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Dionei Schmidt Muraro

SUSCETIBILIDADE A INSETICIDAS EM LINHAGENS DE *Spodoptera frugiperda* (J. E. SMITH, 1797) COM RESISTÊNCIA AS PROTEÍNAS DE *Bacillus thuringiensis* BERLINER EXPRESSAS EM MILHO

Santa Maria, RS
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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-graduação em Agronomia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Mestre em Agronomia**.

Orientador: Prof. Dr. Oderlei Bernardi

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A Deus, pelo dom da vida e por estar sempre ao meu lado em todos os momentos.

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“Quanto mais aumenta nosso conhecimento, mais evidente fica nossa ignorância”.
(John F. Kennedy)

RESUMO

SUSCETIBILIDADE A INSETICIDAS EM LINHAGENS DE *Spodoptera frugiperda* (J. E. SMITH, 1797) COM RESISTÊNCIA AS PROTEÍNAS DE *Bacillus thuringiensis* BERLINER EXPRESSAS EM MILHO

AUTOR: Dionei Schmidt Muraro
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A lagarta-do-cartucho, *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) é um dos mais importante inseto-praga da cultura do milho no Brazil. O controle desta espécie tem sido realizado principalmente com o uso de milho Bt e inseticidas. No entanto, a evolução da resistência de *S. frugiperda* a proteínas Bt e inseticidas tem ameaçado a sustentabilidade dessas táticas de controle. Nesse sentido, para subsidiar o manejo dessa espécie foram realizados estudos com linhagens de *S. frugiperda* resistentes e suscetíveis em milho Bt e não-Bt para avaliar a eficácia de dois tratamentos de sementes (clorantraniliprole e imidacloprido + tiodicarbe) no controle de infestações iniciais e a sua suscetibilidade aos inseticidas foliares (espinetoram e clorfenapir). Para a realização destes estudos foram selecionadas linhagens de *S. frugiperda* resistentes as proteínas Cry1F, Cry1A.105 + Cry2Ab2 e Cry1A.105 + Cry1F + Cry2Ab2. Além disso, uma linhagem suscetível de referência e hetetorozigotos obtidos de cruzamentos recíprocos foram utilizadas nos experimentos. No primeiro estudo, em laboratório, discos foliares foram obtidos de plantas de milho cujas sementes foram tratadas com clorantraniliprole ou imidacloprido + tiodicarbe, os quais foram acondicionados em placas de bioensaio. Aos 7, 14 e 21 dias após a emergência (DAE) em discos foliares oriundos de plantas com tratamento de sementes a sobrevivência de linhagens resistentes, heterozigotos e suscetível reduziu em até 23,2; 24,8 e 28,2%, respectivamente, quando comparado ao mesmo híbrido sem tratamento de sementes. Em condições de campo, os mesmos tratamentos de sementes apresentaram baixa eficácia no controle de *S. frugiperda*. Somente aos 7 DAE houve redução significativamente no número de plantas com danos (nota de dano inferior a 3 na Escala Davis). No segundo estudo, as mesmas linhagens de *S. frugiperda* foram usadas em bioensaios para avaliação da suscetibilidade a inseticidas aplicados na superfície da dieta artificial (laboratório) e sobre as plantas (casa de vegetação e campo). Em laboratório, as lagartas foram criadas em milho Bt e não-Bt até o terceiro ínstar larval quando foram expostas aos inseticidas espinetoram e clorfenapir aplicados na superfície da dieta. As linhagens de *S. frugiperda* resistentes e heterozigotos apresentaram similar suscetibilidade a espinetoram (CL_{50} = 0,16 a 0,18 ug i.a./cm^2) e clorfenapir (CL_{50} = 0,17 a 0,20 ug i.a./cm^2), quando criadas em milho Bt e não-Bt. No entanto, a linhagem Sus teve maior suscetibilidade aos inseticidas quando se desenvolveu em milho não-Bt; LC_{50} = 0,05 (espinetoram) and 0,08 (clorfenapir) ug i.a./cm^2. Entretanto, em casa de vegetação e campo, não foram detectadas diferenças significativas na sobrevivência de *S. frugiperda* quando a dose comercial de ambos os inseticidas foi aplicada em milho Bt e não-Bt. Os resultados demonstram que o tratamento de sementes de milho com clorantraniliprole ou imidacloprido + tiodicarbe possui baixa eficácia de controle de *S. frugiperda*. Observou-se também que linhagens de *S. frugiperda* que se desenvolveram em milho Bt e não Bt possuem similar suscetibilidade aos inseticidas espinetoram e clorfenapir.

Palavras-chave: Lagarta-do-cartucho, controle químico, tratamento de sementes

ABSTRACT

SUSCEPTIBILITY TO INSECTICIDES IN *Spodoptera frugiperda* (J. E. SMITH, 1797) STRAINS WITH RESISTANCE TO *Bacillus thuringiensis* BERLINER PROTEINS EXPRESSED IN MAIZE

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Fall armyworm, *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) is the most important pest of maize in Brazil. The control of this species has been performed with Bt maize and insecticides. However, the resistance evolution in *S. frugiperda* populations to Bt proteins and insecticides has threatened the sustainability of these control tactics. To support the management os this species, studies with resistant and susceptible strains of *S. frugiperda* were carried out in Bt and non-Bt maize technologies to evaluate the effectiveness of two seed treatments (chlorantraniliprole and imidacloprid + thiodicarb) and also its susceptibility to espinetoram and chlorfenapyr in diet-overlay bioassays (laboratory) and foliar sprays (greenhouse and field). *S. frugiperda* strains resistant to Cry1F, Cry1A.105 + Cry2Ab2 and Cry1A.105 + Cry1F + Cry2Ab2 were selected. In addition, a susceptible reference strain (Sus) and heterozygous obtained from reciprocal crosses were also evaluated were used in the experiments. In the first study, in laboratory leaf discs were obtained from maize plants whose seeds were treated with chlorantraniliprole or imidacloprid + thiodicarb. At 7, 14 and 21 days after emergence (DAE), in leaf discs from plants with seed treatment, the survival of resistant, heterozygous and susceptible strains was reduced up to 23.2; 24.8 and 28.2%, respectively, when compared to the same maize hybrid without seed treatment. Under field conditions, the same seed treatments showed low efficacy against natural infestations of *S. frugiperda*. Only at 7 DAE there was a significant reduction in the number of plants with damage caused by *S. frugiperda*. In the second study, the same strains were used in bioassays to evaluate the susceptibility to insecticides applied in the diet surface and plants. In the laboratory, larvae were grown on Bt and not Bt maize until the third larval instar when they were exposed to the insecticides spinetoram and chlorfenapyr in diet-overlay bioassays. Resistant and heterozygous strains showed similar susceptibility to spinetoram (LC_{50} = 0.16 to 0.18 ug a.i./cm^2) and chlorfenapyr (LC_{50} = 0.17 to 0.20 ug a.i./cm^2), when developed on Bt and non-Bt maize. However, Sus strain was more susceptible to both insecticides when fed on non-Bt maize; LC_{50} = 0.05 (spinoteram) and 0.08 (chlorfenapyr) ug a.i./cm^2. However, in greenhouse and field studies, no significant differences were detected in the survival of *S. frugiperda* when the commercial dose of both insecticides was applied in Bt and non-Bt maize. The results demonstrate that maize seed treatments with chlorantraniliprole or imidacloprid + thiodicarb have low control efficacy of *S. frugiperda*. It was also observed that strains of *S. frugiperda* fed on Bt and non-Bt maize have similar susceptibility to spinetoram and chlorfenapyr.

Keywords: Fall armyworm, chemical control, seed treatment

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1 INTRODUÇÃO

No Brasil, a lagarta-do-cartucho, *Spodoptera frugiperda* (J. E. Smith 1797), é considerada a espécie mais destrutiva do milho (*Zea mays* L.) (CRUZ et al., 1999; CRUZ et al., 2012). O sucesso de *S. frugiperda* como praga se deve a elevada capacidade de dispersão dos adultos (SPARKS, 1979; NAGOSHI et al., 2015), polifagia (NAGOSHI et al., 2015), múltiplas gerações por ano (FITT et al., 2006; FARIAS et al., 2014) e alta capacidade reprodutiva (VALICENTE; COSTA, 1991). Além do milho, a lagarta-do-cartucho ataca as culturas do algodão (MARTINELLI et al., 2006), arroz (BOTTON et al., 1998), amendoim (ISIDRO; ALMEIRA; PEREIRA, 1997), soja (MOSCARDI et al., 1985), sorgo (CORTEZ et al., 1997) trigo (TAKAHASHI et al., 1980), etc.

O controle desta espécie na cultura do milho foi por muito tempo realizado quase que exclusivamente pelo uso de inseticidas químicos sintéticos (CRUZ et al., 1995). Entretanto, o uso de pulverizações foliares de inseticidas para o manejo de *S. frugiperda* em milho é dificultado pelo comportamento larval desta espécie, que tem o hábito de se alojar no interior do "cartucho" dificultando o inseticida de atingir o alvo de controle (CARVALHO et al., 2013). Desta forma, o monitoramento desta espécie é de fundamental importância desde o início do estabelecimento da cultura do milho. De acordo com IRAC as aplicações de inseticidas para *S. frugiperda* em milho, devem ser realizadas quando ≥ 20% das plantas com notas de dano ≥ 3 (plantas com 1 a 5 lesões circulares pequenas) (Davis et al., 1992). A utilização adequada de inseticidas no manejo dessa espécie aumenta a eficácia de controle e também auxilia no Manejo da Resistência de Insetos (MRI).

O controle químico de *S. frugiperda* também tem sido recomendado em tratamento de sementes, para o manejo das infestações iniciais (CRUZ et al., 1999; THARP et al., 2000; AZEVEDO et al., 2004; SAPPINGTON et al., 2018). Entretanto, os agricultores estão utilizando o tratamento de sementes principalmente para evitar danos de *Dichelops* spp. (QUINTELA et al., 2006), *Liogenys fuscus* (SANTOS et al., 2008), *Elasmopalpus lignosellus* (VIANA et al., 2011) e *Agrotis ipsilon* (KULLIK et al., 2011). No entanto, alguns inseticidas foram registrados para uso em tratamento de sementes para o manejo de *S. fugiperda*, dentre os quais: clorantraniliprole (Dermacor, Corteva Agrisciences) e imidacloprido + tiodicarbe (CropStar, Bayer CropScience) (AGROFIT, 2018). Clorantraniliprole é um inseticida do grupo químico das diamidas, ligando-se aos receptores de rianodina nas células musculares, fazendo com que o canal se abra e promova saída descontrolada de Cálcio (Ca^{+2}) do reticulo endoplasmático, inicialmente provocando paralisia muscular e consequentemente a morte do inseto (CORDOVA et al., 2006; LAHM et al., 2007). As diamidas possuem boa ação residual, baixa toxicidade a insetos benéficos e mamíferos, provocando menores danos ao meio ambiente (EBBINGHAUS-KINTSCHER, 2006). Por outro lado, o imidacloprido é um neonicotinoide que mimetiza a ação da acetilcolina e não é degradado pela acetilcolinesterase. Esse inseticida liga-se ao receptor da acetilcolina na membrana das células pós-sinápticas, abrindo canais seletivos de íons$^+$ na mesma, com consequente hiperatividade nervosa, seguido de colapso do sistema nervoso (SCHROEDE, 1984). O tiodicarbe é um carbamato que atua inibindo a atividade da acetilcolinesterase, causando um acúmulo de acetilcolina na região sinaptica. A neuroexcitação excessiva ocorre por causa da ligação prolongada da acetilcolina ao seu receptor pós-sináptico. Os sinais de intoxicação incluem inquietação, hiperexcitabilidade, tremores, convulsões e paralisia (FOKUTO, 1990).

Outra tática de controle de *S. frugiperda* foi liberada para cultivo a partir de 2007, quando se iniciou o uso comercial de eventos de milho transgênicos que expressam proteínas de *Bacillus thuringiensis* Berliner (Bt) no Brasil (CTNBIO, 2007). O uso dessa tática de controle aumentou gradativamente, sendo utilizada atualmente em mais de 15 milhões de hectares/ano, representando aproximadamente 90% da área de cultivo de milho no Brasil (CÉLERES, 2018). Atualmente, o uso de milho Bt é uma das principais táticas de controle de *S. frugiperda* em milho no Brasil (WAQUIL et al., 2013; BERNARDI et al., 2015). O uso desta tática de controle tem contribuído para a redução no uso de inseticidas em milho (KLÜMPER; QAIM, 2014; BURTET et al., 2017). Essa redução é importante para o restabelecimento da suscetibilidade a estes produtos em populações dessa espécie-praga (MARTINELLI; OMOTO, 2005). Entretanto, o uso exagerado de inseticidas e a adoção contínua de milho Bt para o controle de *S. frugiperda* associado a não utilização das estratégias de MRI, expos as populações da praga a uma intensa pressão de seleção, favorecendo a evolução da resistência.

