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Source: Florida Entomologist, 102(2) : 347-352

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.102.0209

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Spodoptera frugiperda (Lepidoptera: Noctuidae) strains from northern Argentina: esterases, profiles, and susceptibility to *Bacillus thuringiensis* (Bacillales: Bacillaceae)

*Flavia del Valle Loto[1], Alfonso Emanuel Carrizo[2], Cintia Mariana Romero[1,3], Mario Domingo Baigorí[1,3], and Licia María Pera[1,]**

Abstract

Spray products based on *Bacillus thuringiensis* (*Bt*) (Berliner) (Bacillales: Bacillaceae) are widely used as control agents of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), in some major crops such as corn, cotton, and soy. However, there is little information about the toxicity of *Bt* formulations to control corn and rice-infesting *S. frugiperda* strains. These *S. frugiperda* strains are morphologically indistinguishable, so they are genetically identified as corn and rice *S. frugiperda* strains. The main objective of this study was to evaluate the susceptibility of third instar larvae of both *S. frugiperda* biotypes to either the native *Bt* RT or the reference *Bt* HD1 subsp. *kurstaki* (*Btk*) formulations. In addition, the *S. frugiperda* populations were characterized by their esterase profile in polyacrylamide gels. This study also allowed differentiation of the *S. frugiperda* strains. Concerning the susceptibility assays, a significant difference in mortality values was found within some corn or rice *S. frugiperda* colonies when *Bt* RT treatment was used, whereas there were no significant differences in *Btk* HD1 toxicity within colonies that share the same biotype. When toxicity of either the native *Bt* RT or the reference *Btk* HD1 to each *S. frugiperda* biotype was compared, significant differences in mortality values also were found in the corn and the rice *S. frugiperda* strains. Finally, the statistical analysis of the mortality data revealed that there was a significant difference between the 2 *S. frugiperda* strains, regardless of the *Bt* preparation. These results show that the *S. frugiperda* control could depend not only on the bioinsecticide used, but also on the *S. frugiperda* biotype being treated.

Key Words: fall armyworm; biotypes; esterases; biocontrol; *Bt* formulation; mortality

Resumen

Los insecticidas preparados a base de *Bacillus thuringiensis* (*Bt*) (Berliner) (Bacillales: Bacillaceae) son muy utilizados para el control de *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) en cultivos de gran importancia, tales como el maíz, el algodón y la soja. Sin embargo, hay poca información acerca de la toxicidad de estas formulaciones hacia las cepas maíz y arroz de *S. frugiperda*, las cuales son morfológicamente indistinguibles, y por ello, son identificadas utilizando técnicas de biología molecular. El principal objetivo de este trabajo fue evaluar la susceptibilidad de larvas del tercer estadio de ambos biotipos de *S. frugiperda* hacia formulados elaborados con la cepa nativa *Bt* RT o con la cepa de referencia *Bt* HD1 subsp. *kurstaki* (*Btk*). Asimismo, ambas poblaciones de *S. frugiperda* fueron caracterizadas teniendo en cuenta el perfil de esterasas en geles de polyacrilamnida permitiendo su identificación. Con respecto a los ensayos de susceptibilidad, en los tratamientos realizados con *Bt* RT se observaron diferencias significativas entre los datos de mortalidad de algunas de las colonias correspondientes al biotipo maíz o al biotipo arroz. Pero cuando se utilizó el formulado a base de *Btk* HD1, no se detectó una diferencia significativa entre los valores de mortalidad de las poblaciones que comparten el mismo biotipo de la plaga. Por otro lado, cuando se comparó la toxicidad de la cepa nativa *Bt* RT o la cepa de referencia *Btk* HD1 hacia cada biotipo de *S. frugiperda*, se encontraron diferencias significativas en los valores de mortalidad tanto en la cepa maíz como arroz de *S. frugiperda*. Finalmente, el análisis estadístico de los valores de mortalidad revela que hubo una diferencia significativa entre los dos biotipos de la plaga, independientemente de la preparación de *Bt* utilizada. Estos resultados muestran que el control de *S. frugiperda* podría depender no sólo del bioinsecticida utilizado sino también del biotipo de la plaga que se desea combatir.

