the Buenos Aires population. Results of our study showed that *D. saccharalis* populations from Tucumán and Buenos Aires provinces take approximately 47 and 41 days, respectively, to complete a single generation (from egg to adult) under laboratory conditions. Egg stage duration for both populations was similar to other reports (Wongsiri & Randolph, 1962; Greco, 1995; Navarro *et al.*, 2009, but see King *et al.*, 1975) under similar conditions. Six and five larval instars of Tucumán and Buenos Aires populations, respectively, were found. Wongsiri & Randolph (1962); King *et al.* (1975); Melo (1984); Machado Botelho (1985); Greco (1995) and Navarro *et al.* (2009) observed a minimum of five larval instars in general, and some of them, reported a few individuals undergoing six or seven larval instars. Our results with respect to larval development were similar to those

Table 3. Number (mean \pm SE) of mated females, total fecundity (number of eggs per female), and total fertility (percentage egg hatch) of each *Diatraea saccharalis* cross and backcross using populations collected in Tucumán and Buenos Aires provinces in Argentina and reared at $25 \pm 2^\circ\text{C}$, 70–75% RH and 14L:10D.

Type of crosses	♀	♂	N° paired ¹	Mated female	Fecundity	Fertility
Parental crosses	B	B	15	15	266.8 \pm 20.6 (15)	86.4 \pm 3.1 (15)
Parental crosses	T	T	12	12	323 \pm 30.82 (12)	90.4 \pm 1.1 (12)
Hybrid crosses	B	T	42	21	76.76 \pm 13 (21)	82.4 \pm 5.4 (18)
Hybrid crosses	T	B	43	19	99.16 \pm 18.1 (19)	9.9 \pm 1.7 (17)
Backcrosses	B	F ₁ (B♀ \times T♂)	26	7	2.86 \pm 2.86 (7)	20 \pm 0 (1)
Backcrosses	B	F ₁ (T♀ \times B♂)	26	16	11.87 \pm 3.59 (15)	39.6 \pm 7.4 (7)
Backcrosses	T	F ₁ (B♀ \times T♂)	22	7	0 (7)	–
Backcrosses	T	F ₁ (T♀ \times B♂)	25	17	19.24 \pm 6.46 (17)	79.6 \pm 6.0 (8)
Backcrosses	F ₁ (B♀ \times T♂)	B	28	6	26 \pm 19.89 (6)	72 \pm 6.0 (3)
Backcrosses	F ₁ (B♀ \times T♂)	T	20	3	0 (3)	–
Backcrosses	F ₁ (T♀ \times B♂)	B	25	5	10 \pm 10 (5)	44 \pm 0 (1)
Backcrosses	F ₁ (T♀ \times B♂)	T	30	12	24.67 \pm 10.63 (12)	86.2 \pm 4.1 (5)
Interhybrids mating	F ₁ (T♀ \times B♂)	F ₁ (B♀ \times T♂)	18	7	7.57 \pm 3.73 (7)	72.8 \pm 3.8 (3)
Interhybrids mating	F ₁ (T♀ \times B♂)	F ₁ (T♀ \times B♂)	25	7	0 (7)	–
Interhybrids mating	F ₁ (B♀ \times T♂)	F ₁ (B♀ \times T♂)	26	9	0 (9)	–
Interhybrids mating	F ₁ (B♀ \times T♂)	F ₁ (T♀ \times B♂)	22	4	0 (4)	–

¹N° paired refers a 'triple cage' with two males and one female.

reported by Wongsiri & Randolph (1962) and Greco (1995) but duration was shorter than that reported by King *et al.* (1975) and Navarro *et al.* (2009). Pupal stage duration was also shorter than reported by other studies (Wongsiri & Randolph, 1962; King *et al.*, 1975; Greco, 1995; Navarro *et al.*, 2009). Pupal mass values obtained for both populations were lighter than those reported by King *et al.* (1975). These differences could be attributed to different artificial diet or different host plants used (King *et al.*, 1975; Bessin & Reagan, 1990; Murúa *et al.*, 2003; Murúa & Virla, 2004). Adults from the sugarcane populations had a similar survival time as those reported by Wongsiri & Randolph (1962); King *et al.* (1975); Greco (1995) and Navarro *et al.* (2009). The sex ratio found for the *D. saccharalis* population from Tucumán was similar to that reported by Bessin & Reagan (1990) (1F:1 M) but the sex ratio for the Buenos Aires population was slightly biased toward males (1F: 1.44 M).