Desta forma, o conceito de resistência segundo o IRAC pode ser definido como uma mudança hereditária na suscetibilidade de uma população da praga que se reflete na falha repetida de um produto de atingir o nível de controle esperado, quando utilizado de acordo com a recomendação do rótulo para determinada espécie praga. No Brasil, a resistência de *S. frugiperda* foi reportada para as proteínas Cry1F (milho TC1507) e Cry1Ab (milho MON810) expressas em milho (FARIAS et al., 2014; OMOTO et al., 2016). Em laboratório também foi reportada a resistência de *S. frugiperda* ao milho YieldGard VT PRO que expressa Cry1A.105 e Cry2Ab2 (BERNARDI et al., 2015; SANTOS-AMAYA et al., 2015; HORIKOSHI et al., 2016) e PowerCore com Cry1A.105, Cry1F e Cry2Ab2 (HORIKOSHI et al., 2016; BERNARDI et al., 2017). Além da resistência as proteínas Bt expressas em milho, a evolução da resistência dessa espécie também foi reportada para os inseticidas lambda-cialotrina (DIEZ-RODRÍGUEZ; OMOTO, 2001), clorpirifós (CARVALHO et al., 2013), lufenurom

(NASCIMENTO et al., 2016) e espinosade (OKUMA et al., 2018). Atualmente, mais de 20 ingredientes ativos apresentam relatos de resistência em *S. frugiperda* em todo mundo (IRAC, 2018).

Nesse cenário, o uso de estratégias de MRI é de fundamental importância para a continuidade do uso de plantas Bt e inseticidas no manejo de *S. frugiperda*. Dentre as estratégias de MRI para plantas Bt destaca-se a expressão em alta dose das proteínas inseticidas e a piramidação de genes em associação com áreas de refúgio (GOULD, 1998; BRAVO; SOBERÓN, 2008; HUANG et al., 2011). Entende-se por alta dose a expressão da proteína Bt pelo menos 25 vezes o que seria necessário para matar 99% de uma população suscetível, ou seja, uma concentração de proteína Bt suficiente para tornar a resistência funcionalmente recessiva (TAYLOR; GEORGHIOU, 1979; GOULD, 1998). Essa estratégia proporciona baixa ou nehuma sobrevivência de insetos heterozigotos os quais, no início do processo de evolução da resistência, são os principais carreadores dos alelos da resistência (GOULD, 1998).

As áreas de refúgio servem como um "reservatório" de insetos suscetíveis, na qual podem sobreviver, reproduzir e acasalar-se com os indivíduos sobreviventes em áreas com plantas Bt. Assim a geração subsequente será composta novamente em sua maioria por insetos heterozigotos, os quais serão suscetíveis a (s) proteína (s) Bt, caso o evento atingir os requisitos de alta dose (ANDOW, 1998). A mortalidade dos heterozigotos é fundamental para manter a frequência da resistência baixa (GOULD, 1998; CAPRIO et al., 2000). Por outro lado, a piramidação de genes consiste da expressão de duas ou mais proteínas Bt em plantas de milho com elevada atividade inseticida contra a mesma espécie (GHIMIRE et al., 2011; CARRIÈRE; CRICKMORE; TABASHNIK, 2015). Essa estratégia possibilita que somente os indivíduos homozigotos resistentes à ambas as proteínas Bt expressas na planta possam sobreviver (BREVAULT et al., 2013). O sucesso das plantas piramidadas também depende da disponibilidade de áreas de refúgio, baixa frequência inicial de alelos de resistência a cada proteína Bt na pirâmide e ausência de resistência cruzada entre as proteínas expressas na planta (CARRIÈRE; CRICKMORE; TABASHNIK, 2015). Sendo assim, para o sucesso do manejo de resistência de *S. frugiperda* a proteínas Bt expressas em milho se faz necessário a adoção de áreas de refúgio e isso tem sido negligenciado no Brasil (menos de 20% dos agricultores usam o refúgio).

No que diz respeito ao MRI para inseticidas algumas práticas podem contribuir para evitar ou retardar a evolução da resistência dessa espécie, tais como: rotação de inseticidas com modo de ação distinto, aplicação somente quando atingir o nível de controle, uso de doses recomendadas e aplicação de inseticidas em condições meteorológicas adequadas (ROSA;

MARTINS, 2011). Além disso, o uso de outras táticas de controle como tratamento de sementes (CECCON et al., 2004), fungos entomopatogênicos (THOMAZONI et al., 2014) e baculovírus (THÉZÉ et al., 2015; BENTIVENHA et al., 2018), também podem auxiliar no manejo da resistência de *S. frugiperda* a plantas Bt e inseticidas usados em pulverização foliar.

Portanto, para refinar as estratégias de manejo de *S. frugiperda* na cultura do milho neste estudo objetivou-se:

1) Avaliar a eficácia do tratamento de sementes (clorantraniliprole e imidacloprido + tiodicarbe) aplicado em milho Bt e não Bt no controle de infestações iniciais de *S. frugiperda*.

2) Avaliar a sobrevivência de linhagens de *S. frugiperda* resistentes, heterozigotos e suscetíveis em milho Bt e não Bt e a sua suscetibilidade a inseticidas recomendados em aplicação foliar.

2 ARTIGO 1

Efficacy of seed treatments applied on Bt and non-Bt maize against fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Highlights

The efficacy of seed treatments on Bt and non-Bt maize against fall armyworm (FAW) were evaluated.

The seed treatments tested were chlorantraniliprole alone and imidacloprid and thiodicarb combined.

Laboratory assays using FAW strains and field studies in natural infestation were performed.

The seed treatments on Bt and non-Bt maize present low efficacy against FAW.

ABSTRACT

Fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), is the main pest of maize in Brazil, attacking maize plants from emergence to repropductive stages. We evaluated the efficacy of two seed treatments (chlorantraniliprole alone or imidacloprid combined with thiodicarb) on Bt and non-Bt maize in laboratory assays with distinct FAW strains and in the field against a natural infestation. In the laboratory, leaf-discs from seed treated Bt-maize plants at 7 days after emergence (DAE) increased the mortality of FAW resistant, heterozygote and susceptible strains up to 24.8%, when compared with the respective maize without a seed treatment. In the field, the same seed treatments showed low efficacy against natural infestations of FAW. At 7 and 14 DAE, Bt maize with seed treatment had 31.1% less FAW damage than non-Bt maize with the same seed treatment. At these times, seed treated Bt maize also reduced the number of plants with significant damage (rating ≥ 3), although not significantly different at 14 DAE. No differences in FAW damage was observed between Bt and non-Bt maize grown with and without a seed treatment at 21 DAE. Our results demonstrate that maize seed treated with chlorantraniliprole alone or imidacloprid and thiodicarb combined present low efficacy against FAW strains in laboratory and field conditions.

Keywords: transgenic maize; insecticides; resistance management

1. Introduction

Fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith, 1797) is the main insect pest of maize (*Zea mays* L.) in South America (Cruz et al., 2012; Blanco et al., 2016). This species attacks maize plants from emergence to reproductive stages. Early infestation of FAW on maize can reduce plant population and cause yield losses up to 57% when control strategies are not used (Cruz et al., 1999). For its management, the use of maize hybrids expressing *Bacillus thuringiensis* Berliner (Bt) proteins is the main control tactic used in Brazil (Bernardi et al., 2014). In the first years of commercial Bt maize use, there was a decrease in foliar applications of insecticide against FAW in maize fields (Burtet et al., 2017). However, the low adoption of a refuge with non Bt maize, as part of an Insecticide Resistance Management (IRM) plan, exposed FAW populations to an intense selection pressure, resulting in the evolution of resistance to Bt proteins expressed in maize. In Brazil, field-evolved resistance of FAW to Cry1F and Cry1Ab in maize were reported (Farias et al., 2014a; Omoto et al., 2016). From laboratory assays, FAW has shown resistance to maize expressing Cry1A.105 and Cry2Ab2 (Santos-Amaya et al., 2015; Horikoshi et al., 2016), and maize containing Cry1A.105, Cry2Ab2 and Cry1F (Horikoshi et al., 2016; Bernardi et al., 2017). Resistance to Bt maize by FAW was also reported in Puerto Rico (Storer et al., 2010), some areas of the southeastern region of the United States (Huang et al., 2014) and Argentina (Chandrasena et al., 2017).

In Brazil, since FAW has evolved resistance to Bt maize, there has been an increase in insecticide use against this species in this crop (Burtet et al., 2017). However, the efficacy of insecticides is directly influenced by the larval behavior of FAW. As FAW larvae grow, they move into the maize whorl, reducing their exposure to foliar applications of insecticide (Ceccon et al., 2004). In addition, populations of FAW in Brazil have evolved resistance to the following insecticides: lambda-cyhalothrin (Diez-Rodriguez & Omoto, 2001; Diez-Rodriguez et al., 2011), chlorpyrifos (Carvalho et al., 2013), lufenuron (Nascimento et al., 2016), and spinosad (Okuma et al., 2018).

Additional control tactics are needed for the management of FAW on Bt and non-Bt maize. To manage early infestations of this species, the use of seed-applied insecticides (i.e. a seed treatments) are commonly performed (Cruz et al., 1999; Azevedo et al., 2004; Ceccon et al., 2004; Quintela et al., 2006). Many Brazilian farmers use a seed treatment to prevent damage to maize seeds and seedlings caused by *Dichelops* spp. (Quintela et al., 2006), *Liogenys fusca* (Blanchard) (Santos et al., 2008), *Elasmopalpus lignosellus* (Zeller) (Viana et al., 2011) and *Agrotis ipsilon* (Hufnagel) (Kullik et al., 2011). Specifically, for FAW, previous studies evaluating seed treatment with carbofuran or thiamethoxam demonstrated low efficacy against early infestations of FAW in maize (Azevedo et al., 2004). In contrast, when thiodicarb was used as a seed treatment, the number of maize plants damaged by FAW was reduced (Ceccon et al., 2004). In recent years, a diamide have been suggested as seed treatment for managing FAW, but their efficacy against FAW remains unknown.

Given this knowledge gap, we conducted a series of laboratory and field experiments to determine if the addition of a seed treatment to maize (either with or without Bt) provided protection early to FAW. We hypothesized that the response of FAW to a seed treatment may vary based on the population's susceptibility to Bt-toxins. We evaluated the efficacy of two commercially available seed treatments (chlorantraniliprole alone and imidacloprid combined with thiodicarb) on Bt and non-Bt maize against the FAW. We conducted these evaluations with laboratory bioassays using distinct FAW strains that varied in their susceptibility to Bt toxins (Cry1F, Cry1A.105 + Cry2Ab2 and Cry1F + Cry1A.105 + Cry2Ab2). In addition, we conducted field trails with these seed treatments and maize varieties using natural infestation of FAW.

2. Material and methods

2.1 FAW strains

The F_2 screen method was used to select FAW strains with capability to survive on Bt protein-expressing maize lines (Androw et al., 1998). We selected populations with high survival on maize expressing the following Bt proteins: Cry1F (Herculex; P3779H, DuPont Pioneer, Santa Rosa-RS, Brazil), Cry1A.105 and Cry2Ab2 (YieldGard VT PRO; DKB390PRO, Dekalb, Uberlandia-MG, Brazil) and Cry1A.105, Cry1F and Cry2Ab2 (PowerCore; 2A620PW, Dow AgroSciences, Paracatu-MG, Brazil). The FAW colonies resistant to Cry1F (hereafter H-R), Cry1A.105 and Cry2Ab2 (hereafter Y-R) and Cry1A.105, Cry1F and Cry2Ab2 (hereafter P-R) were selected using leaf tissue bioassays (Bernardi et al., 2015) from a field population collected during the 2016–2017 growing season in Paulínia, São Paulo, Brazil (22°42'38"S and 47°06'26"W). These larvae were reared to adult stage on excised leaves of the respective Bt maize on which they were selected (survival > 80%). The adults were used to establish the resistant colonies. During six generations, resistant colonies were reared from neonate to third instar on the respective Bt maize on which they were selected. Subsequently, third instar larvae were transferred to artificial diet where they remained until the pupae stage (Bernardi et al., 2015). We used a laboratory strain of FAW that has been maintained in the laboratory since 2012 without exposure to Bt-proteins or insecticides. We refer to this colony as a susceptible strain (Sus). To evaluate heterozygous strains, the reciprocal cross between resistant ♀ × susceptible ♂ were performed for all three resistant strains. We only used these heterozygote strains because inheritance of resistance is not a sex-linked trait, and heterozygotes have demonstrated similar mortality-response to Bt proteins in artificial diet and leaf bioassays (Store et al., 2010; Farias et al., 2014b; Bernardi et al., 2015; Santos-Amaya et al., 2015; Horikoshi et al., 2016; Bernardi et al., 2017; Chandrasena et al., 2018).