Palabras Clave: oruga militar tardía; biotipos; esterasas; control biológico; formulados *Bt*; mortalidad

Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a generalist insect that attacks important crops using a diversity of plants as hosts. Two *S. frugiperda* biotypes that show host-plant associated genetic variation have been described. The rice strain is associated with rice (*Oryza sativa* L.; Poaceae) and bermudagrass (*Cynodon dactylon* [L.] Pers.; Poaceae), whereas the

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corn strain predominates on corn (*Zea mays* L.; Poaceae), sorghum (*Sorghum bicolor* [L.] Moench.; Poaceae), and cotton (*Gossypium* spp.; Malvaceae) (Pashley 1986). More recently, Prowell et al. (2004) reported that although host specificity generally is strong, asymmetries in host fidelity occurs, showing a wider range of host use in the rice *S. frugiperda* strain. The presence of both *S. frugiperda* biotypes was reported from southern Canada to Argentina (Starratt & McLeod 1982; Busato et al. 2004; Prowell et al. 2004; Virla et al. 2008; Juárez et al. 2012). These strains also are sympatric and morphologically identical (Pashley et al. 1985). However, there is some evidence that the *S. frugiperda* strains can be differentiated by their wing morphometrics (Cañas-Hoyos et al. 2014). In addition, the corn and rice *S. frugiperda* strains produce different female sex pheromone blends (Groot et al. 2008) and they vary, not only in the timing of the reproductive traits, but also in their temporal patterns of locomotor activity (Schöfl et al. 2009). Nagoshi & Meagher (2004) reported that the 2 *S. frugiperda* strains could display a markedly different response to seasonal environmental cues as well. Besides host specificity, the *S. frugiperda* biotypes also are identified by molecular techniques, such as allozyme markers (Prowell et al. 2004), PCR-RFLP (restriction fragment-length polymorphism) of the mitochondrial COI (cytochrome oxidase subunit I) gene (Levy et al. 2002), AFLP (amplified fragment-length polymorphism) (McMichael & Prowell 1999; Prowell et al. 2004), tandemly repeated DNA sequences (Lu et al. 1994), etc. In addition, the genome of both biotypes was recently sequenced showing that individuals of corn and rice *S. frugiperda* populations were significantly different from each other; specifically, the authors found significant number variations in detoxification and digestion genes between the *S. frugiperda* strains, which are consistent with differential adaptation to different host-plant ranges (Gouin et al. 2017).

The use of bioinsecticides to control *S. frugiperda* represents a promising alternative to the intensive use of chemical insecticides. In this connection, *Bacillus thuringiensis* (*Bt*) (Berliner) (Bacillales: Bacillaceae) is the most widely used entomopathogen, and it also is compatible with sustainable and environmentally friendly agricultural practices (Brar et al. 2007). This Gram-positive bacterium produces crystalline proteinaceous inclusions called Cry toxins that turn *Bt* into a valuable tool for insect pest management (Bravo et al. 2011). In addition, *Bt*-based formulations are highly specific with low toxicity to non-target organisms (Pigott & Ellar 2007).

There is little information about the toxicity of *Bt* formulations against molecularly identified corn and rice *S. frugiperda* strains. Thus, a better understanding of the insect response is of great interest in terms of biopesticide application. Accordingly, the main objective of this study was to assess the susceptibility of third instar larvae of both *S. frugiperda* strains to *Bt* preparations. In addition, the *S. frugiperda* populations were characterized by their esterase isozyme markers to further understand those biotypes.

Materials and Methods

INSECT COLLECTION AND REARING

At least 50 larvae of fall armyworm (*S. frugiperda*) were collected from 6 different localities, such as Charata (27.166666°S, 61.066666°W), El Manantial (26.816666°S, 65.266666°W), El Mollar (26.933333°W, 65.666666°W), Lules (26.900000°S, 65.133333°W), Metan (25.633333°S, 64.933333°W), and Raco (26.616666°S, 65.416666°W) in northern Argentina (Fig. 1). Larvae at different stages were manually collected from the whorl of the plants, and individually

