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Population variability of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in maize (Poales: Poaceae) associated with the use of chemical insecticides

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The fall armyworm, *Spodoptera frugiperda* Smith & Abbot (Lepidoptera: Noctuidae), is a major pest in the western hemisphere, capable of causing substantial damage to corn, sorghum, fodder, grass, rice, cotton, and peanut crops (Nagoshi & Meagher 2004). Approximately 3,000 tons of active ingredient per year are used to control this single pest (Blanco et al. 2014). In several regions of the world, insecticide overuse has resulted in the development of populations highly resistant to these chemicals (Yu et al. 2003; Ahmad & Arif 2010). Consequently, collateral effects such as negative impacts on biological control organisms also occur (Romeis et al. 2006).

Populations of the fall armyworm have been studied in various environments that show various factors can affect their level of resistance to insecticides. Population monitoring of the fall armyworm resistance status has shown that management strategies can minimize the environmental impact of insecticide use (Zenner de Polanía et al. 2007). Insects have demonstrated great genetic plasticity, with more than 500 species now resistant to one or more insecticides (McGaughey 1985; Ferré & Van Rie 2002). In designing resistance management strategies for *S. frugiperda*, we must take into account a few factors. First, we need to genetically characterize the population of interest. Second, studies should be conducted to examine the origins of possible biotypes within the population associated with certain environmental conditions. Third, we need to examine the dynamics of a population's genetic variability through generations.

Molecular markers have become a powerful tool to evaluate the degree of genetic variation in a given population with respect to a given factor over the years. Several types of relevant molecular markers have been identified in insect populations (Batley et al. 2004; Clark et al. 2007; Martinelli et al. 2007; Lindroth 2011), including the inter-simple sequence repeats (ISSR). The ISSR (Zietkiewicz et al. 1994) is a molecular marker that uses microsatellites as primers to amplify multiple loci, mainly repeated nucleotide sequences of several sizes. Some advantages of these types of molecular markers include high reproducibility, acceptable cost, no need for previous sequence information, and high polymorphism (Semagn et al. 2006). The objective of our research was to study the genetic variability of *S. frugiperda* in maize (Poales: Poaceae) from México under various crop management strategies using ISSR molecular markers.

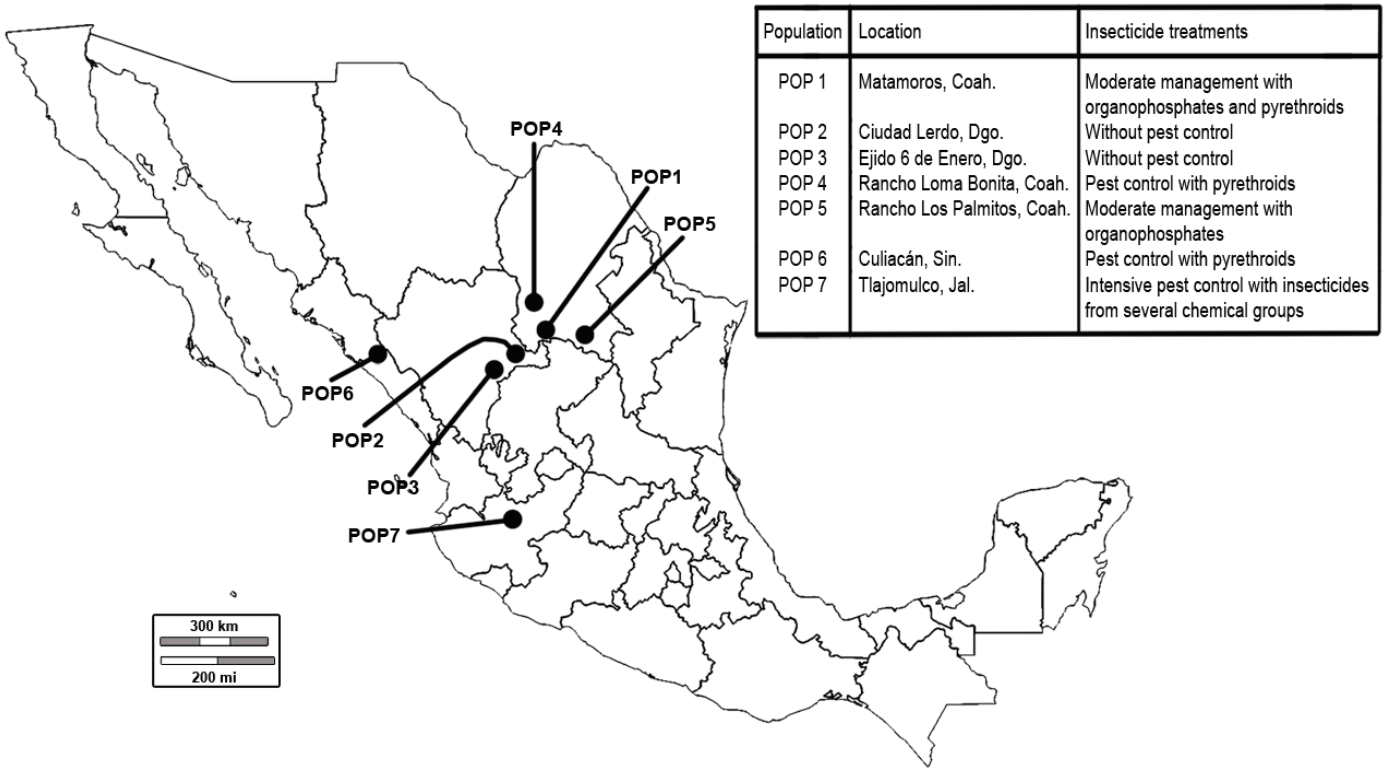
Maize fields in localities of Coahuila, Durango, Jalisco, and Sinaloa states that differed in their pest management strategies were selected, and the results of larval testing were used to compare and contrast the impact of management strategies on the genetic variability. The locations from where *S. frugiperda* populations were collected and the pest management tactics utilized are shown in Fig. 1. A modified protocol of Doyle & Doyle (1991) was used for DNA extraction from the cut mid-section of every selected larva. Polymerase chain reaction (PCR) analysis was performed using ISSR molecular markers. All PCR products were separated on 1% agarose gels for 1.3 h at 65 V. The gels were viewed and photographed under UV illumination and images digitalized in a UVP Mini Darkroom. Amplified bands from every individual were put in a single absence–presence matrix for data organization. Analysis of molecular variance (AMOVA) was carried out using the InfoGen version 2014 statistical software (Balzarini & Di Rienzo 2014). In addition to genetic diversity, Nei's unbiased heterozygosis, mean allele number, and the number of effective alleles were obtained. Data were analyzed to obtain frequency dendrograms.