2.2. Efficacy of seed treatment applied to Bt and non-Bt maize against FAW strains in laboratory bioassays

The following maize hybrids were used in the laboratory bioassays: Herculex expressing Cry1F protein (P3779H, DuPont Pioneer, Santa Rosa, RS, Brazil), YieldGard VT PRO expressing Cry1A.105 and Cry2Ab2 proteins (DKB390PRO, Dekalb, Uberlândia, MG, Brazil) and PowerCore expressing Cry1A.105, Cry1F and Cry2Ab2 proteins (30A37PW, Dow AgroSciences, Jardinópolis, SP, Brazil), and a non-Bt maize (30A37, Dow

AgroSciences, Jardinópolis, SP, Brazil). Each variety was treated with the recommended dose of two maize seed treatments: chlorantraniliprole (Dermacor, Corteva Agriscience, Marinette, WI, USA) at 30 g a.i. per 60,000 seeds, and imidacloprid + thiodicarb (CropStar, Bayer CropScience, Belford Roxo, RJ, BR), at 52.5 + 157.5 g a.i. per 60,000 seeds. Maize seeds were sowed in a field at a density of 90,000 seed/ha with a row spacing of 0.50 m (four rows of 6 m in length, per maize treatment). Maize whorl leaves were collected for use in the bioassays at 7, 14 and 21 days after emergence (DAE) representing the V_2, $V_{3\text{-}4}$, and V_5 maize growth stages, respectively. Leaf tissue from each plant was tested for Bt protein expression using the QuickStix™ Kit (Envirology, Portland, OR, USA) for Cry2A and Cry1F.

In the laboratory, 1.2 cm diameter leaf-discs were cut from the maizwhorl leaf randomly and placed on a 2.5% mixture of water-agar (1ml per well) in plastic plates (CM&CM Comércio de Plásticos, São Paulo, SP, Brazil) with 128 wells. The leaf-discs were separated from the water-agar layer with a disc of filter paper. Subsequently, one FAW neonate larvae (resistant, heterozygous or susceptible strain) was placed on each leaf-disc. Plates were sealed with self-adhesive plastic sheets that allow gas exchange with the external environment and placed in a growth chamber (temperature: 25±5ºC; relative humidity: 60±10%; photoperiod: 14:10 h light: dark). The experimental design in the laboratory was completely randomized with eight replicates per treatment (16 neonates per replicate). Larval survival was evaluated at five days. Larvae were considered dead when they showed no apparent movement after a slight touch with a fine paintbrush. Larval survival data were subjected to studentized residuals analysis to confirm the assumption of normality using Shapiro-Wilk test (PROC UNIVARIATE) and for homogeneity of variances with Bartlett test (PROC GLM) in SAS® 9.1 (SAS Institute, 2002). Data were subjected to analysis of variance (ANOVA) using the PROC GLM procedure in SAS® 9.1 (SAS Institute, 2002). Treatment differences were determined with a least-square means statement (LSMEANS option in PROC GLM) using a Tukey adjustment ($P > 0.05$) in SAS® 9.1 (SAS Institute, 2002).

2.3. Efficacy of a seed treatment on Bt and non-Bt maize against natural infestations of FAW

To evaluate the efficacy of seed treatments against natural infestations of FAW on Bt and non-Bt maize, a field study was performed using the same two seed treatments and four maize varieties described above. Each maize variety was planted on 19 January 2018 at a density of 90,000 seed per ha, with an application of 225 kg per ha of Nitrogen–Phosphorus–Potassium (NPK; 5–20–20). Each combination of maize variety and seed treatment were planted in replicated plots comprised of five rows, 4 m in length and with a spacing of 0.50 m between rows.

Treatments were distributed in a 4 × 3 factorial arrangement, and each block contained 12 plots. The first factor (A) was represented by three Bt maize varieties (Cry1F, Cry1A.105 and Cry2Ab2, and Cry1A.105, Cry1F and Cry2Ab2) and one non-Bt maize. The second factor (B) was composed of two seed treatments (chlorantraniliprole alone and imidacloprid + thiodicarb combined) and without seed treatment. Damages caused by natural infestation of FAW was estimated on 20 plants of the two central rows of each plot at 7, 14 and 21 days after plants emerged. A damage rating was attributed to each plant according to the Davis scale: 0 = no damage to 9 = severe damage (Davis et al., 1992). These results were then converted to percentage of plants damaged by FAW and percentage of plants with damage rating ≥ 3 (circular and/or elongated lesions up to 1.3 cm). The number of plants with a damage rating ≥ 3 has been suggested as a threshold for foliar insecticide applications against FAW in Brazil. If $\geq 20\%$ of the plants within a plot received rating ≥ 3 the plot was considered to be damaged significantly (IRAC, 2018). Data were subjected to two-way ANOVA using the PROC GLM procedure in SAS® 9.1 (SAS Institute, 2002). Seed treatments, maize technologies, and their interactions were considered fixed factors in the model.

3. Results

3.1 Selection of the FAW resistant colonies

FAW resistant strains to Cry1F, Cry1A.105 + Cry2Ab2, and Cry1A.105 + Cry1F + Cry2Ab2 were selected using the F_2 screen method (Andow et al., 1998). In total, 102 isofamilies were screened on each Bt maize and 27 families had surviving larvae (more than 80% survival).

3.2. Efficacy of seed treatment against FAW strains in laboratory bioassays

We observed a different response to seed treatments by the seven strains of FAW. This effect was most noticeable at 7 and 14 DAE. At 7 DAE, seed treatment provided up to 23.2% increase in mortality of Bt-resistant strains of FAW on Bt maize. FAW strain resistant to Cry1A.105 + Cry1F + Cry2Ab2 fed on plant from seed treated non-Bt maize had a significant reduction in survival compared with the control treatment without seed treatment. Significant reductions in the survival of FAW larvae were also observed for the heterozygotes strain (Cry1F) and for the susceptible strains on non-Bt maize. For these two strains, seed treatment significantly reduced FAW survival (Table 1).

At 14 days after plants emergence, seed treatment significantly reduced the survival of the Y-R and P-R strains fed on leaves of Cry1A.105 + Cry2Ab2 and Cry1A.105 + Cry1F + Cry2Ab2 maize, respectively. Cry1A.105 + Cry2Ab2 with chlorantraniliprole alone and imidacloprid + thiodicarb combined reduced larvae survival by 16.3 and 6.4%, respectively. Similarly, Cry1A.105 + Cry1F + Cry2Ab2 reduced larval survival by 17.3% (chlorantraniliprole) and 14.2% (imidacloprid + thiodicarb). Neonates from the H-R heterozygous strain had significant lower survival on Bt-maize with a seed treatment (68.7 and 73.4%; chlorantraniliprole and imidacloprid + thiodicarb, respectively) than without a seed treatment (81.2% survival). Seed treatments with chlorantraniliprole alone and imidacloprid + thiodicarb combined on the Cry1F maize reduced the heterozygote survival, however, this did not differ significantly from the control treatment.

No significant reduction in FAW survival was observed at 21 DAE, regardless of the seed treatment and the FAW strain. However, FAW strains had significant lower survival rates when fed on Bt maize than on non-Bt maize (Table 3). Neonates from Y-R and P-R ♀ × susceptible ♂ did not survive on their respective Cry1A.105 + Cry2Ab2 and Cry1A.105 + Cry1F + Cry2Ab2. Larvae from the susceptible strain also did not survive on Bt maize technologies. However, on non-Bt maize, the susceptible strain had a similar survival on leaf-discs from plants with or without seed treatment (> 89%) (Table 3). These results indicate that at 21 DAE, the seed treatments were not a source of FAW mortality.

3.3. Efficacy of seed treatment applied to Bt and non-Bt maize against natural infestation of FAW

There was no significant seed treatment × Bt maize interaction for the percentage of plants damaged by FAW and plants with significant damage at 7, 14 and 21 DAE (Table 4). However, the number of plants damaged by FAW and plants with significant damage (i.e. rating ≥ 3) at 7 and 14 DAE did vary significantly across the maize varieties. Seed treatment only had significantly effects at 7 DAE for the percentage of plants with damage and plants with significant damage.

At 7 DAE, Cry1A.105 + Cry1F + Cry2Ab2 maize emerged from seeds treated with chlorantraniliprole had fewer plants damaged by FAW (44%) and fewer plants with significant damage (14.2%) than the non-Bt maize with this seed treatment (Table 5). At this time, Cry1F and Cry1A.105 + Cry2Ab2 maize with chlorantraniliprole had a similar amount of damaged plants (61 and 50%, respectively) than the non-Bt variety (66%). However, we observed fewer Cry1A.105 + Cry2Ab2 plants with significant damage (16%) than Cry1F and the non-Bt maize (> 28%). Cry1A.105 + Cry1F + Cry2Ab2 maize treated with chlorantraniliprole had fewer plants damaged than Cry1F, Cry1A.105 + Cry2Ab2 and non-Bt maize with this seed treatment at 14 DAE. The percentage of plants with significant damage was lower on Cry1A.105 + Cry2Ab2 and Cry1A.105 + Cry1F + Cry2Ab2 (40 and 48.7%, respectively) than on the Cry1F and non-Bt maizes (≥ 67% of plants).

When maize seeds were treated with imidacloprid + thiodicarb combined, fewer FAW damaged plants were observed on Cry1A.105 + Cry2Ab2 (41 and 58.7%) and Cry1A.105 + Cry1F + Cry2Ab2 (36 and 56.2%) than on Cry1F (49 and 78.7%) and non-Bt (more than 61% of plants damaged) at 7 and 14 DAE (Table 5). In contrast, Cry1F and non-Bt maize had the percentage of plants with significant damage superior to 20 (at 7 days) and 63% (at 14 days). At 21 days, Bt and non-Bt maize emerged from seeds treated with imidacloprid + thiodicarb did not differ in damage caused by FAW (Table 5).

Only at 7 DAE we observed a significant difference in FAW damage among plants grown with or without a seed treatment (Fig. 1). The use of a seed treatment reduced both the % of plants showing any FAW damage, as well as plants with significant damage. However, there was no significant difference between chlorantraniliprole alone and imidacloprid + thiodicarb combined regardless of the maize variety. Seed with imidacloprid + thiodicarb on Cry1F and non-Bt maize had a significant lower percentage of damaged plants compared with chlorantraniliprole and without seed treatment. The percentage of plants with significant damage was significantly lower when Cry1F, Cry1A.105 + Cry2Ab2 and non-Bt maize were seed treated with chlorantraniliprole alone and imidacloprid + thiodicarb combined. Cry1A.105 + Cry1F + Cry2Ab2 with imidacloprid + thiodicarb combined had significantly lower percentage of plants with significant damage than the control without seed treatment, however, it did not differ significantly from the treatment with clorantraniliprole alone.

4. Discussion

The insecticides chlorantraniliprole (IRAC MoA group 28) alone and imidacloprid (IRAC MoA group 4) + thiodicarb (IRAC MoA group 1) combined used as seed treatment in Bt and non-Bt maize significantly affected the survivorship of FAW strains. In the laboratory bioassays, at 7 and 14 DAE, a higher mortality of FAW strains on leaves of Bt and non-Bt maize obtained from plants grown with a seed treatment was observed. Only the heterozygote strain from H-R ♀ × susceptible ♂ survived on Cry1F maize. This occurs because Cry1F maize did not meet the high-dose concept for FAW (Farias et al., 2016; Santos-Amaya et al., 2016). The heterozygote strain also had low mortality when exposed to Cry1F maize grown with a seed treatment. In contrast, we observed no survival by heterozygous neonates exposed to leaves of Cry1A.105 + Cry2Ab2 and Cry1A.105 + Cry1F + Cry2Ab2, with and without seed treatments.

In field conditions, at 7 DAE, Bt maize with seed treatments presented significant lower damage by FAW than non-Bt maize without seed treatment. At these times, seed treated Cry1A.105 + Cry2Ab2 and Cry1A.105 + Cry1F + Cry2Ab2 with chlorantraniliprole alone or imidacloprid + thiodicarb combined showed less FAW damage than Cry1F and non-Bt with the same seed treatments. This suggests that foliar insecticide sprays against FAW can be delayed when seed treatments are used in these pyramided maize events. However, the reduction in FAW damages was not sufficient to maintain the damages below the economic threshold level after 7 DAE (Cruz et al., 1995).

In previous studies, chlorantraniliprole in seed treatment on soybean and maize also showed low efficacy against FAW (Trash et al., 2013) and *Mythimna unipuncta* Haworth (Carscallen et al., 2018). A low efficacy of thiamethoxam, carbofuran, imidacloprid and fipronil applied in maize seeds against FAW was also reported (Ceccon et al., 2004). Furthermore, carbofuran and thiamethoxan applied in seed treatment did not reduce FAW damage in non-Bt maize plants (Azevedo et al., 2004). Seed treatment with imidacloprid +

thiodicarb on non-Bt maize was not effective against *Diatraea saccharalis* (Fabricius) (Farias et al., 2013).

The effects of seed treatments against insects that feed on leaves may be associated with insecticide translocation in the plant (Stamm et al., 2016; Lanka et al., 2013). Indeed, the insecticides chlorantraniliprole and imidacloprid when applied as seed treatment have been shown to be transported upward throughout the plant via xylem (Lahm et al., 2007; Stamm et al., 2015; Chen et al., 2015; Carscallen et al., 2018). However, insecticide uptake and translocation may vary across plant species and growth stages (Lanka et al., 2013; Chen et al., 2015). For example, clothianidin used as seed treatment in maize had low translocation in the plants throughout the growing season and has been associated with reports of inconsistent efficacy against early infestation of pest species (Alford et al., 2017). In addition, the soil conditions of each region may alter the efficacy of seed treatment (Stamm et al., 2016). This might explain the low efficacy of seed treatment against early infestations of FAW in maize observed in our study.