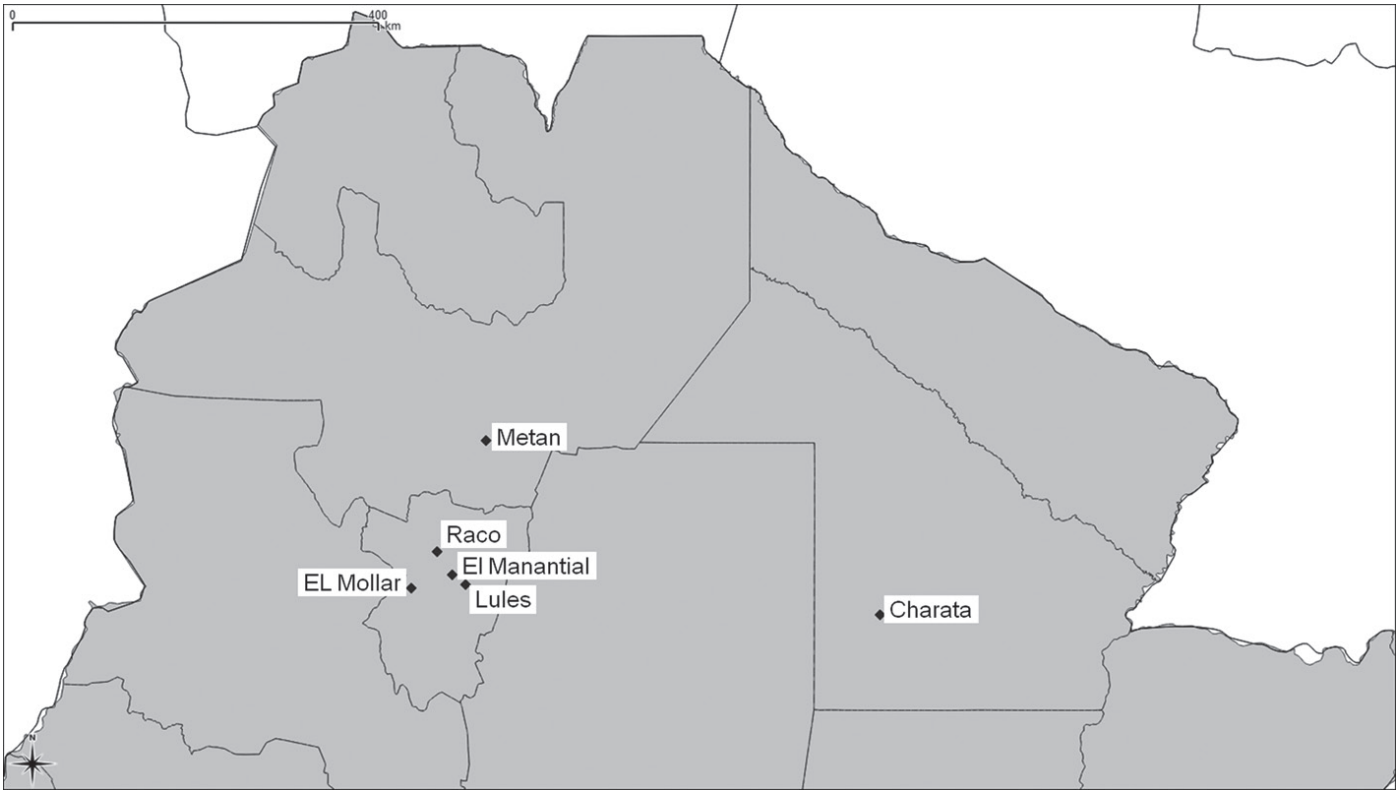


Fig. 1. Map of locations in northern Argentina where *Spodoptera frugiperda* larvae were collected. Populations from El Mollar, Lules, and Charata were genetically identified as corn *S. frugiperda* strains, whereas those from El Manantial, Raco, and Metán were typified as rice *S. frugiperda* strains.

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placed in glass tubes (12 cm length × 1.5 cm diam) (I.V.A., Buenos Aires, Argentina) containing host leaves of rice (*Oryza sativa* L.) or corn (*Zea mays* L.) as appropriate. Adults were reared in recycled and colorless plastic bottles (30 cm high × 10 cm diam) (M&S, Villa Del Parque, CABA, Buenos Aires, Argentina). For aeration, the top was closed with a nylon mesh cloth (locally acquired). These cages contained pieces of paper that allowed the females to oviposit. Food was provided via a cotton plug saturated with a mixture of honey and water (1:1 vol/vol). The cages were checked daily for oviposition and adult mortality. Egg masses were collected and deposited in glass tubes as mentioned above. Once emerged, neonate larvae were placed in 250 cc plastic pots (locally acquired) covered with a nylon mesh cloth until they reached the third instar. These third instar larvae were isolated in glass tubes to prevent cannibalism. All cultures were separately maintained in the laboratory rooms at 25.0 ± 0.5 °C, 14:10 h (L: D) artificial photoperiod, and 70 ± 15% RH. Larvae were fed with artificial diet that contained (in g per L^{-1}): 150 bean flour; 35 wheat germ; 30 brewer's yeast; 6 ascorbic acid; 1.6 sorbic acid; 2 nipagin; 22 agar; and 2 mL formaldehyde as described by Osores et al. (1982).