The number of spermatophores found in dissected females was generally one for both populations. This observation was reported by Peairs & Saunders (1980).

The periods of preoviposition, oviposition, postoviposition and fertility were similar, but fecundity differed compared with other reports (Wongsiri & Randolph, 1962; King *et al.*, 1975; Greco, 1995). King *et al.* (1975) and Bessin & Reagan (1990) reported higher fecundity of *D. saccharalis* than those recorded in our study. However our fecundity results were similar to those found by Wongsiri & Randolph (1962) and Greco (1995).

Intra and inter-population crosses revealed that *D. saccharalis* populations from the northwestern and Pampas regions in Argentina presented evidence of prezygotic and postzygotic incompatibility. Such a result is likely to be the product of an interruption of gene flow produced by either geographic or host plant-associated isolation, suggesting that Tucumán and Buenos Aires populations of *D. saccharalis* are possibly an incipient species. As it has been mentioned, the occurrence of distinct genetic structure of sugarcane borer populations has been confirmed in several regions of North and South America. Most of these studies used molecular tools (Lange *et al.*, 2004; Cortés *et al.*, 2010; Joyce *et al.*, 2014) and only Box (1951, 1956), and Pashley *et al.* (1990) reported morphological, physiological and behavioral differences among sugarcane

populations. Morphological variation (size and color) has been reported within *D. saccharalis* populations in México and Venezuela and it was attributed to differences in host plant affiliation (Box, 1951, 1956). Pashley *et al.* (1990) found that populations of *D. saccharalis* from Louisiana and México are genetically divergent from those in Brazil, and likely consist of two distinct species. However, crosses between Louisiana and Brazilian populations revealed no differences among parental, interhybrid and backcrosses. These authors suggested that while reproductive barriers do inhibit the development of hybrids, the fitness of these hybrids may be compromised in nature. Successful interbreeding of laboratory colonies does not necessarily imply that there are no reproductive isolating mechanisms in nature.

Studies by Prieto *et al.* (2008) with Tucumán populations of *D. saccharalis*, indicated that this species prefers sugarcane instead of corn when both crops are present in the same time and place. However, Parody (2011), using molecular markers (AFLP and random amplified polymorphic (RAPDs)), showed that the level of genetic structure of different sugarcane borer populations in Argentina is low and that most of the genetic diversity is at the individual level within populations and did not find a relationship with the host plant where the insects were collected. According to these results, she suggested that in the context of evolution of resistance to Bt corn, the lack of host race differentiation would suggest no mating restriction among insects from different host plants or crops, which could serve as a non-structural refuge. Although results from the Parody (2011) study contradict ours, the Parody (2011) study found inconsistencies in the data analysis with the molecular markers (AFLP and RAPDs). Use of COI sequences for a preliminary analysis showed that there was genetic structure of sugarcane borer populations. In another study, Alves Lopes *et al.* (2014) using RAPD polymorphisms found higher genetic diversity within populations than among populations, suggesting that gene flow occurs more between populations than within populations.

This study is the first record of reproductive isolation between *D. saccharalis* populations from Argentina using reproductive parameters and mating compatibility. Our data show evidence of reproductive isolation possibly as a result of

interbreeding or migration interruption between the two populations, suggesting that Tucumán and Buenos Aires populations of *D. saccharalis* are distinct genotypes and possibly cryptic species.

In opposition to results mentioned by Parody (2011) and Alves Lopes *et al.* (2014), and according to Prieto *et al.* (2008), it is possible that *D. saccharalis* contains morphologically indistinguishable subpopulations that are defined by their host plant preferences as exemplified by *Spodoptera frugiperda* (J. E. Smith) and *Rhagoletis pomonella* (Walsh) (Bush, 1969; Pashley *et al.*, 1985; Pashley, 1986). Future studies should further examine the genetic structure of *D. saccharalis* populations because of the implications for management of resistant insects to transgenic crops. The management decision for a natural refuge will depend of host race preferences for specific host plants to ensure mating among insects emerging from Bt and non Bt crops. The ability to characterize and identify genotypes of *D. saccharalis* will improve management efforts against this pest. Additional research on the population genetics of sugarcane borer in other regions and crops from Argentina will further increase our understanding of its geographic population structure and clarify the possible existence of host races or cryptic species.

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