Our results demonstrated that in the early growth stages of maize, seed treatments with chlorantraniliprole or imidacloprid + thiodicarb can reduce FAW damage. However, this may not be sufficient to delay or reduce foliar insecticide sprays to prevent damage by FAW. The field experiment was conducted in the late planting season and a higher infestation of FAW after plants emergence was observed, which may have impact on the efficacy of seed treatments against early infestations of FAW. Indeed, higher infestations of FAW in maize have been observed in the late planted maize in southern Brazil and up to four foliar insecticides sprays were necessary to prevent yield loss (Burtet et al., 2017).

Monitoring both the presence of FAW larvae and damage on Bt and non-Bt maize plants is essential for supporting decision making regarding the use of synthetic insecticides to prevent economic losses. According to the Insect Resistance Action Committee, the use of insecticides against FAW on Bt maize and non-Bt maize is recommended when 20% of the plants show a damage rating ≥ 3 (IRAC, 2018). Bt maize could also be integrated with other control tactic such as biological control with baculovirus. Recently, formulated insecticide with *Spodoptera frugiperda* nucleopolyhedrovirus was released for commercial use in Brazil and showed high efficacy against FAW strains (Bentivenha et al., 2018). This would represent the resumption of IPM against FAW in maize in Brazil and would contribute to the IRM programs.

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Table 1

Survival of FAW neonates (% ± Standard Error) on Bt and non-Bt maize with and without a seed treatment at 7 days after plant emergence in laboratory bioassays.

Seed treatment	**Resistant strain**[a,b]		**Heterozygote strain**[a,b]		**Susceptible strain**[a]	
	Cry1F maize	Non-Bt maize	Cry1F maize	Non-Bt maize	Cry1F maize	Non-Bt maize[c]
Chlorantraniliprole	81.0 ± 4.2 aA	92.1 ± 1.5 aB	68.2 ± 2.6 aA	89.0 ± 1.5 aB	0.0 ± 0.0 aA	68.7 ± 2.2 aB
Imidacloprid + thiodicarb	79.6 ± 1.5 aA	92.1 ± 2.9 aB	59.5 ± 4.4 aA	93.7 ± 4.4 aB	0.0 ± 0.0 aA	83.0 ± 1.6 bB
Control	96.8 ± 1.8 bA	94.3 ± 6.0 aA	84.3 ± 1.8 bA	99.3 ± 0.0 aB	0.0 ± 0.0 aA	96.9 ± 1.2 cB
	Cry1A.105 + Cry2Ab maize	Non-Bt maize	Cry1A.105 + Cry2Ab maize	Non-Bt maize	Cry1A.105 + Cry2Ab maize	Non-Bt maize
Chlorantraniliprole	75.0 ± 5.7 abA	87.5 ± 3.6 aB	0.0 ± 0.0 aA	89.1 ± 3.5 aB	0.0 ± 0.0 aA	68.7 ± 2.2 aB
Imidacloprid + thiodicarb	67.1 ± 1.5 aA	92.1 ± 2.7 aB	0.0 ± 0.0 aA	89.8 ± 3.3 aB	0.0 ± 0.0 aA	83.0 ± 1.6 bB
Control	86.0 ± 3.0 bA	99.2 ± 0.6 aB	0.0 ± 0.0 aA	99.0 ± 0.2 aB	0.0 ± 0.0 aA	96.9 ± 1.2 cB
	Cry1A.105 + Cry1F + Cry2Ab2 maize	Non-Bt maize	Cry1A.105 + Cry1F + Cry2Ab2 maize	Non-Bt maize	Cry1A.105 + Cry1F + Cry2Ab2 maize	Non-Bt maize
Chlorantraniliprole	63.2 ± 1.4 aA	68.7 ± 0.2 aA	0.0 ± 0.0 aA	85.2 ± 3.8 aB	0.0 ± 0.0 aA	68.7 ± 2.2 aB
Imidacloprid + thiodicarb	70.5 ± 3.9 bA	79.0 ± 1.5 bB	0.0 ± 0.0 aA	93.2 ± 1.2 aB	0.0 ± 0.0 aA	83.0 ± 1.6 bB
Control	86.4 ± 1.7 cA	99.8 ± 0.3 cB	0.0 ± 0.0 aA	98.4 ± 1.5 aB	0.0 ± 0.0 aA	96.9 ± 1.2 cB

[a] Means within a column followed by the same lowercase letter for each maize and in a row followed by the same uppercase letter for each FAW strain in Bt and non-Bt maize are not significantly different (LSMEANS with Tukey's adjustment; *P* > 0.05).

[b] Neonates from resistant and heterozygous strains were exposed to insecticides applied in seed treatment only in the respective Bt maize which were selected (H-R strain on Cry1F, Y-R strain on Cry1A.105 + Cry2Ab and P-R strain on Cry1A.105 + Cry1F + Cry2Ab2).

[C] Only one susceptible strain and one non-Bt maize were used in the bioassays.

Table 2

Survival of FAW neonates (% ± Standard Error) on Bt and non-Bt maize with and without seed treatments at 14 days after plant emergence in laboratory bioassays.

Seed treatment	Resistant strain[a,b]		Heterozygote strain[a,b]		Susceptible strain[a]	
	Cry1F maize	Non-Bt maize	Cry1F maize	Non-Bt maize	Cry1F maize	Non-Bt maize[c]
Chlorantraniliprole	89.0 ± 2.5 aA	89.8 ± 1.7 aA	68.7 ± 0.8 aA	95.3 ± 1.6 aB	0.0 ± 0.0 aA	96.8 ± 1.2 aB
Imidacloprid + thiodicarb	90.0 ± 3.5 aA	92.2 ± 1.9 aA	73.4 ± 1.0 aA	96.8 ± 1.7 aB	0.0 ± 0.0 aA	97.6 ± 1.1 aB
Control	93.6 ± 1.6 aA	96.8 ± 1.1 aA	81.2 ± 1.6 bA	96.8 ± 1.2 aB	0.0 ± 0.0 aA	99.0 ± 0.6 aB
	Cry1A.105 + Cry2Ab maize	Non-Bt maize	Cry1A.105 + Cry2Ab maize	Non-Bt maize	Cry1A.105 + Cry2Ab maize	Non-Bt maize
Chlorantraniliprole	68.0 ± 3.3 aA	94.5 ± 2.7 aB	0.0 ± 0.0 Aa	95.3 ± 1.6 aB	0.0 ± 0.0 aA	96.9 ± 1.2 aB
Imidacloprid + thiodicarb	77.9 ± 2.4 bA	93.7 ± 2.3 aB	0.0 ± 0.0 aA	96.1 ± 1.1 aB	0.0 ± 0.0 aA	97.8 ± 1.1 aB
Control	84.3 ± 3.3 bA	98.4 ± 1.0 aB	0.0 ± 0.0 aA	99.2 ± 0.3 aB	0.0 ± 0.0 aA	99.0 ± 0.6 aB
	Cry1A.105 + Cry1F + Cry2Ab2 maize	Non-Bt maize	Cry1A.105 + Cry1F + Cry2Ab2 maize	Non-Bt maize	Cry1A.105 + Cry1F + Cry2Ab2 maize	Non-Bt maize
Chlorantraniliprole	67.9 ± 2.9 aA	94.5 ± 2.7 aB	0.0 ± 0.0 aA	97.6 ± 1.1 aB	0.0 ± 0.0 aA	96.9 ± 1.2 aB
Imidacloprid + thiodicarb	71.0 ± 5.1 aA	88.6 ± 2.1 aB	0.0 ± 0.0 aA	99.2 ± 0.8 aB	0.0 ± 0.0 aA	97.8 ± 1.1 aB
Control	85.2 ± 1.0 bA	93.7 ± 1.7 aB	0.0 ± 0.0 aA	99.4 ± 0.3 aB	0.0 ± 0.0 aA	99.0 ± 0.6 aB

[a] Means within a column followed by the same lowercase letter for each maize and in a row followed by the same uppercase letter for each FAW strain in Bt and non-Bt maize are not significantly different (LSMEANS with Tukey's adjustment; *P* > 0.05).

[b] Neonates from resistant and heterozygous strains were exposed to insecticides applied in seed treatment only in the respective Bt maize which were selected (H-R strain on Cry1F; Y-R strain on Cry1A.105 + Cry2Ab; and P-R strain on Cry1A.105 + Cry1F + Cry2Ab2).

[C] Only one susceptible strain and one non-Bbt maize were used in the bioassays.

Table 3

Survival of FAW neonates (% ± Standard Error) on Bt and non-Bt maize with and without a seed treatment at 21 days after plant emergence in laboratory bioassays.

Seed treatment	**Resistant strain[a,b]**		**Heterozygote strain[a,b]**		**Susceptible strain[a]**	
	Cry1F maize	Non-Bt maize	Cry1F maize	Non-Bt maize	Cry1F maize	Non-Bt maize[c]
Chlorantraniliprole	89.8 ± 3.5 aA[a]	87.5 ± 3.9 aA	63.2 ± 2.2 aA	88.3 ± 1.8 aB	0.0 ± 0.0 aA	91.4 ± 2.3 aB
Imidacloprid + thiodicarb	89.0 ± 2.3 aA	90.9 ± 2.6 aA	60.9 ± 1.6 aA	87.5 ± 2.9 aB	0.0 ± 0.0 aA	90.8 ± 3.7 aB
Control	91.4 ± 2.6 aA	89.0 ± 2.0 aA	64.8 ± 2.3 aA	90.8 ± 1.2 aB	0.0 ± 0.0 aA	89.1 ± 1.0 aB
	Cry1A.105 + Cry2Ab maize	Non-Bt maize	Cry1A.105 + Cry2Ab maize	Non-Bt maize	Cry1A.105 + Cry2Ab maize	Non-Bt maize
Chlorantraniliprole	72.6 ± 2.6 aA	89.8 ± 2.3 aB	0.0 ± 0.0 aA	96.5 ± 2.7 aB	0.0 ± 0.0 aA	91.4 ± 2.3 aB
Imidacloprid + thiodicarb	76.5 ± 2.2 aA	92.1 ± 1.6 aB	0.0 ± 0.0 aA	94.5 ± 3.2 aB	0.0 ± 0.0 aA	90.8 ± 3.7 aB
Control	79.6 ± 1.9 aA	90.6 ± 3.5 aB	0.0 ± 0.0 aA	92.1 ± 3.1 aB	0.0 ± 0.0 aA	89.1 ± 1.0 aB
	Cry1A.105 + Cry1F + Cry2Ab2 maize	Non-Bt maize	Cry1A.105 + Cry1F + Cry2Ab2 maize	Non-Bt maize	Cry1A.105 + Cry1F + Cry2Ab2 maize	Non-Bt maize
Chlorantraniliprole	71.5 ± 2.1 aA	90.6 ± 2.9 aB	0.0 ± 0.0 aA	99.2 ± 0.8 aB	0.0 ± 0.0 aA	91.4 ± 2.3 aB
Imidacloprid + thiodicarb	74.2 ± 2.9 aA	93.2 ± 1.6 aB	0.0 ± 0.0 aA	96.0 ± 1.8 aB	0.0 ± 0.0 aA	90.8 ± 3.7 aB
Control	78.1 ± 1.6 aA	87.5 ± 1.7 aB	0.0 ± 0.0 aA	90.6 ± 2.6 aB	0.0 ± 0.0 aA	89.1 ± 1.0 aB

[a] Means within a column followed by the same lowercase letter for each maize and in a row followed by the same uppercase letter for each FAW strain in Bt (Cry1F, Cry1A.105 + Cry2Ab and Cry1A.105 + Cry1F + Cry2Ab2) and non-Bt maize are not significantly different (LSMEANS with Tukey's adjustment; $P > 0.05$).

[b] Neonates from resistant and heterozygous strains were exposed to insecticides applied in seed treatment only in the respective Bt maize which were selected (H-R strain on Cry1F, Y-R strain on Cry1A.105 + Cry2Ab and P-R strain on Cry1A.105 + Cry1F + Cry2Ab2).

[c] Only one susceptible strain and one non-Bt maize were used in the bioassays.

Table 4

Summary of ANOVA regarding the effect of seed treatment, Bt-maize and their interactions on plants damaged by FAW under field conditions.