All biological assays were done using third instar larvae of an F_2 laboratory generation. This procedure was conducted to minimize disease and eliminate parasitoids (Perkins 1979). So newly emerged caterpillars obtained from eggs were individualized and kept in transparent tubes. Until the third instar, the daily procedures were food exchange, recipient cleanings, and collection of cephalic capsules.

MOLECULAR IDENTIFICATION

All *S. frugiperda* populations were tested by PCR-RFLP of the *cytochrome oxidase I* COI gene. Extraction of genomic DNA was performed using a cetyl trimethyl ammonium bromide (CTAB) based extraction protocol as described by Clark et al. (2007). Its PCR amplification was conducted in a 25 µL reaction mix containing 2.5 µL 10 X STR reaction buffer (Promega Corporation, Madison, Wisconsin, USA), 20 ng total DNA, 20 pmol L^{-1} of JM76 and JM77 primers, and 2 units of Taq DNA polymerase (Promega Corporation, Madison, Wisconsin, USA). Amplification was performed on a DNA thermocycler (Perkin-Elmer, Waltham, Massachusetts, USA) with the following program: an initial incubation at 94 °C (5 min), followed by 35 cycles of 94 °C (1 min), 58 °C (1 min), 72 °C (2 min), and a final segment of 72 °C for 7 min. Samples were electrophoresed in 7% PAGE (polyacrylamide gel electrophoresis) gel, then stained with ethidium bromide. The PCR amplified DNA products (0.5 µL) were digested in separated reactions with either *Sac*I or *Msp*I restriction endonuclease (Promega Corporation, Madison, Wisconsin, USA). Samples were incubated at 37 °C for 1 h. The restriction enzyme profiles also were visualized with ethidium bromide in 8.0% PAGE gel. Primers were synthesized by Tecnolab S.A. (Buenos Aires, Argentina). They included JM76 (5'-GAGCTGAATTAGG(G/A)ACTCCAGG-3') and JM77(5'-ATCACCTCC(A/T)CCTGCAGGATC-3') (Levy et al. 2002).

NON-DENATURING PAGE OF EXTRACTS FROM LARVAE AND ESTERASE STAINING

Third instar larvae of F_1 laboratory generation were macerated in 10 mM Tris-HCl buffer (pH 8.0). Homogenates were centrifuged at 12,000 g for 5 min at 4 °C, and supernatants were used immediately for electrophoresis. Extracts were separated by non-denaturing PAGE using a 10% (wt vol^{-1}) polyacrylamide gel. The molecular weight estimation of each esterase was determined by comparing its electrophoretic mobility with that of proteins contained in the high molecular weight calibration kit from GE Healthcare (Little Chalfont, Amersham, Buckinghamshire, United Kingdom). Esterase activity was assayed using 1.3 mM of α-naphthyl acetate (C2) as a substrate. Released naphthol was bound with 1 mM Fast Blue to give a colored product. Reactions were carried out at 37 °C in shaken plates containing 100 mM phosphate buffer (pH 7).

BACTERIAL SUSPENSION

The native *Bt* RT strain from our own culture collection was used throughout this study. Its 16 S rRNA partial nucleotide sequence was deposited in GenBank database under the accession number EF638795. The reference strain *Btk* HD1 was kindly provided by Daniel Zaigler, Bacillus Genetic Stock Center, Columbus, Ohio, USA. Spore-crystal suspensions were prepared by suspending in sterilized water a 5-d culture grown on LB agar at 30 °C. The presence of crystal proteins was checked with Coomasie blue reagent (Sharif & Alaeddinoğlu 1988). To determine protein concentration, 1 mL of the suspension was washed twice with NaCl 0.14 M 0.01% Triton X100. The pellet was suspended in 1 mL of NaOH 0.02 N for 5 h. Samples were centrifuged at 12,000 g for 10 min. At the end, the protein content was measured in the supernatant by the Lowry method (Lowry et al. 1951) using bovine serum albumin as the standard.

BIOASSAYS

All experiments were conducted in a climate-controlled room at 25.0 ± 0.5 °C, 70 ± 15% RH, and a photoperiod of 14:10 h (L:D). Ten replicates of 10 third instar larvae were fed the artificial diet (approximately 0.25 cm^2), previously soaked with 40 µL of a *Bt* spore-crystal suspension containing a protein concentration of 0.75 mg mL^{-1}. Before the start of the bioassay, those pieces were dried for 30 min under sterile conditions. Control groups were conducted with a piece of artificial diet soaked with 40 µL of sterile distilled water.