The results of PCR analysis using ISSR molecular markers produced a total of 105 bands or loci, of which 92.59% were polymorphic and provided enough information to group populations into several nodes. Results from AMOVA (Table 1) show that *P*-values were small (P = 0.0175 and 0.0075) and genetic variability was greater within populations (96.27%) than among populations (3.73%). These data indicate that the collected populations were essentially panmictic, and there was little genetic differentiation among them, further suggesting that there was high gene flow among the populations. However, the ISSR molecular markers showed a great number of polymorphic loci, which supported placement of populations in separate groups. This genetic plasticity in *S. frugiperda* was noted in other studies using amplified fragment length polymorphism–type molecular markers for investigation of *S. frugiperda* population genetic structure (Martinelli et al. 2007; Lobo-Hernández & Saldamando-Benjumea 2012). Other workers on genetic variability in *S. frugiperda* and other widely distributed species reported that greater variability occurs within than between populations (Clark et al. 2007; Roux et al. 2007; Fuentes-Contreras et al. 2008), which agrees with results found in our research.

The dendrogram (Fig. 2) shows the formation of 4 groups, each containing between 1 and 3 populations (POP): group 1 (POP 1); group 2 (POP 2 and POP 3); group 3 (POP 4, POP 5, and POP 6), and

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Population	Location	Insecticide treatments
POP 1	Matamoros, Coah.	Moderate management with organophosphates and pyrethroids
POP 2	Ciudad Lerdo, Dgo.	Without pest control
POP 3	Ejido 6 de Enero, Dgo.	Without pest control
POP 4	Rancho Loma Bonita, Coah.	Pest control with pyrethroids
POP 5	Rancho Los Palmitos, Coah.	Moderate management with organophosphates
POP 6	Culiacán, Sin.	Pest control with pyrethroids
POP 7	Tlajomulco, Jal.	Intensive pest control with insecticides from several chemical groups

Fig. 1. *Spodoptera frugiperda* populations in cultivated maize in various parts of Mexico from which larvae were collected to study molecular genetic variation.

group 4 (POP 7). Population 7 had a genetic composition significantly different from other populations in this study. Population 3 was collected from a maize crop without insect control, whereas population 7 was collected from a corn crop where insecticides of various toxicological groups from organophosphates to some relatively new materials, such as neonicotinoids, had intensively been used for years (INIFAP 2007).

