

**University of São Paulo  
“Luiz de Queiroz” College of Agriculture**

**Resistance risk assessment of *Spodoptera frugiperda* (J.E. Smith)  
(Lepidoptera: Noctuidae) to Cry1F protein from *Bacillus  
thuringiensis* Berliner in Brazil**

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Thesis submitted in order to obtain the degree of  
Doctor in Science. Area: Entomology

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*I offer to my family: ANTÃO, LORENA (parents), LUIS CARLOS, JAIRO, ÁLVARO, ADRIANO (brothers), LÉIA, SILVANA, CÁTIA, ANDRESSA (sister-in-law), CAROLINE, VITÓRIA, ALENCAR and JOÃO MIGUEL (nephews) for their support and understanding during this stage of my life*



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## ABSTRACT

### **Resistance risk assessment of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) to Cry1F protein from *Bacillus thuringiensis* Berliner in Brazil**

The event TC1507 maize with *cry1F* gene from the bacterium *Bacillus thuringiensis* Berliner (Bt) was approved for commercial release in Brazil in 2008. The evolution of pest resistance to Bt plants has been a great concern to preserve the lifetime of this technology. Therefore, in this study we assess the risk of evolution of resistance to Cry1F protein in *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) populations from major maize-growing regions in Brazil. The baseline susceptibility to Cry1F was determined with diet overlay bioassay for susceptible reference population and four field populations of *S. frugiperda*. Then, we monitored 43 populations of *S. frugiperda* sampled in nine different States of Brazil during 2010/2011, 2011/2012 and 2012/2013 crop seasons. Only 4-fold variation in susceptibility to Cry1F was detected among *S. frugiperda* from field populations in the baseline susceptibility study. Diagnostic concentration of 2,000 ng cm<sup>-2</sup> was defined for monitoring the susceptibility to Cry1F in *S. frugiperda* populations. Survival at 2,000 ng cm<sup>-2</sup> of Cry1F protein increased significantly throughout crop seasons in populations from São Paulo, Santa Catarina, Rio Grande do Sul, Bahia, Mato Grosso, Goiás, Mato Grosso do Sul, and Paraná, but not in Minas Gerais. We also sampled a population of *S. frugiperda* in TC1507 field failures in Bahia in October, 2011. This population was selected in laboratory with Cry1F protein up to 20,000 ng cm<sup>-2</sup> and the resistance ratio of the selected resistant population (BA25R) was > 5,000-fold. This resistant population was able to survive in Cry1F maize from neonate till pupa and produce normal adult. The inheritance of *S. frugiperda* resistance to Cry1F protein was autosomal. To test the functional dominance, neonate larvae obtained from the cross of resistant and susceptible populations were tested in leaf bioassay, and around 8% of heterozygotes were able to survive and complete the larval development and produce normal adults on TC1507 leaves while susceptible larvae could not survive for up to five days after infestation. Dominance was estimated to be 0.15 ± 0.09, suggesting that resistance to Cry1F in TC1507 maize was incompletely recessive. We also conducted resistance selection studies in other seven *S. frugiperda* populations from six different Brazilian states to test whether the resistance alleles were at same locus or not. The F<sub>1</sub> larvae obtained from the cross between resistant population (BA25R) and each of the seven selected resistant populations were able to survive at 2,000 ng cm<sup>-2</sup> of Cry1F protein in diet bioassay, and therefore they shared the same locus of resistance to Cry1F protein. We estimated the frequency of resistance allele to Cry1F protein in populations of *S. frugiperda* of main crop season 2011/2012 from five states. We established 517 isofemale lines using F<sub>2</sub> screen method. The total frequency of Cry1F resistance allele in Brazil was 0.088 with 95% confidence interval between 0.077 and 0.100. Based on results obtained in this study, the risk of resistance evolution to Cry1F protein by *S. frugiperda* is high in Brazil.