Larvae that could not crawl after being touched with a brush were considered dead. Mortality was scored every 24 h for 7 d.

DATA ANALYSIS

The analysis of the variance (ANOVA) and the subsequent Tukey's post-hoc test were performed using the Minitab (version 14; Minitab Inc., State College, Pennsylvania, USA) software for windows. Differences were accepted as significant when $P < 0.05$. Esterase activity bands were individually identified by their specific migration rates in the electrophoretic assay. Once bands were properly and distinctively identified, binary (0/1) matrices were constructed to compare the patterns. Simple matching similarity coefficients were generated by the SIMQUAL subroutine from the NTSYS-pc 2.02f (Applied Biostatistics, Inc., Setauket, New York, USA). Clusters were then constructed by the unweighted pair group method with arithmetic average (UPGMA) algorithm with the NTSYS program (Rohlf 1998).

Results

INSECT COLLECTION AND MOLECULAR IDENTIFICATION

As shown in Figure 1, *S. frugiperda* larvae were collected from corn fields and grass plants located in northern Argentina. They were molecularly characterized by the presence of diagnostic mitochondrial markers. Briefly, amplified products (569 base pairs) obtained with the JM-76/JM-77 primers, were separately digested with *Sac*I and *Msp*I restriction enzymes. The amplified product from the rice *S. frugiperda* strain was cut once by *Sac*I but not by *Msp*I, whereas the corn *S. frugiperda* strain showed a reciprocal pattern. According to these molecular

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markers, populations from Charata, Lules, and El Mollar were identified as corn strains, whereas those from Metán, Raco, and El Manantial were found to be rice strains.

ESTERASE PATTERN OF *SPODOPTERA FRUGIPERDA* BIOTYPES

To further knowledge about these *S. frugiperda* biotypes, the regional populations were characterized by their esterase isozyme markers. Esterase patterns of third instar larvae from both *S. frugiperda* biotypes are shown in Figure 2. Two distinct esterase bands groups called EST 1 and EST 2 were detected. A group of lower molecular weight esterase (EST 1) was found in both strains, marking differences between individuals. The group of heavier esterase bands (EST 2) displayed differences between biotypes, allowing their differentiation. A band of 220 kDa was found exclusively in rice biotype larvae. In the same way, for the corn biotype, 2 polymorphic esterase bands of either 86 or 112 kDa were observed.

The degree of similarity among of *S. frugiperda* populations based on their respective esterase profile (Fig. 2) is depicted in Figure 3. The numerical analysis clearly revealed 2 major clusters at a similarity level of 51%. Cluster *a* comprised corn strains, while cluster *b* included rice strains.

BIOASSAYS

The susceptibility of 6 *S. frugiperda* populations (100 individuals per population) to *Bt* preparations was evaluated. As mentioned above, 3 colonies of rice, as well as corn, *S. frugiperda* strains were studied by using third instar larvae of each biotype exposed to either *Bt* RT or *Btk* HD1 formulations. The mean ± SD of mortality values obtained for each treatment are shown in Table 1. It was observed that a significant difference in mortality values was found within some corn or rice colonies when *Bt* RT treatment was used, whereas there were no significant differences in *Btk* HD1 toxicity within colonies that share the same biotype. In addition, no larvae death was observed in the control group.

On the other hand, when toxicity of either *Bt* RT or *Btk* HD1 to each biotype (300 individuals per biotype) was compared, significant differences were found in corn ($F = 39.93$; $P < 0.001$) and rice ($F = 6.83$; $P = 0.011$) *S. frugiperda* strains. For *S. frugiperda* corn colonies, the percentage of larvae that died due to *Bt* RT and *Btk* HD1 was 71 ± 11 and 52 ± 11, respectively, whereas for *S. frugiperda* rice colonies, the percentage of larvae that died due to *Bt* RT and *Btk* HD1 was 83 ± 11 and 74 ± 14, respectively. According to these results, the native *Bt* RT was more toxic to *S. frugiperda* populations than the reference strain *Btk* HD1.