Variable	Source of variation	Type III SS	Df	Mean square	*F*	*P*
7 DAE						
% plants damaged	Maize × seed treatment	238.70	6	39.78	0.31	0.9254
	Maize	3118.22	3	1039.40	8.18	<0.0003
	Seed treatment	1489.29	2	744.64	5.86	<0.0066
	Block	172.72	3	57.57	0.45	0.9254
	Model (total)	5018..95	14	358.49	2.82	<0.0071
	Error	4190.02	33	126.97		
	Corrected total	9208.97	47			
% plants with damage rating ≥3	Maize × seed treatment	148.29	6	24.70	0.68	0.6625
	Maize	2761.22	3	920.40	25.54	<0.0001
	Seed treatment	2346.79	2	1173.39	32.56	<0.0001
	Block	108.72	3	36.24	1.01	0.4025
	Model (total)	5364.95	14	383.21	10.69	<0.0001
	Error	1189.02	33	36.03		
	Corrected total	6553.97	47			
14 DAE						
% plants damaged	Maize × seed treatment	66.79	6	11.13	0.20	0.9756
	Maize	3562.08	3	1187.36	20.93	<0.0001
	Seed treatment	80.37	2	40.18	0.70	0.4997
	Block	648.41	3	216.13	3.81	<0.0189
	Model (total)	4357.67	14	311.26	4.44	<0.0021
	Error	1871.58	33	70.00		
	Corrected total	6229.25	47			
% plants with damage rating ≥3	Maize × seed treatment	161.70	6	26.95	0.35	0.9055
	Maize	5517.41	3	1839.13	23.80	<0.0001
	Seed treatment	73.29	2	36.64	0.47	0.6265
	Block	201.75	3	67.25	0.87	0.4663
	Model (total)	5954.16	14	425.29	5.50	<0.0001
	Error	2549.75	33	77.26		
	Corrected total	8503.91	47			
21 DAE						
% plants damaged	Maize × seed treatment	89.20	6	14.86	0.05	0.9993
	Maize	1273.22	3	424.40	1.49	0.2333
	Seed treatment	6.29	2	3.14	0.01	0.9890
	Block	4442.72	3	1480.90	5.25	0.0046
	Model (total)	5811.25	14	415.08	1.46	0.1814
	Error	9352.72	33	283.40		
	Corrected total	15163.97	47			
% plants with damage rating ≥3	Maize × seed treatment	84.16	6	14.02	0.06	0.9991
	Maize	1230.39	3	410.13	1.67	0.1930
	Seed treatment	232.16	2	116.08	0.47	0.6280
	Block	2034.22	3	678.07	2.75	0.0579
	Model (total)	3580.95	14	255.78	1.04	0.4414
	Error	8119.02	33	246.06		
	Corrected total	11699.97	47			

Table 5

Percentage of damaged plants (% ± Standard Error) and plants with significant damage (Davis scale) caused by natural infestations of FAW on Bt and non-Bt maize grown with and without a seed treatment at 7, 14 and 21 days after emergence (DAE) in a field experiment.

Maize	% plants damaged[b]			% plants with significant damage[ab]		
	7 DAE	14 DAE	21 DAE	7 DAE	14 DAE	21 DAE
Chlorantraniliprole						
Cry1F	61.0 ± 5.4 ab	75.2 ± 2.3 b	57.5 ± 3.2 a	28.7 ± 4.9 b	67.2 ± 3.0 b	46.2 ± 6.4 a
Cry1A.105 + Cry2Ab	50.0 ± 2.0 ab	61.2 ± 3.1 ab	47.5 ± 5.0 a	16.0 ± 2.6 a	48.7 ± 5.5 a	35.7 ± 5.5 a
Cry1A.105 + Cry1F + Cry2Ab2	44.0 ± 3.2 a	58.0 ± 3.8 a	43.0 ± 6.3 a	14.2 ± 1.2 a	40.0 ± 5.6 a	33.7 ± 6.2 a
Non-Bt	66.0 ± 6.1 b	76.0 ± 2.3 b	55.0 ± 4.8 a	34.7 ± 1.8 b	67.5 ± 4.3 b	47.5 ± 3.2 a
Imidacloprid + thiodicarb						
Cry1F	49.0 ± 0.7 ab	78.7 ± 3.1 b	55.7 ± 5.6 a	20.0 ± 2.8 b	65.0 ± 5.4 b	46.2 ± 3.2 a
Cry1A.105 + Cry2Ab	41.0 ± 2.4 a	58.7 ± 1.2 a	47.5 ± 7.2 a	13.5 ± 2.1 a	46.0 ± 3.2 a	43.2 ± 5.8 a
Cry1A.105 + Cry1F + Cry2Ab2	36.0 ± 1.5 a	56.2 ± 2.3 a	47.5 ± 4.7 a	14.0 ± 1.5 a	44.0 ± 2.3 a	37.5 ± 4.7 a
Non-Bt	61.7 ± 2.3 b	73.7 ± 3.1 b	58.5 ± 3.2 a	31.2 ± 2.3 b	63.2 ± 3.1 b	48.7 ± 4.2 a
Without seed treatment						
Cry1F	70.0 ± 3.5 b	79.0 ± 6.6 b	60.0 ± 2.0 a	44.2 ± 3.6 b	71.2 ± 5.1 b	52.0 ± 4.6 a
Cry1A.105 + Cry2Ab	55.0 ± 3.3 a	65.7 ± 3.8 ab	48.5 ± 4.9 a	28.7 ± 3.7 a	50.0 ± 3.5 a	42.0 ± 5.8 a
Cry1A.105 + Cry1F + Cry2Ab2	52.2 ± 7.9 a	62.5 ± 6.3 a	48.2 ± 4.8 a	25.7 ± 0.4 a	47.0 ± 2.8 a	37.5 ± 4.0 a
Non-Bt	76.2 ± 3.7 b	77.5 ± 4.7 b	58.5 ± 3.9 a	46.2 ± 3.7 b	66.5 ± 6.3 b	53.7 ± 5.5 a

[a] Signficant damage is based on a plant receiving a score ≥ 3 (% ± SE) on the Davis scale.

[b] Means within a column for Bt and non-Bt maize followed by the same letter are not significantly different (LSMEANS with Tukey's adjustment; $P > 0.05$).

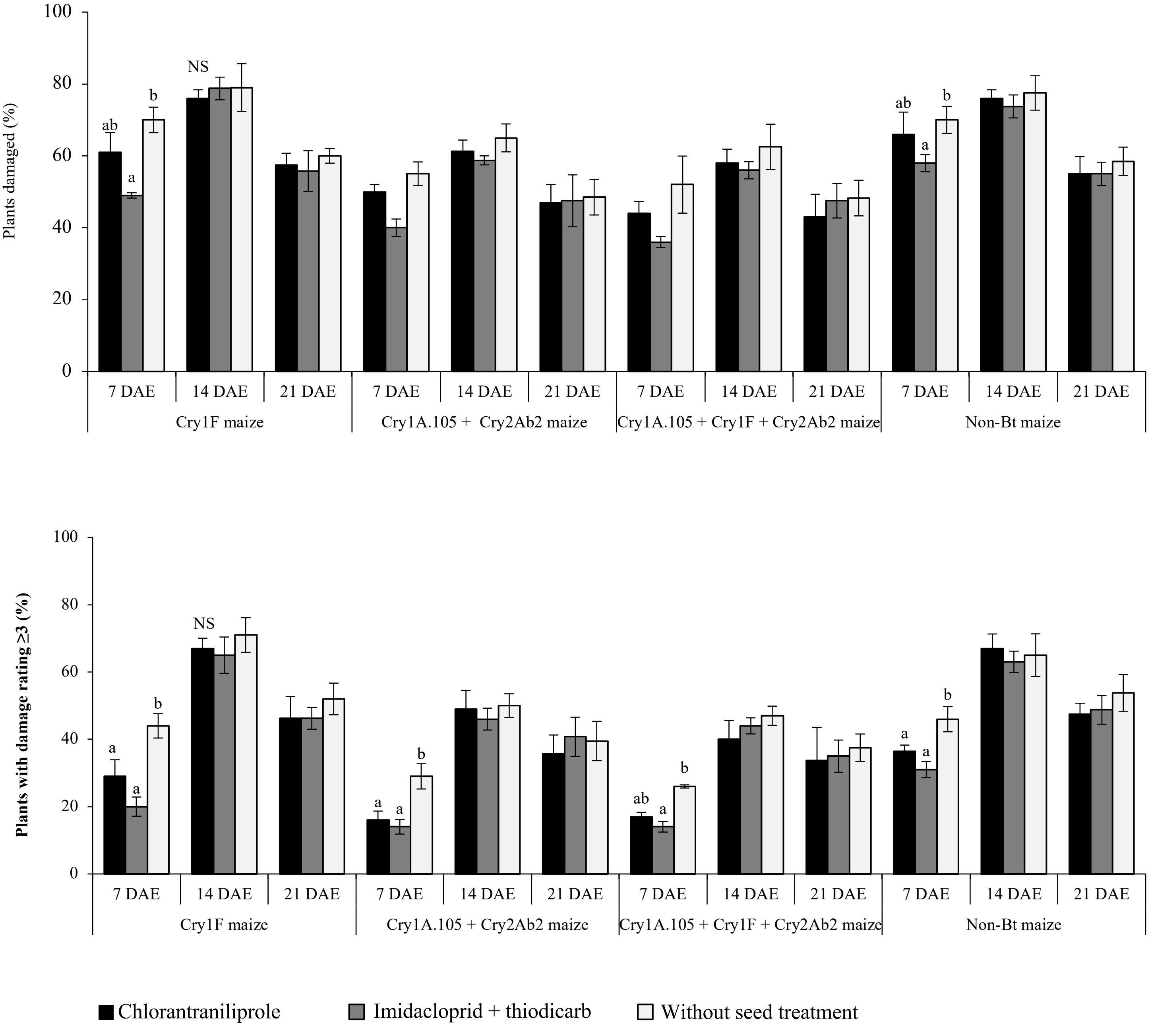

Fig 1. Percentage of damaged plants and plants with damage rating ≥3 (Davis scale) caused by FAW in Bt and non-Bt maize with and without seed treatments in field experiment. Group of bars (± SE – Standard Error) with the same letter for each maize and evaluation time did not differ (NS) from each other (LSMEANS with Tukey's adjustment; *P* > 0.05).

3 ARTIGO 2

Laboratory and field survival of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on Bt and non-Bt maize and its susceptibility to insecticides

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Section: Pest Management Science

Abstract

BACKGROUND: Field-evolved resistance of fall armyworm (FAW), *Spodoptera frugiperda* (Smith), has been reported to Bt maize technologies in Brazil. The control failures of FAW by Bt maize increased the use of insecticides for their control. However, no information is available on the interaction between resistant FAW and their response to insecticides. Here, we evaluated the survival of FAW strains on Bt and non-Bt maize in laboratory and field conditions and its susceptibility to insecticides.

RESULTS: In the laboratory, resistant FAW larvae reared on Bt and non-Bt maize showed a similar susceptibility to spinetoram (LC_{50} = 0.16 to 0.18 µg a.i. cm^{-2}) and chlorfenapyr (LC_{50}

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= 0.17 to 0.20 µg a.i. cm^{-2}). However, their susceptibility was lower than the susceptible strain reared on non-Bt maize; LC$_{50}$ = 0.05 (spinetoram) and 0.08 (chlorfenapyr) µg a.i. cm^{-2}. In contrast, heterozygous strains had similar susceptibility to the susceptible strain. In field trials, no differences in FAW survival were detected between strains when the commercial dose of the two insecticides were applied in Bt and non-Bt maize.

CONCLUSION: FAW strains surviving on Bt and non-Bt maize, at the same development stage, have similar susceptibility to insecticides. The IPM and IRM importance of these results are discussed.

Keywords: fall armyworm; Bt proteins; chemical control; resistance management

1 INTRODUCTION

Fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith, 1797), is a primary pest of maize in Brazil and other South American countries.[1,2] The management of this pest in maize growing areas has been accomplished with Bt maize technologies and chemical control.[3] Currently, 90% of maize grown in Brazil contains Bt proteins.[4] However, low compliance of resistance management strategies as refuge areas (less than 20% of growers adopted refuge) has contributed to the evolution of FAW resistance to Bt proteins.[5,6] Field-evolved resistance of FAW to Bt proteins has been reported to the Cry1F protein expressed in Herculex maize[5] and to the Cry1Ab protein expressed in MON810 maize.[6] Laboratory studies have also indicated resistance of FAW to YieldGard VT PRO maize which expresses Cry1A.105 and Cry2Ab2,[7–9] PowerCore containing Cry1A.105, Cry2Ab2 and Cry1F[9,10], Agrisure Viptera and Agrisure Viptera 3 that express Vip3Aa20 and Vip3Aa20/Cry1Ab, respectively.[11] FAW also evolved resistance to Cry1F maize in Puerto Rico,[12] in some areas of the southeastern United States,[13] and Argentina.[14]

In many cases, foliar chemical control used against FAW in Bt and non-Bt maize has presented unsatisfactory efficacy.[3] This may be in part due to the behavior of FAW larvae which stays inside the maize whorl, thus decreasing insecticide contact. In addition, resistance of Brazilian populations of FAW have been reported to lambda-cyhalothrin,[15,16] chlorpyrifos,[16] lufenuron,[17] and spinosad.[18] In Brazil, the resistance evolution of FAW to Bt maize and insecticides is a consequence of an intensive crop production system (two maize seasons per year) and bioecological characteristics of the species, which has high population density and overlapping generations in the agroecosystem.[3,8,19,20] Furthermore, the limited use of alternative Integrated Pest Management (IPM) practices and Insect Resistance Management (IRM) strategies also favors the rapid evolution of resistance.[21]

Control failures of some Bt-expressing maize varieties have required insecticidal sprays to complement FAW management.[3] In southern Brazil, up to four insecticidal sprays are used on Cry1 and Cry2-expressing maize varieties to effectively control FAW.[3] In contrast, Vip3Aa20-expressing maize lines remain effective against this species.[3,22] According to growers and consultants, FAW larvae that survive on Bt maize in field conditions are more difficult to control with insecticides than larvae surviving on non-Bt maize (refuge or conventional maize areas) when insecticidal sprays are performed at the same time. To determine if resistant and susceptible FAW strains differ in survival after insecticide application, we performed laboratory, greenhouse and field studies on Bt and non-Bt maize and evaluated FAW susceptibility to common insecticides.