Keywords: Fall armyworm; *Bacillus thuringiensis*; Resistance management; TC1507; High-dose; Genetic basis



## RESUMO

### **Avaliação do risco de resistência de *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) à proteína Cry1F de *Bacillus thuringiensis* Berliner no Brasil**

O evento de milho TC1507 com gene *cry1F* da bactéria *Bacillus thuringiensis* Berliner foi aprovado comercialmente no Brasil em 2008. A evolução da resistência de pragas a plantas Bt tem sido uma grande preocupação na preservação desta tecnologia. Portanto, neste estudo foi avaliado o risco de evolução da resistência à proteína Cry1F em populações de *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) das principais regiões de cultivo de milho no Brasil. A linha-básica de suscetibilidade à proteína Cry1F foi determinada em bioensaio de aplicação superficial na dieta para a população suscetível de referência e quatro populações de campo de *S. frugiperda*. Posteriormente, a suscetibilidade a Cry1F foi monitorada em 43 populações de *S. frugiperda* coletadas em nove Estados do Brasil nas safras agrícolas de 2010/2011, 2011/2012 e 2012/2013. A variação na suscetibilidade foi de apenas quatro vezes para Cry1F entre as populações de campo na linha-básica de suscetibilidade. A concentração diagnóstica de 2.000 ng cm<sup>-2</sup> de proteína Cry1F foi definida para o monitoramento da suscetibilidade. A sobrevivência em 2.000 ng cm<sup>-2</sup> de proteína Cry1F aumentou significativamente no decorrer das safras em populações de São Paulo, Santa Catarina, Rio Grande do Sul, Bahia, Mato Grosso, Goiás, Mato Grosso do Sul e Paraná, mas não em Minas Gerais. Além disso, uma população de *S. frugiperda* foi coletada em milho TC1507 com falha de controle na Bahia em outubro de 2011. Esta população foi selecionada no laboratório com a proteína Cry1F até 20.000 ng cm<sup>-2</sup>, obtendo-se uma população resistente (BA25R) com razão de resistência >5000 vezes. Esta população resistente foi capaz de sobreviver no milho TC1507 desde larva neonata até a fase de pupa e com emergência de adultos normais. O padrão de herança da resistência de *S. frugiperda* a Cry1F foi autossômica. Para testar a dominância funcional, as larvas neonatas do cruzamento entre a população resistente e suscetível foram testadas em folhas do evento TC1507 e cerca de 8% dos heterozigotos foram capazes de sobreviver, completar o desenvolvimento e produzir adultos normais, enquanto as larvas da linhagem suscetível não sobreviveram por mais de cinco dias após a infestação. A dominância foi estimada em 0,15 ± 0,09; portanto, a resistência à proteína Cry1F no milho TC1507 foi incompletamente recessiva. A resistência foi selecionada para outras sete populações de seis Estados brasileiros para testar se os alelos de resistência estavam no mesmo locus. As larvas F<sub>1</sub> obtidas do cruzamento entre a população resistente (BA25R) e cada uma das sete populações selecionadas sobreviveram na concentração de 2.000 ng cm<sup>-2</sup> de proteína Cry1F e, portanto, essas populações compartilharam o mesmo locus de resistência à proteína Cry1F. A frequência do alelo resistente à proteína Cry1F foi estimada em populações de *S. frugiperda* coletadas em cinco Estados na safra 2011/2012. Foram estabelecidas 517 isolinhas utilizando o método de "F<sub>2</sub> screen". A frequência total do alelo de resistência à proteína Cry1F no Brasil foi de 0,088, com intervalo de confiança de 95% entre 0,077 e 0,100. Com base nos resultados, o risco de evolução da resistência à proteína Cry1F por *S. frugiperda* é elevada no Brasil.