An additional statistical analysis of mortality data from 3 populations (600 individuals; 300 treated with *Bt* Rt, and 300 treated with *Btk* HD1) of each biotype also revealed that there was a significant difference between the 2 *S. frugiperda* strains ($F = 45.24$; $P < 0.001$). The mortality values for rice and corn *S. frugiperda* strains were 79 ± 13 and 62 ± 14 %, respectively. Those mortality values showed that rice *S. frugiperda* strains were more sensitive than corn *S. frugiperda* strains, regardless of the *Bt* preparation used.

Discussion

The susceptibility of *S. frugiperda* populations to *Bt* products has been explored in different ways. One was the incidence of the geographical region. In an interesting work, Monnerat et al. (2006) found differences among 3 *S. frugiperda* populations from Latin American countries such as Mexico, Colombia, and Brazil when *Bt* strains or pure Cry1B, Cry1C, and Cry1D toxins were studied. Additionally, by using the random amplification of polymorphic DNA-PCR technique, the authors detected

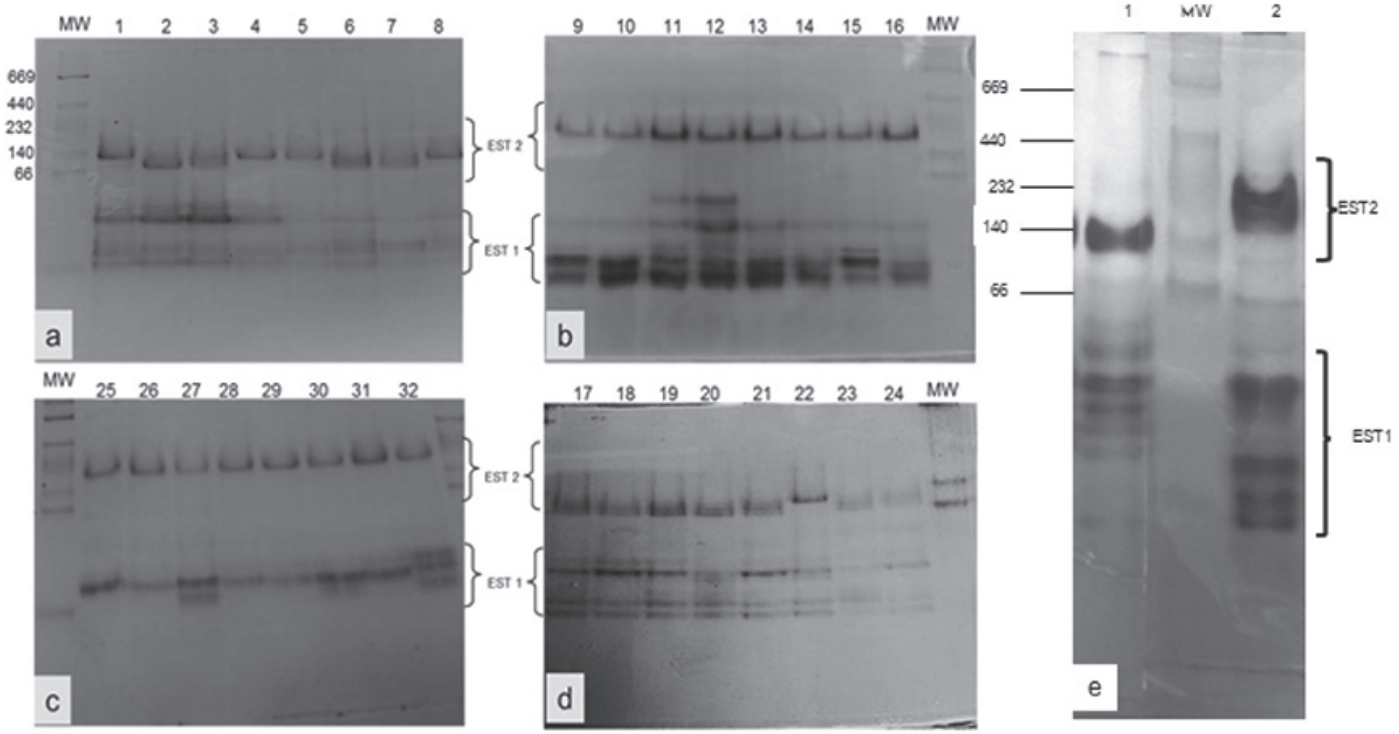


Fig. 2. Esterase profiles of corn and rice *S. frugiperda* strains collected from corn fields and grass plants located in northern Argentina. Individuals from El Mollar (a), El Manantial (b), Raco (c), and Charata (d). Populations from El Mollar and Charata were genetically identified as corn *S. frugiperda* strains, whereas those from El Manantial and Raco were identified as rice *S. frugiperda* strains. For comparison, 1 individual from corn (1) and rice (2) *S. frugiperda* strain also was shown (e). Molecular weight markers (MWM, kDa).

