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Strains of *Spodoptera frugiperda* (J.E.Smith) (Noctuidae) in the states of Paraná and São Paulo, Brazil

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Abstract. Two strains of *Spodoptera frugiperda* (J.E. Smith) were first described in the United States; in Brazil, in the states of Rio Grande do Sul, Mato Grosso, and western Paraná they have also been reported. This study was aimed at identifying these strains in Norte Pioneiro and Campos Gerais, in the states of Paraná and southwestern São Paulo. Larvae of *S. frugiperda* were collected in the cities of Ponta Grossa, Tibagi, Arapoti, and Wenceslau Braz in Paraná, and in the city of Itaberá, in São Paulo. PCR-RFLP genotyping of the COI gene was carried out using sixty-six specimens. Based on their electrophoretic pattern, 51 individuals were identified as corn strain, five as rice strain, and 10 as hybrids (Rice in MspI and Maize in SacI). Our findings indicate that both *S. frugiperda* strains are present the study areas.

Keywords: COI; Hybrids; MspI; RFLP; SacI.

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith), is widely distributed in the western hemisphere, occurring from central-southern Canada to southern Argentina (Pogue 2002). Recently, this pest has become a new invasive species in West and Central Africa, where it was first recorded in early 2016 (Goergen *et al.* 2016; Jeger *et al.* 2017).

Two strains of *S. frugiperda* were first described in the United States (Pashley 1986), and since then they have also been reported in Mexico (Rosas-García *et al.* 2016), Colombia (Cano-Calle *et al.* 2015), Argentina (Murúa *et al.* 2015), Paraguay (Juárez *et al.* 2012), and Africa (Cock *et al.* 2017).

In Brazil, studies have confirmed the presence of these two *S. frugiperda* strains especially in Rio Grande do Sul (Busato *et al.* 2002, 2004, 2005; Machado *et al.* 2008), but also in Mato Grosso (Campo Verde and Primavera do Leste) and Paraná (Palotina) (Nagoshi *et al.* 2007).

Several studies have also reported distinct susceptibilities of *S. frugiperda* strains to chemical pesticides (Busato *et al.* 2006; Hay-Roe *et al.* 2011; Ríos-Díez & Saldamando-Benjumea 2011) and Bt plants (Ríos-Díez *et al.* 2012). In order to account for these differences in the control of this pest, our objective was to identify these strains in different crops in areas of Norte Pioneiro and Campos Gerais, in Paraná and southwestern São Paulo.

MATERIAL AND METHODS

The study was carried out at the Laboratory of Entomology and Phytopathology (LabEF) at the ABC Foundation - Agricultural Research and Development, in the city of Castro, Paraná. Control specimens of the corn and rice strains were obtained from the Federal University of Mato Grosso (UFMT) and the United States Department of Agriculture - Agricultural Research Service (USDA-ARS), respectively.

Larvae of *S. frugiperda* were collected in the cites of Ponta Grossa, Tibagi, Arapoti, and Wenceslau Braz in Paraná, and in the cities of Itaberá, in São Paulo. Individuals were maintained under laboratory conditions in a climate room at a temperature of 25 ± 1° C, 70 ± 10% relative humidity, and 14-h photoperiod, and fed an artificial diet (Hoffmann-Campo *et al*. 1985) until reaching the pupal stage, when they were transferred to a freezer (-20° C) for later DNA extraction.

Spodoptera frugiperda DNA was extracted using the CTAB method with modifications, followed by PCR-RFLP of the mitochondrial gene COI for insect genotyping (Cano-Calle *et al*. 2015). The amplification was performed in a 25 µL reaction mix containing 2.5 µL of 10X reaction buffer, 0.75 µL of 50 mM MgCl 2, 0.5 µL of 10 mM dNTPs, 1.0 µL of the forward primer JM76 (5' GAGCTGAATTAGG(G/A)ACTCCAGG 3'), 0.5 µL (5 U/µL) of Taq DNA polymerase, 13.75 µL of sterile ultrapure water, and 5.0 µL of DNA (10 ng/µL). The thermocycling conditions were as follows: the first cycle started with a temperature of 94° C for 3 min, followed by 30 cycles at 94° C for 1 min, 62° C for 1 min, and 72° C for 1 min, and a final extension cycle

at 72° C for 10 min. Digestion was then carried out with the restriction enzymes MspI and SacI. For each enzyme, 300 ng of PCR product were transferred to new tubes, 2 µL of 10x Buffer (Invitrogen) and 30 µL of sterile ultrapure water were added to the solution, followed by 10 units of MspI (0.5 µL) or 10 units of SacI (0.5 µL), and incubated at 37° C for 2 h. Samples were run on a 2% agarose gel before and after digestion with restriction enzymes.