Palavras-chave: Lagarta-do-cartucho; *Bacillus thuringiensis*; Manejo da resistência; TC1507; Alta-dose; Base genética



## 1 INTRODUCTION

Genes *cry* from bacterium *Bacillus thuringiensis* Berliner have been introduced in some plants to control insect pests in agriculture (SOBERÓN; GILL; BRAVO, 2009; JAMES, 2011). Cry proteins are produced continuously in tissues of Bt plants and protecting from degradation by environmental factors and increasing the insect control. Bt plants revolutionized the agriculture by replacing insecticides and reducing environmental impact (SOBERÓN; GILL; BRAVO, 2009). The event TC1507 maize with *cry1F* gene from *B. thuringiensis* that codes for Cry1F protein has been approved for commercial release in Brazil in 2008.

One of the target pests of the event TC1507 is the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), a serious insect pest of maize in tropical region of America (ETCHEVERRY, 1957; ESTRADA, 1960; HOROVITZ, 1960; SIFUENTES, 1967; CRUZ, 1995). Many economically important crops such as cotton, rice, sugarcane, beans, maize, wheat, sorghum and soybeans are attacked by *S. frugiperda* (SILVA et al., 1968). There is a high genetic variability in this species (NAGOSHI; MEAGHER, 2008) and two host races have already been characterized (PASHLEY, 1986; BUSATO et al., 2004). Before releasing of Bt maize, *S. frugiperda* was controlled mainly with the use of insecticides in Brazil, but with low efficacy of this strategy because of high and multiple infestations of this species in different crops (SILVA, 1999) and evolution of resistance to major insecticides (DIEZ-RODRIGUEZ; OMOTO, 2001; CARVALHO et al., 2013). With the approval of Bt maize in Brazil, this technology has been an excellent option for *S. frugiperda* control.

The continuous expression of *cry* genes in Bt plants entails in strong selection for resistance in insect pest (McGAUGHEY; WHALON, 1992). Field evolution of resistance to Bt plants has been reported in many countries (VAN RENSBURG, 2007; STORER et al., 2010; DHURUA; GUJAR, 2011; GASSMANN et al., 2011), including *S. frugiperda* to Cry1F in Puerto Rico (STORER et al., 2010). To confirm the resistance, resistant insects to Bt crop should have the ability to survive on Bt plant, from egg to adult stage and produce viable offspring (ANDOW, 2008). But to prove that field failures are caused by resistance, it is necessary to show more details. The first step is show economic control of the insect by Bt crop. For example, Cry1F maize hybrids showed excellent control of *S. frugiperda* in the released commercial period (SIEBERT et al., 2008). The second step is necessary to show

economic damage in the Bt crop. There were reports in Puerto Rico about the unusual damage caused by *S. frugiperda* to Cry1F maize hybrids (STORER et al., 2010). The third step, the resistant insect needs to have the ability to survive in Bt plant. Resistant *S. frugiperda* was not shown to survive in Cry 1F maize yet, but resistant *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) larvae were able to survive in Cry1Ab maize (VAN RENSBURG, 2007). The last step, the resistance needs to be genetically inherited. *S. frugiperda* resistant from Puerto Rico showed inheritance autosomal, and incomplete recessive to Cry1F protein (STORER et al., 2010).

Evolution of resistance is one of major limitations to continued use of Bt crops under field conditions. The main goal of resistance management strategy is to delay or prevent the occurrence of field failures (ANDOW, 2008). High-dose/refuge is the main strategy to prevent Bt field failures. This strategy is a combination of high-dose plants and refuge areas (SHELTON et al., 2000). High-dose is the protein concentration sufficiently high to make the resistance functionally recessive (TAYLOR; GEORGHIOU, 1979; GOULD, 1998). Refuge is non-Bt field that provide susceptible individuals for mating with resistant (McGAUGHEY; WHALON, 1992; SHELTON et al., 2000). Besides, the initial frequency of allele resistance should be less  $1 \times 10^{-3}$  (ROUSH, 1994). If at least one these assumptions fail, resistance evolution will be faster. Besides, others parameters can have influence on evolution of resistance.