This insecticide use may not be completely responsible for all of the genetic differences observed in population 7, although reports on other Lepidoptera exposed to continuous insecticide exposures show similar genetic changes (Endersby et al. 2006; Franck et al. 2007). Population 4 (Rancho Loma Bonita, Coahuila) and population 6 (Culiacán, Sinaloa) were collected on crops with intensive use of pyrethroid insecticides for pest control, and both populations were sorted into the same group. These management differences suggest that populations intensively exposed to insecticides are more genetically diverse; such variability suggests that chemical treatment affects population allele diversity, which has also been shown previously (Franck et al. 2007). The herein used ISSR molecular markers provide new tools to investigate complex resistance problems under field conditions, and finer-grained interpretations of results using them are expected as larger databases become available.

Summary

The objective of this research was to determine the genetic variability of *Spodoptera frugiperda* Smith & Abbot (Lepidoptera: Noctuidae) larvae from populations collected on maize (Poales: Poaceae) crops from several locations (Sinaloa, Jalisco, Coahuila, and Durango) in Mexico grown under various types of pest management using inter-simple sequence repeats (ISSR) type molecular markers. These ISSR markers amplified 105 loci, with 92.59% being polymorphic, which genetically characterized these populations and related this variability with factors such as pest control management. The most divergent population was from Tlajomulco, Jalisco. This location had the most intense use of chemical insecticides with diverse modes of action, which may account for much of the difference in genetic variability observed among sites in this study.

Key Words: ISSR; genetic flow; insecticide

Sumario

El objetivo de esta investigación fue estudiar la variabilidad genética de gusano soldado *Spodoptera frugiperda* Smith & Abbot (Lepidóptera: Noctuidae) a partir de larvas de poblaciones recolectadas en

Table 1. Analysis of molecular variance (AMOVA) for genetic variability in *Spodoptera frugiperda* populations using 3 inter-simple sequence repeat (ISSR) molecular markers.

Variability source	Sum of squares	df	Mean square	*P*-value	No. of iterations	Variability composition	Variability (%)
Between	22.10	6	3.68	0.0175	400	0.09	3.73
Within	227.47	98	2.32	0.0075	400	2.32	96.27
Total	249.56	104	2.40	—	—	2.41	100.00

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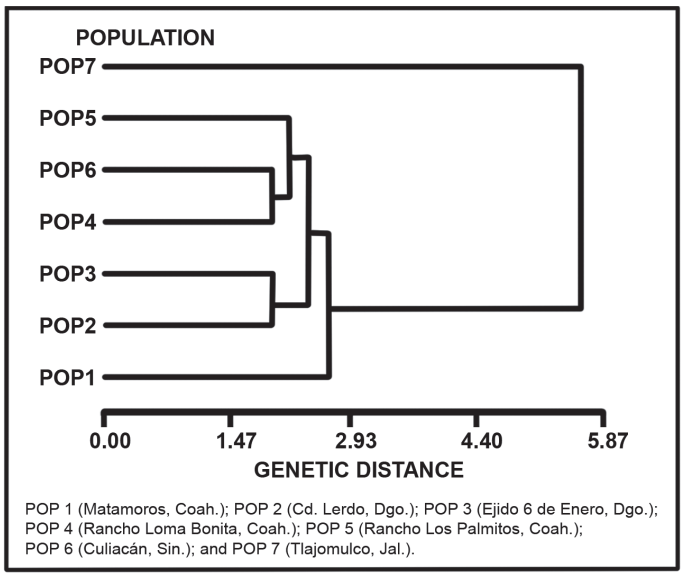


Fig. 2. Dendrogram of genetic distance among *Spodoptera frugiperda* populations analyzed using ISSR molecular markers.

cultivos de maíz (Poales: Poaceae) de distintas localidades (Sinaloa, Jalisco, Coahuila y Durango) de Mexico bajo diferentes tipos de manejo del cultivo, utilizando marcadores moleculares tipo ISSR. Estos marcadores amplificaron un total de 105 loci, de los siendo el 92.59% polimórficos, lo cual permitió caracterizar las poblaciones genéticamente y relacionar esta variabilidad con factores como estrategias de control de la plaga. La población más diferenciada, población 7, fue la de Tlajomulco, Jalisco; la localidad con un uso más intensivo de insecticidas químicos con diversos modos de acción, lo que puede explicar gran parte de las diferencias de variabilidad genética observada entre sitios en este estudio.

Palabras Clave: ISSR; flujo genetico; insecticidas

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