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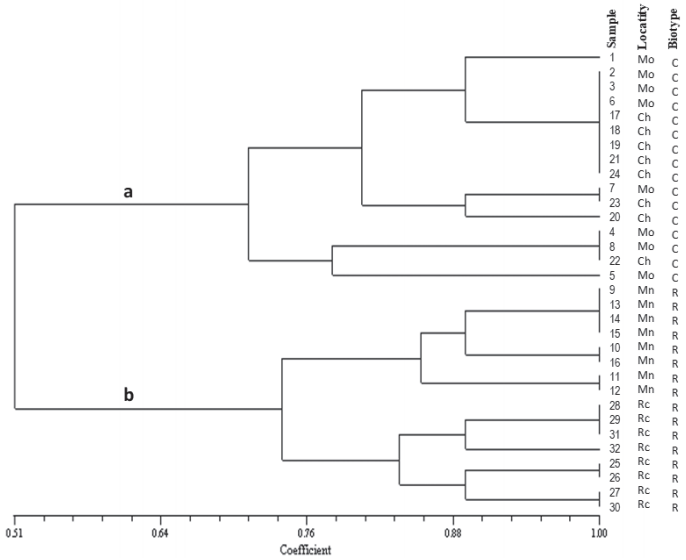


Fig. 3. Dendrogram showing clustering and relationships of *S. frugiperda* samples based on the presence or absence of esterase activity bands in non-denaturing polyacrylamide gel. Associations were produced by using the UPGMA clustering method. Major clusters are denoted as (a) and (b). Sample numbers correspond to individuals from El Mollar (Mo), El Manantial (Mn), Raco (Rc), and Charata (Ch) shown in Figure 2. Populations were genetically identified as corn (C) or rice (R) *S. frugiperda* strains.

a genetic variability among *S. frugiperda* colonies that allows the clustering of *S. frugiperda* populations associated with corn according to their geographical origin. Another scenario was the evaluation of the susceptibility of *S. frugiperda* host-associated strains to the δ-endotoxin present in transgenic crops. For example, *S. frugiperda* larvae collected from bermudagrass (*Cynodon dactylon* [L.] Pers; Poaceae) and browntop millet (*Urochloa ramosa* [L.] Nguyen; Poaceae) were significantly more susceptible to transgenic *Bt* cotton that expresses the Cry1Ac protein than larvae collected from field corn (Adamczyk et al. 1997). In our research, it was shown that *S. frugiperda* colonies molecularly identified as rice biotype displayed a higher susceptibility to *Bt* formulates than the corn biotype in populations from northern Argentina. These results are compatible with those reported by Ingber et al. (2018) using diet-based bioassays, in which corn and hybrid populations also were more tolerant to the *Bt* toxins than the rice *S. frugiperda* strain.

Additionally, it is necessary to mention that there are some reports that fail to show associations between molecularly identified *S. frugiperda* biotypes and their respective hosts (Martinelli et al. 2007; Virla et al. 2008; Salinas-Hernandez & Saldamando-Benjumea 2011; Rosas-García et al. 2016). In fact, our results also showed 2 cases where the rice *S. frugiperda* biotypes were collected from corn host plants (populations from Metán and El Manantial) (Table 1). Thus, because a host plant is not always determinant for the identification of the colonizing *S. frugiperda* strain, its molecular characterization is highly recommended before studying any aspect of the pest. Accordingly, in this work, both *S. frugiperda* biotypes were properly identified by the presence of diagnostic mitochondrial markers before the susceptibility assays were conducted. It is interesting to note that although these results were obtained using isolated DNA, the strain characterization also may be done by amplifying DNA directly from *S. frugiperda* tissues, such as eggs or neonate larva that is either frozen or ethanol preserved (Loto et al. 2013). The use of ethanol-preserved larvae is very attractive, especially in those cases where the sample is required to maintain optimum conditions for extended periods, e.g., during the collection of insect specimens.