High-dose is a measure of dominance that is estimated by similarity of the heterozygote in relation of two homozygotes resistant (WRIGHT, 1929). Therefore, high-dose is the capacity of Bt event to kill all or nearly all heterozygous insect (TABASHNIK; CROFT, 1982; GOULD, 1998; TABASHNIK et al., 2004). Mathematical models have shown slower resistance evolution in high-dose events than low-dose (TABASHNIK; CROFT, 1982; TABASHNIK et al., 2004). Typically dominance has been measure at a fixed mortality, normally 50% or at a given Bt protein concentration (STONE, 1968; CURTIS; COOK; WOOD, 1978). The two ways do not have a huge importance for evolution of resistance. The best way is measure the fitness of three genotypes at plant concentration that is most relevant for resistance evolution (BOURGUET; GENISSEL; RAYMOND, 2000). Many methods have been proposed to estimate the high-dose concept in absence of a resistant population. The most accepted is based on dilution of plant tissue at 25-fold in artificial diet. In this dilution, mortality should be higher than 99% of susceptible

individuals at seven days after infestation of neonate larvae (US-EPA, 1998). Caprio; Sumerford and Sims (2000) suggested being 50-fold. Few works has showed the dominance with plant dilutions. Cry1Ac soybean events TIC107 and MON 87701 × MON 89788 showed to be high-dose for *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), but were not able to kill all susceptible larvae of *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae) in 25-fold tissues dilution, and therefore these events cannot be considered high-dose for this species (MacRAE et al., 2005; BERNARDI et al., 2012).

Resistance management strategies can be more accurately designed if the genetic basis of resistance to Bt crops are better understood (GOULD, 1998; FERRÉ; VAN RIE, 2002). Characterization of resistance to Cry proteins has shown dominance from recessive to incompletely recessive, and genes in autosomal chromosome (MAHON et al., 2007; PEREIRA; STORER; SIEGFRIED, 2008; PETZOLD-MAXWELL et al., 2012; ZHANG et al., 2012). *S. frugiperda* resistant to Cry1F from Puerto Rico was incomplete recessive and autosomal (STORER et al., 2010). In general, resistance to Cry protein has been determined as monogenic (TABASHNIK et al., 1997a; MAHON et al., 2007; PEREIRA; STORER; SIEGFRIED, 2008). Additionally, resistant populations could share the same resistant locus for the same Bt protein in many insect species (TABASHNIK et al., 1997b; TABASHNIK et al., 2004; MAHON; OLSEN; DOWNES, 2008; MAHON et al., 2010; FABRICK; TABASHNIK, 2012; MAHON; DOWNES; JAMES, 2012; ZHANG et al., 2012).

The key element to predict the rate of evolution of resistance is the frequency of resistance allele (FERRÉ; VAN RIE, 2002). The method called F<sub>2</sub> screen can detect the resistance allele even in low frequency (ANDOW; ALSTAD, 1998). This method is based on maintenance of genetic variation in isofemale line, and the resistance alleles are concentrating in homozygote genotype, where they are detected. The limitation of this method is labor intensive and expensive rearing requirements. Besides, this method is useful to determine the frequency of the resistance allele in order to evaluate of resistance management strategies (SIEGFRIED et al., 2007). Frequency of resistance allele to Bt proteins has been estimated by using F<sub>2</sub> screen in many insect species. In *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) in U.S.A., the frequency of major resistance allele to Cry1Ab protein was estimated from 0.0023 to less than 0.0027 (HUANG; LEONARD; ANDOW, 2007; HUANG et al., 2008; HUANG et al., 2009). The estimated

frequency in *H. armigera* was 0.0146 to 0.052 to Cry1Ac in China (XU et al., 2009; ZHANG et al., 2012), and 0.0006 in Australia (DOWNES; MAHON; OLSEN, 2007).