The digestion with the restriction enzyme MspI produces a band of approximately 569 bp that characterizes the rice strain, while in the corn strain, two fragments of approximately 497 and 72 bp are observed (Nagoshi & Meagher 2003a). After digestion with the enzyme SacI, two fragments of approximately 500 bp and 69 bp are present in the rice strain, while only a fragment of approximately 569 bp is observed in the corn strain (Nagoshi *et al.* 2006).

RESULTS AND DISCUSSION

Sixty-six specimens of *S. frugiperda* collected in the study areas and 27 control specimens of the corn and rice strains were genotyped. The electrophoretic patterns found for each strain were similar to those reported by Nagoshi & Meagher (2003a, 2003b) and Nagoshi *et al.* (2006). Fifty-one individuals were identified as corn strain, five as rice strain, and 10 as hybrids (Rice in MspI and Corn in SacI) (Figure 1).

The presence of hybrids has been reported in different studies (Nagoshi & Meagher 2003a, 2003b; Vélez-Arango *et al*. 2008, Salinas-Hernandez & Saldamando-Benjumea 2011) based on RFLP markers of the MspI enzyme and PCR of the FR sequence. Saldamando & Vélez-Arango (2010) described two types of hybrids: individuals characterized by fragments produced by the digestion with the MspI enzyme and amplification with FR primers (referred to as hybrids + / +) and individuals distinguished by the absence of fragments produced with the MspI enzyme or amplification products with FR primers (referred to as hybrids - / -).

Cano-Calle *et al.* (2015) reported that hybrids were also identified with SacI; however, this restriction enzyme was only used when the FR fragment was difficult to amplify in the sample.

Hybrids may be the product of interstrain matings, indicating the occurrence of gene flow in the study region. Nagoshi & Meagher (2003a) suggested that hybrids result from the unidirectional mating between rice-strain females and corn-strain males, since mating between corn-strain females and rice-strain males was not observed under laboratory conditions. In contrast, Prowell *et al.* (2004) found evidence of bi-directional breeding in nature, with 54% of hybrids as the offspring of rice-strain females and corn-strain males and 46% of reciprocal mating.

Rice-strain individuals are commonly found in habitats occupied by corn-strain populations (Prowell *et al*. 2004), indicating that they use the corn habitat more often than the opposite, and consequently, hybrids are mostly found in corn fields. Prowell *et al.* (2004) pointed out that 62% of hybrids in their study were collected in corn fields. Similarly, Saldamando & Vélez-Arango (2010) obtained the highest percentage (41%) of hybrids in the corn habitat. In the present study, most of the hybrids were found in a single egg cluster on wheat, therefore it cannot be inferred that most of them were found in wheat, as this crop was not sampled as the corn habitat.

Our study did not examine the habitats used by the rice strain described in the literature; however, rice-strain individuals were collected in the corn-strain habitat. Prowell *et al.* (2004) reported that only 2% of the individuals collected in the rice habitat were identified as corn strain, while 18% of individuals collected in the corn habitat were assigned as rice strain. On the other hand, of the two populations collected in black oat, the corn strain predominated.

Both *S. frugiperda* strains were found in the study areas and the occurrence of hybrids indicates the existence of gene flow between strains. Despite the presence of rice-strain individuals, most specimens were identified as corn strain, which predominates in the region.

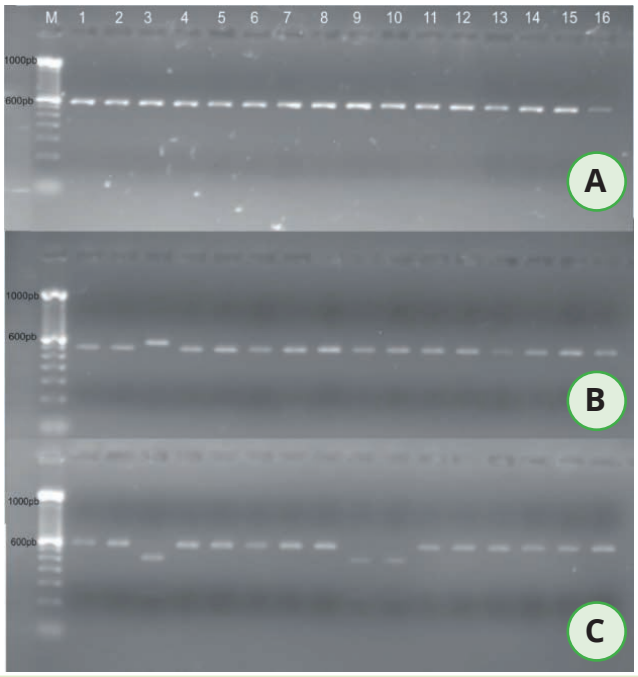

Figure 1. A – Undigested PCR-RFLP product. B - Digestion with the restriction enzyme MspI. C – Digestion with the restriction enzyme SacI. Individual 3 assigned as rice strain, individuals 9 and 10 designated as hybrids, and the remainder identified as corn strain. M- Molecular marker (100pb). 2% agarose gel.

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