Furthermore, allozymes studies allowed the differentiation between related species (Stuchi et al. 2012) and biotypes (Coats et al. 1994) when they were used as molecular makers. Additionally, zymographic analysis also is frequently applied for the identification of species and biotypes of insect populations (Loxdale & Brookes 1989). Here, *S. frugiperda* larvae collected from corn fields and grass plants located in northern Argentina also were analyzed by using their esterase activities. Non-denaturing polyacrylamide gel electrophoresis showed different esterase patterns among the strains and between individuals. The presence of enzymes from the EST 1 group was repeated in populations from different localities. However, bands grouped in EST 2 were biotype specific. An esterase of 220 kDa was found exclusively in *S. frugiperda* rice populations, while a band of 86 kDa or 112 kDa was specific for the corn biotype. Thus, according to the esterase activity data, the *S. frugiperda* biotypes also were differentiated. Furthermore, in addition to its application as a molecular marker, the functionality of these enzymes in insects is typically associated with insecticide resistance (Li et al. 2007). Resistant *S. frugiperda* strains usually have a metabolism of esterases higher than their susceptible counterparts (Yu et al. 2003). So, the presence of differential esterase patterns in both *S. frugiperda* biotypes also could explain part of the differences in *Bt* susceptibility. In addition, the genome comparison of both *S. frugiperda* strains showed differences in detoxification genes, including esterases, glicosyltransferases, and Cytochrome P450 enzymes. The corn strain presents 6 esterase genes that are absent in the rice strain. Meanwhile, 1 esterase gene is present only in the rice strain genome (Gouin et al. 2017).

Concerning the susceptibility of third instar larvae of both *S. frugiperda* strains to *Bt* preparations, the native *Bt* RT was found to be highly pathogenic, even more so than the reference strain *Bt*k HD1. In this connection, it was previously reported that each *Bt* strain shows a different combination of *cry* genes. Under the same assays conditions, *Btk* HD1 amplifies for *cry*1Aa, *cry*1Ab, *cry*1Ac, *cry*2Aa, and *cry*2Ab, whereas *Bt* RT amplifies only for *cry1*Ab, *cry*1Ac, and *cry*2Ab. However, it also was observed that the native *Bt* RT strain has a significantly higher specific

Table 1. Origin of *Spodoptera frugiperda* populations and their susceptibility to *Bacillus thuringiensis* preparations in bioassays done with third instar larvae.

				Mortality ± SD (%)	
Locality (province)	Year of collection	Host plant	Strain	*Bt* RT	*Btk* HD1
Charata (Chaco)	2010	Corn	Corn	66 ± 14 (a)	45 ± 12 (a)
Lules (Tucumán)	2011	Corn	Corn	67 ± 7 (a)	56 ± 8 (a)
El Mollar (Tucumán)	2010	Corn	Corn	80 ± 7 (b)	56 ± 11 (a)
Metán (Salta)	2010	Corn	Rice	78 ± 11 (A)	71 ± 17 (A)
Raco (Tucumán)	2011	Grass	Rice	81± 10 (AB)	69 ± 14 (A)
El Manantial (Tucumán)	2010	Corn	Rice	90 ± 9 (B)	83 ± 8 (A)

Mortality values in a column followed by the same letter are not significantly different ($P \leq 0.05$). Note that lower and upper case letters were used to compare the susceptibility to *Bt* RT or *Btk* HD1 within corn or rice *S. frugiperda* strains, respectively.

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biomass-bound protease activity (1,988 ± 98 U g dry wt^{-1}) than the reference *Btk* HD1 strain (946 ± 14 U g dry wt^{-1}) (Álvarez et al. 2009). Although it is known that Cry toxins are solubilized and activated by host proteases once ingested (Pigott & Ellar 2007), these results and those reviewed by Brar et al. (2007), who discussed the role of *Bt* proteases as a potential indicator of entomotoxicity, could be an alternative or complementary toxicity mechanism of *Bt*-based biopesticides.

As a conclusion to this research, *S. frugiperda* control could depend not only on the bioinsecticide used, but also on the *S. frugiperda* biotype being treated. Thus, the bioassays conducted in this work showed that third instar larvae of rice *S. frugiperda* strains were more sensitive than those of corn *S. frugiperda* strains, regardless of the *Bt* preparation used. In this connection, the *S. frugiperda* characterization by either genetic or esterase isozyme markers is highly recommended before studying any aspect of the pest. Additionally, the search for novel *Bt* strains such as the native *Bt* RT also will expand the repertoire of *Bt*-based biopesticides.

Acknowledgments

This work was supported by grants PICT 2015 2596 Fondo para la Investigación Científica y Tecnológica (*FONCyT*), and PIP 339 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

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