2 MATERIAL AND METHODS

2.1 Selection of FAW resistant colonies

To select FAW strains with capability to survive on Bt protein-expressing maize lines, the F_2 screen method was used.[23] We selected populations with high survival on Herculex maize (Cry1F; P3779H, Dupont Pioneer, Santa Rosa, RS, Brazil), YieldGard VT PRO (Cry1A.105

and Cry2Ab2; DKB 390 PRO, Dekalb, Uberlandia, MG, Brazil) and PowerCore (Cry1A.105, Cry1F and Cry2Ab2; 2A620PW, Dow AgroSciences, Paracatu, MG, Brazil).The FAW resistant to Herculex (hereafter H-R), YieldGard VT PRO(hereafter Y-R) and PowerCore (hereafter P-R) were selected in leaf tissue bioassays[8] from a field population collected during the 2016–2017 growing season in Paulínia, São Paulo, Brazil (22°42'38"S and 47°06'26"W). A susceptible strain (Sus), maintained in laboratory since 2012 in absence of selection pressure by Bt proteins or insecticides, was used as source of susceptible insects. To evaluate heterozygous strains, reciprocal crosses between resistant strains × susceptible were also performed (H-R × Sus, Y-R × Sus, and P-R × Sus).

2.2 Survival and development of FAW strains on Bt maize

Laboratory studies were performed to evaluate the survival of FAW strains on leaves of Bt maize listed above and non-Bt maize (30F53, Dupont Pioneer, Santa Rosa, RS, Brazil) to demonstrate that the selected strains were resistant. Maize plants were cultivated in 5-liter pots in a greenhouse at temperature 25 ± 5°C, 50 ± 10% relative humidity, and 14:10 [L:D] hours photoperiod. From V_4 to V_6 growth stages, leaves were removed from the maize whorls and leaf discs measuring 5 cm in diameter were cut using a metallic cutter. Leaf discs were placed on a gelled mixture of 2.5% agar-water (20 ml/cup) in 100 ml plastic cups. Leaves were separated from the agar-water layer by a filter paper. One hundred neonates/strain (10 replicates of 10 neonates) were individually reared on leaves of the respective maize from which they were selected and non-Bt maize, until pupae stage. To demonstrate phenotypic resistance, adults were pair-mated to evaluate their ability to produce viable offspring. Data on larval survival, pupation and adult emergence, eggs/female and number of neonates were subjected to studentized residuals analysis to verify the assumption of normality using Shapiro-Wilk test (PROC UNIVARIATE) and for homogeneity of variances with Bartlett test

(PROC GLM) in SAS® 9.1.[24] Statistical differences were determined with Scott-Knott test in SAS® 9.1.[24]

2.3 Susceptibility of FAW strains to insecticides in diet-overlay bioassays

The susceptibility of FAW strains surviving on Bt and non-Bt maize to spinetoram (Exalt 120 g a.i. L^{-1}, Corteva Agriscience, Marinette, WI, USA) and chlorfenapyr (Pirate 240 g a.i. L^{-1}, BASF Corporation, Nova Jersey, NJ, USA) were evaluated. These insecticides were used because there are no current cases of resistance or reduced susceptibility for these products reported in Brazilian populations of this species. Neonates from resistant, heterozygous and susceptible strains were fed Bt or non-Bt maize until the third instar. Then, surviving larvae were exposed to insecticides applied on the diet surface[25] in 24-well acrylic plates (Costar, São Paulo, SP, Brazil). Each insecticide was diluted in distillated water containing 0.1% Triton X-100 surfactant to obtain a uniform spread of the solution over the diet surface. The control treatment was composed of distilled water + surfactant. For each strain, 5–8 concentrations per insecticide were used, and applied on 2.4 cm^2 diet surface at a volume of 30 µl $well^{-1}$. After a drying period, one third instar larvae was placed into each well and plates were sealed with a plate cover and placed in a controlled climate room at temperature 25 ± 1°C, 60 ± 10% relative humidity, and 14:10 [L:D] hours photoperiod. Bioassays were repeated twice per strain, with each concentration being repeated twice per bioassay (four replications of 24 larvae per concentration). Mortality was evaluated at 48 and 96 hours for spinetoram and chorfenapyr, respectively. Larvae were considered dead when they showed no apparent movement after a slight touch with a fine paintbrush. To estimate the lethal concentration (LC_{50} and LC_{90}) and the respective confidence intervals, the concentration-mortality data were submitted to Probit analysis (PROC PROBIT) in SAS® 9.1.[24] A likelihood ratio test was conducted to test the hypothesis that the LCp values were equal. If the hypothesis was rejected, pairwise comparisons were performed, and the significance was declared if confidence intervals did not overlap.[26] Resistance ratio were calculated by dividing the LC_{50} values of resistant and heterozygous strains by the corresponding parameter for the susceptible strain.

2.4 Susceptibility of FAW strains to insecticides in leaf bioassays

Leaf bioassays were performed to evaluate the survival of FAW strains on Bt and non-Bt maize and its susceptibility to insecticides. Greenhouse plants were cultivated in 5-liter pots (one plant per pot). At the V_6 growth stage, Bt and non-Bt maize were sprayed with spinetoram (12 g a.i ha^{-1}) or chlorfenapyr (192 g a.i ha^{-1}) diluted in 150 liters of water using an automatic spray camera (Generation III Sprayer, DeVries Manufacturing, Hollandale, MN, USA) equipped with XR 110.02 fan-type nozzle tips (Teejet Technologies Co., Glendale Heights, Illinois, IL, USA). These doses corresponded to the commercial recommendation of each insecticide for FAW control on maize. Unsprayed plants were used as control treatment. After 4 hours, leaves of the maize whorls were removed and cut to 5 cm^2 pieces. Then, leaves were individually placed on a gelled mixture of 2.5% agar-water in 32-well plastic plates (12 cm^2) (Advento do Brasil, São Paulo, SP, Brazil). Each leaf was infested with one third instar larva of resistant, heterozygous or susceptible strain (four replications of 16 larvae/strain/treatment). The larva survival was evaluated at 48 and 96 hours in spinetoram and chorfenapyr, respectively. Survival data was subjected to studentized residuals analysis to confirm the assumption of normality with Shapiro-Wilk test (PROC UNIVARIATE) and for homogeneity of variances with Bartlett test (PROC GLM) in SAS® 9.1.[24] Statistical differences were determined with Scott-Knott test in SAS® 9.1.[24]

2.5 Susceptibility of FAW to insecticides in field trials

Bt and non-Bt maize technologies mentioned above were cultivated under field conditions during the cropping season of 2017–2018. Planting was performed on 07 December 2017 at a density 80.000 seed ha^{-1}. At sowing, 225 kg ha^{-1} of Nitrogen–Phosphorus–Potassium (NPK; 5–20–20) was applied. At the V_4 and V_8 growth stage, 120 kg N ha^{-1} was also applied. Maize was sown in four identical blocks arranged in a randomized design. Treatments were distributed in a 4 × 3 factorial arrangement, and each block contained 12 plots (each plot comprised of five maize rows of 4 m in length and with a spacing of 0.50 m between rows). Factor A was represented by three Bt maize technologies (Herculex, YieldGard VT PRO and PowerCore) and one non-Bt. Factor B was composed of two insecticide treatments (spinetoram 12 g a.i ha^{-1} or chlorfenapyr 192 g a.i ha^{-1}) and the control (without insecticide). Damage caused by natural infestations of FAW in Bt and non-Bt maize was evaluated every 5 days in the leaf whorls of 20 consecutive plants per plot. A damage rating was attributed to each plant in accordance with the Davis scale.[27] Spraying with insecticides was carried out whenever 20% of the quantified plants in each sampling showed a damage rating ≥ 3. The number of plants with a damage rating ≥ 3 has been suggested as a criterion for using insecticides against FAW on Bt and non-Bt maize in Brazil.[28] Insecticides were applied using a pressurized-CO_2 backpack sprayer with a 2-m bar and 0.5-m nozzle spacing (XR 110.02 fan-type nozzle tips) (150 l ha^{-1}). These results were then converted to percentage of plants with a damage rating ≥ 3. Plants with some damage were also counted (converted into the percentage of plants with leaf damage). Grain yield was evaluated by harvesting the ears in 3 m^2 of the two central rows of each plot. The ears were threshed and grain moisture was standardized at 13% to estimate yield per hectare. Data were subjected to two-way analysis of variance (ANOVA) using the PROC GLM procedure in SAS® 9.1.[24] Maize, insecticide, and their interactions were considered fixed factors in the model. For grain yield, we compared the yields of each maize with and without (control) insecticidal spray against FAW. Comparisons among maize hybrids for grain yield were not performed because their variation in genetic background. Treatment differences were determined with Scott-Knott test in SAS® 9.1.[24]

3 RESULTS

3.1 Selection of the FAW resistant colonies

The F_2 screen method[23] was used to select FAW larvae capable of surviving on Herculex, YieldGard VT PRO and PowerCore maize. A total of 102 isofamilies were screened on each Bt maize. From these, 27 families had surviving larvae (more than 80% survival). The surviving larvae from these families were reared from neonates to adults on excised leaves of the respective Bt maize which they were selected. The adults were used to establish the resistant colonies called H-R, Y-R and P-R. Resistant colonies in the following six generations were reared from neonate to third instar on the respective Bt maize on which they were selected (survival greater than 85%), and then transferred to artificial diet,[25] where they remained until the pupae stage. After this time, the following studies were started.

3.2 Survival and development of FAW strains on Bt maize leaves

Here, we demonstrate the ability of larvae from selected FAW strains to survive from neonates to adults on Bt maize and to produce viable offspring. Resistant FAW strains (H-R, Y-R and P-R) showed similar larval survivorship at 10 days on Bt and non-Bt maize (76.0 – 78.2, 82.0 – 88.0, and 72.3 – 78.7%, respectively) compared with Sus strain on non-Bt maize (81.3%) (Table 1). Only the progeny from reciprocal crosses among H-R × Sus on Herculex had lower survival (50.4 to 57.2%) than the same strain and Sus on non-Bt maize. In contrast, the F_1 progeny from reciprocal crosses among Y-R × Sus, P-R × Sus and Sus did not survive on YieldGard VT PRO and PowerCore, indicating that resistance is phenotypically recessive. These heterozygous strains showed higher than 94% larval survival on non-Bt maize, not differing from Sus. H-R pupae had lower survivorship (40%) on Herculex than H-R and Sus pupae on non-Bt maize (51 and 67%, respectively) (Table 1). Pupae from Y-R and P-R strains had similar survivorship (51 to 60% survival) on Bt and non-Bt maize, however, the progeny from Y-R × Sus and P-R × Sus on non-Bt maize showed higher pupae survival than parent strains (Table 1). Resistant strains reared on Bt and non-Bt maize yielded similar percentage of adults (32 to 52%) compared with Sus on non-Bt maize, but the progeny from H-R × Sus on Bt and non-Bt maize, Y-R × Sus and P-R × Sus on non-Bt maize generated a higher number of adults than parental strains. H-R and Y-R females from Bt and non-Bt maize had similar number of eggs and neonates than Sus strain on non-Bt maize (Table 1). In contrast, P-R females from Bt and non-Bt maize produced a higher number of eggs and neonates than H-R and Y-R strains. Heterozygous females from H-R × Sus and Y-R × Sus on non-Bt maize also yielded similar number of eggs and neonates as Sus. However, females from H-R × Sus on Herculex and P-R × Sus on non-Bt had a higher egg number and neonates than Sus. These results indicated lack of relevant fitness costs associated with the resistance to Bt maize evaluated.

3.3 Susceptibility of FAW strains to insecticides in diet-overlay bioassays

Spinetoram insecticide showed high biological activity against FAW strains (Table 2). Third instar larvae from H-R, Y-R and P-R strains reared on Bt and non-Bt maize showed a similar susceptibility to spinetoram with LC_{50} and LC_{90} values ranging from 0.16 to 0.18 and 0.40 to 0.47 µg a.i. cm^{-2}, respectively. The progeny from reciprocal crosses reared on Bt (only H-R × Sus survived in Herculex) and non-Bt maize had a higher susceptibility to spinetoram than previous strains, with LC_{50} and LC_{90} ranging from 0.06 to 0.07 and 0.21 to 0.25 µg a.i. cm^{-2}, respectively. However, these heterozygous larvae had similar susceptibility to spinetoram as Sus strain (LC_{50} = 0.05 µg a.i. cm^{-2}). The variation in susceptibility in resistant strains relative to reciprocal crosses and Sus strain, based in LC_{50} values, demonstrates a resistance ratio ranging from 3.2 to 3.6-fold.