The main field-evolved resistance was reported in Puerto Rico for *S. frugiperda* to Cry1F maize. After four years from commercial release, the commercial sale of Cry1F maize in Puerto Rico was suspended. Evolution of resistance to Cry1F in Puerto Rico may have been due to the isolation of the island, tropical climate, high pest population and drought conditions in 2006/2007 crop season, which reduced the availability of alternative hosts for *S. frugiperda* (STORER et al., 2010). Most of Brazilian agriculture regions have similar climate conditions from Puerto Rico that allowed to cultivate crops all year round (STORER et al., 2012), and this scenario increases the risk of field evolution of resistance. In the Central-west and Western Bahia regions in Brazil, the winter season is dry and hot, but crops, including maize, can be cultivated under center pivot irrigation systems. In this agricultural scenario, *S. frugiperda* is able to have continuous generations throughout the year which increases the pest problem (MARTINELLI et al., 2006; 2007). In Southern Brazil, despite cold winters, rainfall allows crop production during the winter in some regions, which again enables *S. frugiperda* to build up high population densities at the beginning of the summer season. These conditions that favor year-round use of Bt maize in Brazil, the risk of resistance evolution of *S. frugiperda* to Bt maize is high. Therefore, to assess the resistance risk of *S. frugiperda* to Cry1F and implement an Insect Resistance Management (IRM) program in Brazil, the major goals of this research were:

- To establish the baseline susceptibility and to monitor Cry1F protein susceptibility in Brazilian populations of *S. frugiperda* collected from different locations and crop seasons;
- To demonstrate that field failures in TC1507 event in Brazil were associated with field-evolved resistance to Cry1F protein in *S. frugiperda*;
- To determine the dominance of resistance of *S. frugiperda* to Cry1F directly (leaf bioassays) and indirectly (leaf tissue dilution in artificial diet);

- To test if Cry1F resistant *S. frugiperda* populations from different states of Brazil share the same locus of resistance;
- To conduct a F<sub>2</sub> screen to estimate the frequency of resistance allele to Cry1F protein in *S. frugiperda* in Brazil.

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## 2 GEOGRAPHICAL AND TEMPORAL VARIABILITY IN SUSCEPTIBILITY TO CRY1F PROTEIN FROM *Bacillus thuringiensis* IN *Spodoptera frugiperda* (LEPIDOPTERA: NOCTUIDAE) POPULATIONS IN BRAZIL

### Abstract

The genetically modified maize TC1507 event with *cry1F* gene has been used to control *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in Brazil since 2009/2010 cropping season. As part of the Insect Resistance Management program, we conducted studies to determine the baseline susceptibility to Cry1F prior to widespread plantings of TC1507 maize. Subsequently we evaluated the geographical and temporal variability of susceptibility to this protein in populations of *S. frugiperda* collected from major maize-growing regions in Brazil. The baseline susceptibility to Cry1F was determined with diet-overlay bioassay for a susceptible reference population and four field populations of *S. frugiperda*. Then, we monitored the susceptibility to Cry1F in 43 populations of *S. frugiperda* sampled in nine States of Brazil between 2011 and 2013. In the baseline study, the MIC<sub>50</sub> (concentration that inhibits molting to second instar in 50% of individuals) ranged from 3.59 to 72.47 ng of Cry1F protein cm<sup>-2</sup>. Based on the upper limit of the MIC<sub>99</sub> value of the joint analysis from the baseline susceptibility data, the concentration of 2,000 ng of Cry1F protein cm<sup>-2</sup> was defined as a diagnostic concentration for potentially resistant individuals and used in monitoring the susceptibility of *S. frugiperda* to Cry1F. Survival at 2,000 ng of Cry1F protein cm<sup>-2</sup> increased significantly throughout cropping seasons in *S. frugiperda* populations from São Paulo, Santa Catarina, Rio Grande do Sul, Bahia, Mato Grosso, Goiás, Mato Grosso do Sul, and Paraná. Highest survival (> 50%) was reached in populations collected from Bahia, Mato Grosso, Goiás, Mato Grosso do Sul, and Paraná during 2012/2013 cropping season. Therefore, significant decrease in susceptibility to Cry1F was detected in *S. frugiperda* throughout cropping seasons, especially in regions with intensive maize production in Brazil.

