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 (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 (Campos Verde and Primaverá 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; Rios-Diez & Salamandao-Benuea 2011) and Bt plants (Rios-Diez 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 Pionheiro 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 cities of Ponta Grossa, Tibagi, Arapoti, and Wenceslau Braz in Paraná, and in the city 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 (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 MgCl₂, 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 of 72°C for 10 min.
Richardt et al. (2003a) suggested that hybrids result from the
interactions of different strains. 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 Mspl and Corn in SacI) (Figure 1).

The presence of hybrids has been reported in different studies (Nagoshi & Meagher 2003a, 2003b; Vélez-Arangó et al. 2008, Salinas-Hernández & Salamamdo-Benjumea 2011) based on RFLP markers of the Mspl enzyme and PCR of the FR sequence. Salamamdo & Vélez-Arangó (2010) described two types of hybrids: individuals characterized by fragments produced by the digestion with the Mspl enzyme and amplification with FR primers (referred to as hybrids + / -) and individuals distinguished by the absence of fragments produced with the Mspl 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, Proyellow 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 (Proyellow et al. 2004), indicating that they use the corn habitat more often than the opposite, and consequently, hybrids are mostly found in corn fields. Proyellow et al. (2004) pointed out that 62% of hybrids in their study were collected in corn fields. Similarly, Salamamdo & Vélez-Arangó (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. Proyellow 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 Mspl. 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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