Chlorfenapyr also demonstrated high toxicity against third instar larvae of FAW strains (Table 3). Third instar larvae from H-R, Y-R and P-R strains reared on Bt and non-Bt maize had similar LC_{50} and LC_{90} values to chlorfenapyr, ranging from 0.17 to 0.20 and 0.41 and

0.49 µg a.i. cm^{-2}, respectively. Larvae from reciprocal crosses on Bt (only H-R × Sus survived in Herculex) and non-Bt maize showed similar susceptibility to chlorfenapyr compared with resistant and Sus strains, with LC_{50} and LC_{90} values ranging from 0.10 to 0.17 and 0.31 to 0.44 µg a.i. cm^{-2}, respectively. However, resistant strains reared on Bt and non-Bt maize had significantly lower LC_{50} values than Sus strain (LC_{50} = 0.08 µg a.i. cm^{-2}), but similar LC_{90} values. The differences in susceptibility to chlorfenapyr in resistant strains relative to Sus strain, based in LC_{50} values, indicated a resistance ratio ranging from 2.1 to 2.5-fold.

3.4 Susceptibility of FAW strains to insecticides in leaf bioassays

Third instar larvae from FAW strains reared on Bt or non-Bt maize when exposed to the commercial dose of spinetoram sprayed on Bt and non-Bt maize plants had similar susceptibility (survival lower than 6.2%) (Table 4). In contrast, in the respective maize without spinetoram spray, the larval survival was greater than 87%. Larvae from Y-R × Sus, P-R × Sus and Sus strains on YieldGard VT PRO, PowerCore and Herculex, respectively, did not survive until third instar. A similar susceptibility among FAW strains was also observed in third instar larvae exposed to the commercial dose of chlorfenapyr sprayed on Bt and non-Bt maize, with survival lower than 13.5% (Table 4). However, in the respective maize without insecticide, the survival was greater than 84%. These results indicate that FAW strains surviving on Bt maize, present similar susceptibility to spinetoram and chlorfenapyr as the same strain developing on non-Bt maize.

3.5 Field efficacy of insecticides against FAW larvae surviving on Bt and non-Bt maize

A significant interaction was detected between Bt maize and insecticide for the percentage of plants with leaf damage and plants with damage rating ≥ 3, but there was no interaction among Bt maize × insecticide for grain yield (Table 5). There was also a significant effect of individual factors in all variables measured.

According to damage rating scale, Herculex, YielGard VT PRO, PowerCore and non-Bt maize required 4 to 6 insecticidal sprays to supplement FAW control. After sprays, the percentage of plants with leaf damage (40 to 69%) and plants with damage rating ≥ 3 (31 a 60%) were significantly lower in Bt and non-Bt maize sprayed with spinetoram or chlorfenapyr than control treatments (without insecticide) (Fig. 1). No significant differences in the percentage of plants with leaf damage were detected when spinetoram or chlorfenapyr was applied in the same Bt or non-Bt maize hybrid. In contrast, a lower number of plants with damage rating ≥ 3 was observed on Herculex and non-Bt maize sprayed with spinetoram than the same maize plants sprayed with cholfenapyr. This difference was not observed on YielGard VT PRO and PowerCore. However, chlorfenapyr had a better performance against FAW when applied over YielGard VT PRO and PowerCore than Herculex and non-Bt maize.

3.6 Grain yield

No differences in grain yield were detected on Herculex maize with or without spinetoram and chlorfenapyr sprays against FAW (Table 6). Nevertheless, when insecticides were applied in Herculex, the grain yield increased over 1300 kg ha^{-1}. YieldGard VT PRO, PowerCore and non-Bt maize presented higher grain yield when insecticides were used to supplement the FAW Bt control than the same hybrids without insecticide sprays. In these hybrids, the grain yield increase ranged from 1044 to 2317 kg ha^{-1}.

4 DISCUSSION

Phenotypic resistance is demonstrated by the larval survival on Bt maize leaves and the ability of adults to generated viable offspring.[29] Here, we demonstrated that selected FAW-resistant colonies survive on leaves of Bt maize and generated pupae and normal adults. Similar or higher survival from neonate to adult was previous reported in resistant FAW strains developing on Herculex,[5,9,13] YieldGard VT PRO,[8,9] PowerCore,[9,10] and Viptera maize technologies.[9,11] We also demonstrated that resistant strains reared on Bt and non-Bt maize leaves had a similar or higher reproductive performance as Sus strain on non-Bt maize, indicating lack of relevant fitness costs. These results corroborate previous studies that reported lack of strong fitness costs associated with resistance of FAW to Bt maize expressing Cry1 and Cry2 proteins.[8,30,31] Only the progeny from reciprocal crosses between H-R × Sus survived on Herculex maize leaves, showing that plant expression of Cry1F protein does not meet the high-dose concept, as demonstrated in previous studies.[9,31,34-35] In contrast, progeny from Y-R × Sus and P-R × Sus did not survive on YieldGard VT PRO and PowerCore, respectively, showing that resistance is functionally recessive and Bt proteins expressed in these events meets the definition of high-dose.[8,10,36]

The FAW-resistant strains that were reared on Bt or non-Bt maize and then exposed to spinetoram (IRAC MoA group 5) and chlorfenapyr (IRAC MoA group 13) in diet-overlay bioassays showed lower susceptibility than Sus strain, while the F_1 progeny from reciprocal crosses had similar susceptibility to the Sus strain. The variation in the susceptibility to chemical or microbial insecticides among distinct populations is expected when bioassays are repeated.[26] Therefore, we believe that the variation in susceptibility among FAW strain can be attributed to the natural variation in the response to insecticides and not a consequence of the resistance to Bt proteins, since they have distinct mode of action. Differences in the susceptibility of FAW populations or strains to insecticides were also reported to methomyl (2.7 to 6-fold),[37] lambda-cyhalothrin (> 12-fold),[15,37] lufenuron (> 20-fold),[17] chlorpyrifos (>18-fold)[16]. Another hypothesis for the variation in the susceptibility of FAW strains can be associated to the lab-generation of selected resistant colonies (six generations) which was lower than Sus strain (> 40 generations).

The FAW strains that developed on Bt and non-Bt maize and exposed to the commercial dose of spinetoram and chlorfenapyr in greenhouse and field trials also showed similar susceptibility. These results indicate that insecticides applied over Bt or non-Bt maize (refuge or conventional areas) against resistant strains of FAW had similar effectiveness, when applied against larvae of same age. Laboratory studies have demonstrated that the susceptibility to Bt proteins or insecticides are reduced in advanced instar FAW larvae.[36,37-39] In field conditions, later instar larvae penetrate in the maize whorl, then produce excrements that may prevent insecticides from reaching the insect, making them difficult to control.[16,40-41] In field trials it was observed that although Herculex, YieldGard VT PRO and PowerCore maize present control failures against FAW, the use of these products remain an important control strategy because they reduce FAW damage, decrease insecticidal sprays and increase grain yield.

In the IPM and IRM context, the low variation in the susceptibility to insecticides among FAW strains indicated that a similar efficacy is expected when insecticides are applied against FAW larvae surviving in Bt and non-Bt maize areas. Thus, the use recommended dose of insecticides is essential to obtain similar mortality-response in all strains. These results highlight the importance of following the recommendations of the Insect Resistance Action Committee[28] for insecticide sprays in Bt and non-Bt maize (conventional and refuge areas). Insecticide use is recommended when 20% of the plants show a damage rating ≥ 3. In refuge areas, insecticides should be sprayed only until the V_6 growth stage to allow the survival of Bt-susceptible insects.[28] The use of insecticides with different modes of action is also important to delay or prevent evolution of resistance to insecticides. Therefore, monitoring the presence of FAW and its damage in Bt and non-Bt maize is essential to support the decision making for insecticide use. Bt maize and insecticides can be also integrated with biological control agents (e.g. *Spodoptera frugiperda* multiple nucleopolyhedrovirus (SfMNPV) a registered baculovirus insecticide for use in maize)[42] in which would represent the resumption of IPM in maize in Brazil.

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Table 1. Survivorship (% ± SE – Standard Error) of FAW strains from neonate to adult, number of eggs and neonates per female on Bt and non-Bt maize leaves in laboratory.

FAW strain	Host plant	Survivorship (%)			Eggs/female[b]	Neonates/female[b]
		Larvae[a,c]	**Pupae[b]**	**Adults[b]**		
H-R	Herculex	76.0 ± 4.1 a	40.0 ± 15.1 b	32.0 ± 11.9 b	688.7 ± 100.1 b	529.4 ± 80.1 b
	Non-Bt	78.2 ± 6.4 a	51.0 ± 12.3 b	38.0 ± 11.7 b	744.4 ± 155.4 b	587.4 ± 132.4b
H-R♂ × Sus♀	Herculex	57.2 ± 5.3 b	36.0 ± 15.3b	30.0 ± 12.4b	1201.2 ± 59.6a	1043.9 ± 89.2a
	Non-Bt	90.2 ± 7.5 a	85.0 ± 10.7 a	73.0 ± 9.9 a	885.2 ± 61.5 b	676.7 ± 37.7 b
H-R♀ × Sus♂	Herculex	50.4 ± 6.3 b	32.8 ± 14.8 b	28.0 ± 11.7b	1231.7 ± 72.2a	1053.3 ± 80.9a
	Non-Bt	96.3 ± 4.6 a	94.8 ± 10.0a	84.8 ± 9.5a	893.1 ± 51.6 b	642.2 ± 59.2b
Y-R	YieldGard VT PRO	82.0 ± 4.6 a	57.0 ± 15.7 b	44.0 ± 14.3 b	926.9 ± 182.2 b	808.3 ± 123.2 b
	Non-Bt	88.0 ± 5.2 a	60.0 ± 16.3b	52.0 ± 14.6 b	1099.3 ± 158.4 b	943.4 ± 185.7 b
Y-R♂ × Sus♀	YieldGard VT PRO	0.0 ± 0.0*				
	Non-Bt	98.0 ± 8.3 a	92.0 ± 9.2a	78.0 ± 9.3 a	853.8 ± 108.5 b	765.5 ± 105.7 b
Y-R♀ × Sus♂	YieldGard VT PRO	0.0 ± 0.0*				
	Non-Bt	96.4 ± 5.4 a	94.0 ± 12.6a	85.8 ± 8.8 a	917.4 ± 98.6 b	834.8 ± 87.8 b
P-R	PowerCore	72.3 ± 8.9 a	51.0 ± 15.3 b	40.0 ± 13.0 b	1597.0 ± 204.6a	1407.0 ± 167.6 a
	Non-Bt	78.7 ± 4.3 a	55.0 ± 13.4b	45.0 ± 1.6 b	1284.2 ± 112.2 a	1257.0 ± 94.8 a
P-R♂ × Sus♀	PowerCore	0.0 ± 0.0*				
	Non-Bt	94.0 ± 6.3 a	87.0 ± 13.1 a	71.0 ± 10.8 a	1240.0 ± 145.5 a	1139.2 ± 122.7 a
P-R♀ × Sus♂	PowerCore	0.0 ± 0.0*				
	Non-Bt	92.2 ± 5.4 a	82.0 ± 12.0 a	70.0 ± 11.3 a	1380.1 ± 123.6 a	1278.2 ± 154.8 a
Sus	Herculex	0.0 ± 0.0*				
	YieldGard VT PRO	0.0 ± 0.0*				
	PowerCore	0.0 ± 0.0*				
	Non-Bt	81.3 ± 4.9 a	67.0 ± 14.1 b	51.0 ± 12.6 b	829.2 ± 69.4 b	601.3 ± 62.2 b

[a] Survivorship at 10 days. The progeny from Y-R × Sus and P-R × Sus on YieldGard VT PRO and PowerCore maize, respectively, and Sus strain in all Bt maize did not survive until 10 days.

[b] Means within a column followed by the same letter are not significantly different (Scott-Knott; $P > 0.05$).

[c] An asterisk (*) indicates that data were excluded from the analysis as there was no variability.

Table 2. Concentration-mortality response (LC; µg a.i. cm^{-2}) of third instar larvae of FAW strains reared on Bt and non-Bt maize to spinetoram in diet-overlay bioassays.