Keywords: Fall armyworm; *Bacillus thuringiensis*; Diagnostic concentration; Insect resistance management



### 3 FIELD-EVOLVED RESISTANCE TO CRY1F MAIZE BY *Spodoptera frugiperda* (J.E. SMITH) (LEPIDOPTERA: NOCTUIDAE) IN BRAZIL

#### Abstract

Evolution of resistance may be a potential limiting factor to continued use of Bt proteins in plants. In Brazil, the main target insect of Bt maize has been *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). The Cry1F protein expressed in event TC1507 maize has been one of the most effective ways to control *S. frugiperda*. However, this species has a wide range of biological variability and has evolved resistance to Cry1F under field conditions in Puerto Rico. After reports of reduced effectiveness in areas of Brazil, research was undertaken to investigate if damage in Cry1F maize was caused by resistant *S. frugiperda*. Additional investigations into which methods can be used to define field-evolved resistance were also conducted. A population of *S. frugiperda* was collected from TC1507 maize fields with unexpected damage in Bahia state and divided into two subpopulations. One was subjected to further laboratory selection while the other was not subjected to artificial selection. Both sub-populations were able to survive on Cry1F maize as neonates and subsequently produce normal adults, the artificial selection enhancing the level of resistance. A comparison of bioassay methods indicated that survival of Cry1F-susceptible *S. frugiperda* on non-Bt maize was significantly higher in leaf than plant bioassays. Resistance ratio was higher in overlay bioassays than when Cry1F protein was incorporated into diet. Cry1F resistant *S. frugiperda* showed autosomal inheritance for alleles involved in resistance to Cry1F protein. Progeny of *S. frugiperda* collected in Cry1F maize fields that showed unexpected damage were able to survive on Cry1F maize plants under laboratory conditions from neonate to adult. The ability to survive on the plant showed a genetic basis indicating that the survivors were resistant to Cry1F. Leaf bioassays and diet-overlay bioassays are suitable for characterizing field-evolved resistance by *S. frugiperda*.

Keywords: Fall armyworm; *Bacillus thuringiensis*; Plant survival; Genetic basis; Detection methods



#### 4 DOMINANCE OF CRY1F RESISTANCE ALLELE IN *Spodoptera frugiperda* (J.E. SMITH) (LEPIDOPTERA: NOCTUIDAE) FROM BRAZIL

##### Abstract

Dominance of resistance has been one of major parameters affecting the rate of evolution of resistance to Bt crops. If resistance is functionally recessive, then a Bt crop is high-dose. High-dose is the capacity of Bt crops to kill heterozygous insects and has been an essential component of the most successful strategy to manage resistance to these crops. Experiments were conducted to evaluate directly and indirectly if TC1507 event is high-dose to *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). Some heterozygote neonate larvae were able to survive, complete development and emerge as normal adults on TC1507 leaves, but susceptible larvae could not survive for five days. The estimated dominance of resistance was significantly higher than zero; therefore the resistance to Cry1F expressed in TC1507 was not completely recessive. Dominance was estimated to be  $0.15 \pm 0.09$ . A 25 fold dilution of TC1507 maize leaf tissue was able to cause a maximum mortality of 37% and to inhibit growth of 82% at seven days after infestation. These values were significantly less than 99%. Resistance to Cry1F in TC1507 maize is incompletely recessive in *S. frugiperda*. TC1507 maize is not high-dose for *S. frugiperda*.

Keywords: High-dose; Fall armyworm; *Bacillus thuringiensis*; Resistance management; Recessive; TC1507



## 5 COMPLEMENTATION TEST FOR ALLELISM IN CRY1F RESISTANT *Spodoptera frugiperda* (J.E. SMITH) (LEPIDOPTERA: NOCTUIDAE) POPULATIONS FROM BRAZIL

### Abstract

Field-evolved resistance to Bt plants has been recorded in many countries, insect species and Bt proteins, and resistance has been found to be usually monogenic, autosomal, and from recessive to incompletely recessive alleles. However, it has not been determined if resistance allele were at same locus in the field-evolved resistance populations. Field-evolved resistance was already reported to *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in Puerto Rico and Brazil. Here we propose to test if Cry1F resistance in *S. frugiperda* populations from different states of Brazil shares the same locus. Bioassays were conducted with eight resistant populations and one susceptible population. We first measured the dominance of resistance at 2,000 ng cm<sup>-2</sup> of Cry1F protein in a diet overlay bioassay using two resistant populations. The hybrids between susceptible and two resistant populations were all dead at seven days after infestation with 2,000 ng cm<sup>-2</sup> of Cry1F protein in the overlay bioassay. Therefore, resistance in these two resistant populations was recessive at this Cry1F concentration; in other words this concentration was discriminatory. In complementation tests one resistant population was cross with the other seven resistant populations. F<sub>1</sub> larvae from each cross had the same survival with or without 2,000 ng cm<sup>-2</sup> of Cry1F protein, demonstrating that they are all complementary. The results from the complementation tests showed geographically widespread resistance to Cry1F in *S. frugiperda* in Brazil and strongly suggested that Cry1F resistance alleles in all eight populations were at the same locus.

Keywords: Resistance locus; Fall armyworm; *Bacillus thuringiensis*; Genetic basis; Allele



## 6 FREQUENCY OF CRY1F RESISTANCE ALLELES IN *Spodoptera frugiperda* (J.E. SMITH) (LEPIDOPTERA: NOCTUIDAE) IN BRAZIL

### Abstract

The frequency of resistance alleles is a major factor influencing the rate of evolution of resistance. Although several methods have been proposed to estimate the frequency of resistance alleles, the  $F_2$  screen is the most efficient when resistance is nearly recessive and the resistance allele is at low frequency. This method concentrates a resistance allele in an isofemale line, where it can be detected in the homozygous state. Here we develop a  $F_2$  screen procedure for *Spodoptera frugiperda* (J.E. Smith), and estimate the frequency of resistance allele to Cry1F protein in *S. frugiperda* in Brazil. It was possible to establish 953 isofemale lines in the  $P_0$  generation, and 587 isofemale lines produced eggs in the  $F_2$  generation, and of these 517 isofemale lines were screened in Cry1F protein in overlay bioassay. Populations from Bahia showed the highest frequency of Cry1F resistance allele. The frequency in Bahia was 0.192 with 95% confidence interval (CI) between 0.163 and 0.220. The lowest frequency was in populations from Paraná with 0.042 and 95% CI between 0.025 and 0.063. The frequency of Cry1F resistance allele in Brazil was 0.088 with 95% CI between 0.077 and 0.100.  $F_2$  screen protocol was able to produce enough  $F_2$  larvae to estimate the frequency of resistance allele. Cry1F resistance alleles were not rare, and found at frequencies which may compromise the useful life of TC1507 event in Brazil in the absence of improved resistance management strategies.

Keywords: Resistance; Fall armyworm; *Bacillus thuringiensis*; Evolution; Monitoring



## 7 CONCLUSIONS

- There is a high natural variability in the susceptibility to Cry1F among *S. frugiperda* populations in Brazil. The susceptibility to Cry1F in *S. frugiperda* populations is decreasing throughout crop seasons (2011 to 2013) in Brazil, especially in regions with intensive maize production.
- Progeny of *S. frugiperda* collected in Cry1F maize in field failure had the ability to survive on the plant indicating that the survivors were resistant to Cry1F. Leaf bioassay and diet-overlay bioassay are suitable for characterizing field-evolved resistance to Cry1F in *S. frugiperda*.
- TC1507 maize is not high-dose event for *S. frugiperda*.
- Cry1F resistance alleles from geographically distinct *S. frugiperda* populations in Brazil share the same locus.
- The frequency of Cry1F allele is high in Brazil.