FAW strain	Host plant[a]	Generation	*n*	Slope (± SE)	LC_{50} (95%FL)[b,c]	LC_{90} (95% FL)[b,c]	χ^2 (df)[d]	RR[e]
H-R	Herculex	F_6	700	2.92 (± 0.27)	0.17 (0.14 – 0.20) a	0.47 (0.40 – 0.57) a	2.09 (3)	3.4
	Non-Bt	F_6	576	2.82 (± 0.45)	0.16 (0.11 – 0.20) a	0.46 (0.38 – 0.60) a	2.16 (3)	3.2
H-R♂ × Sus♀	Herculex	F_7	648	2.38 (± 0.22)	0.07 (0.05 – 0.09) b	0.23 (0.16 – 0.37) b	9.22 (4)	1.4
	Non-Bt	F_7	804	1.89 (± 0.29)	0.07 (0.05 – 0.09) b	0.24 (0.20 – 0.29) b	7.47 (4)	1.4
H-R♀ × Sus♂	Herculex	F_7	636	2.40 (± 0.29)	0.06 (0.05 – 0.09) b	0.23 (0.19 – 0.36) b	5.45 (4)	1.2
	Non-Bt	F_7	804	2.61 (± 0.25)	0.07 (0.06 – 0.09) b	0.22 (0.17 – 0.34) b	7.95 (4)	1.4
Y-R	YieldGard VT PRO	F_6	791	2.98 (± 0.30)	0.16 (0.14 – 0.19) a	0.44 (0.38 – 0.54) a	2.55 (3)	3.2
	Non-Bt	F_6	828	3.35 (± 0.21)	0.18 (0.16 – 0.19) a	0.40 (0.37 – 0.45) a	6.07 (3)	3.6
Y-R♂ × Sus♀	Non-Bt	F_7	648	2.37 (± 0.27)	0.07 (0.05 – 0.09) b	0.21 (0.18 – 0.26) b	5.71 (4)	1.4
Y-R♀ × Sus♂	Non-Bt	F_7	636	2.29 (± 0.27)	0.06 (0.04 – 0.08) b	0.22 (0.18 – 0.28) b	5.66 (4)	1.2
P-R	PowerCore	F_6	576	3.20 (± 0.47)	0.18 (0.13 – 0.22) a	0.44 (0.38 – 0.52) a	3.41(3)	3.6
	Non-Bt	F_6	576	2.87 (± 0.44)	0.16 (0.12 – 0.20) a	0.46 (0.38 – 0.60) a	3.45(3)	3.2
P-R♂ × Sus♀	Non-Bt	F_7	672	2.57 (± 0.29)	0.08 (0.06 – 0.11) b	0.25 (0.19 – 0.37) b	8.87(4)	1.6
P-R♀ × Sus♂	Non-Bt	F_7	840	2.47 (± 0.16)	0.07 (0.07 – 0.09) b	0.25 (0.21 – 0.31) b	5.86(4)	1.4
Sus	Non-Bt	-	816	1.89 (± 0.29)	0.05 (0.02 – 0.08) b	0.24 (0.13 – 0.36) b	13.68(3)	-

[a] The progeny from Y-R × Sus and P-R × Sus were not evaluated on YieldGard VT PRO and PowerCore maize, respectively, because they did not survive until third instar.

[b] LC_{50} and LC_{90} values designated by different letters within a column are significantly different from each other through non-overlap of 95% fiducial limits.

[c] LC_{50}: concentration of spinetoram (µg a.i. cm^{-2}) required to kill 50% of insects in the observation period of 2 days.LC_{90} is the concentration of spinetoram required to kill 90% of larvae tested.

[d] $P > 0.05$ in the goodness-of-fit test.

[e] Resistance Ratio (RR_{50}) = (LC_{50} of indicated strain)/ (LC_{50} of Sus strain).

Table 3. Concentration-mortality response (LC; µg a.i. cm^{-2}) of third instar larvae of FAW strains reared on Bt and non-Bt maize to chlorfenapyr in diet-overlay bioassays.

FAW strain	Host plant[a]	Generation	*N*	Slope (± SE)	LC_{50} (95% FL)[b,c]	LC_{90} (95% FL)[b,c]	χ^2 (df)[d]	RR
H-R	Herculex	F_6	736	2.73 (± 0.40)	0.19 (0.12 - 0.23) a	0.41 (0.31 - 0.81) a	6.59 (3)	2.4
	Non-Bt	F_6	810	2.54 (± 0.37)	0.18 (0.11 - 0.24) a	0.44 (0.33 - 0.90) a	7.55 (3)	2.2
H-R♂ × Sus♀	Herculex	F_7	528	2.74 (± 0.27)	0.10 (0.10 - 0.13) ab	0.29 (0.25 - 0.35) a	2.39 (4)	1.2
	Non-Bt	F_7	552	2.69 (± 0.21)	0.11 (0.10 - 0.13) ab	0.33 (0.28 - 0.40) a	1.30 (4)	1.4
H-R♀ × Sus♂	Herculex	F_7	626	2.78 (± 0.23)	0.11 (0.10- 0.14) ab	0.31 (0.26 - 0.38) a	1.23 (4)	1.4
	Non-Bt	F_7	892	2.81 (± 0.27)	0.11 (0.09 - 0.14) ab	0.47 (0.40 - 0.57) a	2.09 (4)	1.4
Y-R	YieldGard VT PRO	F_6	600	2.58 (± 0.22)	0.18 (0.15 - 0.21) a	0.42 (0.37 - 0.51) a	4.53 (3)	2.2
	Non-Bt	F_6	719	2.43 (± 0.40)	0.17 (0.16 - 0.24) a	0.44 (0.31 - 0.82) a	9.31 (3)	2.1
Y-R♂ × Sus♀	Non-Bt	F_6	936	3.02 (± 0.20)	0.11 (0.09 - 0.14) ab	0.27 (0.20 - 0.38) a	5.59 (3)	1.4
Y-R♀ × Sus♂	Non-Bt	F_6	628	2.94 (± 0.29)	0.11 (0.08 - 0.13) ab	0.31 (0.26 - 0.38) a	1.23 (3)	1.4
P-R	PowerCore	F_6	888	3.23 (± 0.61)	0.20 (0.11 - 0.26) a	0.49 (0.35 - 0.97) a	7.59 (3)	2.5
	Non-Bt	F_6	864	3.26 (± 0.42)	0.17 (0.13 - 0.20) a	0.47 (0.40 - 0.57) a	7.73 (3)	2.1
P-R♂ × Sus♀	Non-Bt	F_6	926	3.31 (± 0.20)	0.12 (0.10 - 0.13) ab	0.31 (0.27 - 0.35) a	3.02 (4)	1.5
P-R♀ × Sus♂	Non-Bt	F_6	916	3.07 (± 0.28)	0.12 (0.10 - 0.15) ab	0.33 (0.25 - 0.47) a	9.16 (4)	1.5
Sus	Non-Bt	-	984	2.40 (± 0.22)	0.08 (0.062 - 0.10) b	0.31 (0.23 - 0.50) a	9.02 (4)	-

[a] The progeny from Y-R × Sus and P-R × Sus were not evaluated on YieldGard VT PRO and PowerCore maize, respectively, because they did not survive until third instar.

[b] LC_{50} and LC_{90} values designated by different letters within a column are significantly different from each other through non-overlap of 95% fiducial limits.

[c] LC_{50}: concentration of chlorfenapyr (µg a.i. cm^{-2}) required to kill 50% of insects in the observation period of 4 days.LC_{90} is the concentration of chlorfenapyr required to kill 90% of larvae tested.

[d] $P > 0.05$ in the goodness-of-fit test.

[e] Resistance Ratio (RR) = (LC_{50} of indicated strain)/ (LC_{50} of Sus strain).

Table 4. Survivorship (% ± SE – Standard Error) of third instar larvae of FAW strains in leaves of Bt and non-Bt maize sprayed with spinetoram and chlorfenapyr insecticides.

FAW strain	Host plant[a]	Spinetoram[b]		Chlorfenapyr[b]	
		With Insecticide	Without insecticide	With insecticide	Without insecticide
H-R	Herculex	6.2 ± 1.0 aB	90.8 ± 2.2 aA	11.3 ± 3.2 aB	89.7 ± 1.0 aA
	Non-Bt	5.2 ± 1.0 aB	100.0 ± 0.0 aA	8.8 ± 1.9 aB	100.0 ± 0.0 aA
H-R♂ × Sus♀	Herculex	3.1 ± 1.8 aB	87.2 ± 1.0 aA	10.3 ± 1.3 aB	84.4 ± 3.7 aA
	Non-Bt	2.1 ± 1.0 aB	96.8 ± 1.8 aA	10.2 ± 1.6 aB	96.8 ± 1.8 aA
H-R♀ × Sus♂	Herculex	2.0 ± 1.0 aB	89.5 ± 1.8 aA	9.3 ± 1.2 aB	86.3 ± 1.8 aA
	Non-Bt	2.0 ± 2.0 aB	96.8 ± 1.8 aA	8.5 ± 1.2 aB	96.8 ± 1.8 aA
Y-R	YieldGard VT PRO	5.0 ± 1.0 aB	94.8 ± 1.0 aA	12.0 ± 2.2 aB	95.8 ± 1.0 aA
	Non-Bt	4.1 ± 2.0 aB	98.9 ± 1.0 aA	12.5 ± 3.1 aB	98.9 ± 1.0 aA
Y-R♂ × Sus♀	Non-Bt	4.1 ± 1.0 aB	98.2 ± 0.6 aA	13.5 ± 1.0 aB	100.0 ± 0.0 aA
Y-R♀ × Sus♂	Non-Bt	1.2 ± 1.0 aB	99.5 ± 0.8 aA	8.1 ± 2.1 aB	95.8 ± 1.0 aA
P-R	PowerCore	5.2 ± 1.0 aB	92.8 ± 1.8 aA	12.5 ± 1.8 aB	92.7 ± 1.0 aA
	Non-Bt	5.2 ± 1.0 aB	100 ± 0.0 aA	10.4 ± 2.7 aB	96.9 ± 1.0 aA
P-R♂ × Sus♀	Non-Bt	3.1 ± 1.9 aB	98.9 ± 1.8 aA	9.6 ± 3.6 aB	98.9 ±1.0 aA
P-R♀ × Sus♂	Non-Bt	4.1 ± 1.4 aB	98.7 ± 0.3 aA	9.4 ± 2.7 aB	96.8 ± 1.8 aA
Sus	Non-Bt	1.2 ± 1.0 aB	97.2 ± 1.1 aA	9.1 ± 1.0 aB	95.8 ± 1.0 aA

[a] The progeny from Y-R × Sus and P-R × Sus were not evaluated on YieldGard VT PRO and PowerCore maize, respectively, because they did not survive until third instar.

[b] Means within a column followed by the same lowercase letter and in each row followed by the same uppercase letter for an insecticide are not significantly different (Scott-Knott; *P* > 0.05).

Table 5. Two-away ANOVA results of the effect of Bt maize, insecticide and the interaction on plants damaged by FAW and grain yield.

Variable	Source of variation	Type III SS	df	Mean square	*F*	*P*
% plants with leaf damage	Maize technology × insecticide	13949.07	6	2324.84	5.90	< 0.0001
	Maize technology	20995.37	3	6998.45	17.76	< 0.0001
	Insecticide	100883.79	2	50441.89	128.06	< 0.0001
	Block	14898.61	3	4966.20	6.86	< 0.0001
	Model (total)	150726.86	14	10766.20	14.88	< 0.0001
	Error	301638.88	417	723.35		
	Corrected total	452365.74	431			
% plants with damage rating ≥ 3	Maize technology × insecticide	8267.93	6	1377.98	3.30	< 0.0035
	Maize technology	24300.63	3	8100.21	19.40	< 0.0001
	Insecticide	146594.56	2	73297.28	175.61	< 0.0001
	Block	30115.45	3	10038.48	13.72	< 0.0001
	Model (total)	209278.58	14	14948.47	20.44	< 0.0001
	Error	304963.02	417	731.32		
	Corrected total	514241.60	431			
Grain yield	Maize technology × insecticide	2542616.29	6	423769.38	0.32	0.9214
	Maize technology	51309071.04	3	17103023.68	12.94	< 0.0001
	Insecticide	30747565.16	2	15373782.58	11.63	< 0.0001
	Block	1998441.89	3	666147.29	1.15	0.3413
	Model (total)	77526190.57	14	5537585.04	4.19	< 0.0001
	Error	43596985.29	33	1321120.76		
	Corrected total	121123175.86	47			

Table 6. Average grain yields of Bt and non-Bt maize under different insecticide sprays against FAW during the 2017–2018 growing season in Santa Maria, RS, Brazil.

Maize	**Treatment**	**Sprays (*n*)**	**Yield (kg ha^{-1})[a]**
Herculex (P3779H)	Spinetoram	6	6322.22 ± 721.22 a
	Chlorfenapyr	6	5622.22 ± 599.74 a
	Without insecticide	-	4933.33 ± 300.92 a
YielGard VT PRO (DKB390PRO)	Spinetoram	5	9133.33 ± 338.57 a
	Chlorfenapyr	5	8516.22 ± 408.36 a
	Without insecticide	-	7472.22 ± 355.36 b
PowerCore (2A620PW)	Spinetoram	4	9291.55 ± 312.60 a
	Chlorfenapyr	5	8155.55 ± 328.30 a
	Without insecticide	-	6811.10 ± 348.16 b
Non-Bt (30F53)	Spinetoram	6	8408.77 ± 322.13 a
	Chlorfenapyr	6	7138.88 ± 57.66 a
	Without insecticide	-	6091.88 ± 456.51 b

[a]Means within the column for each maize technology followed by the same letter are not significantly different by Scott-Knott test ($P > 0.05$).

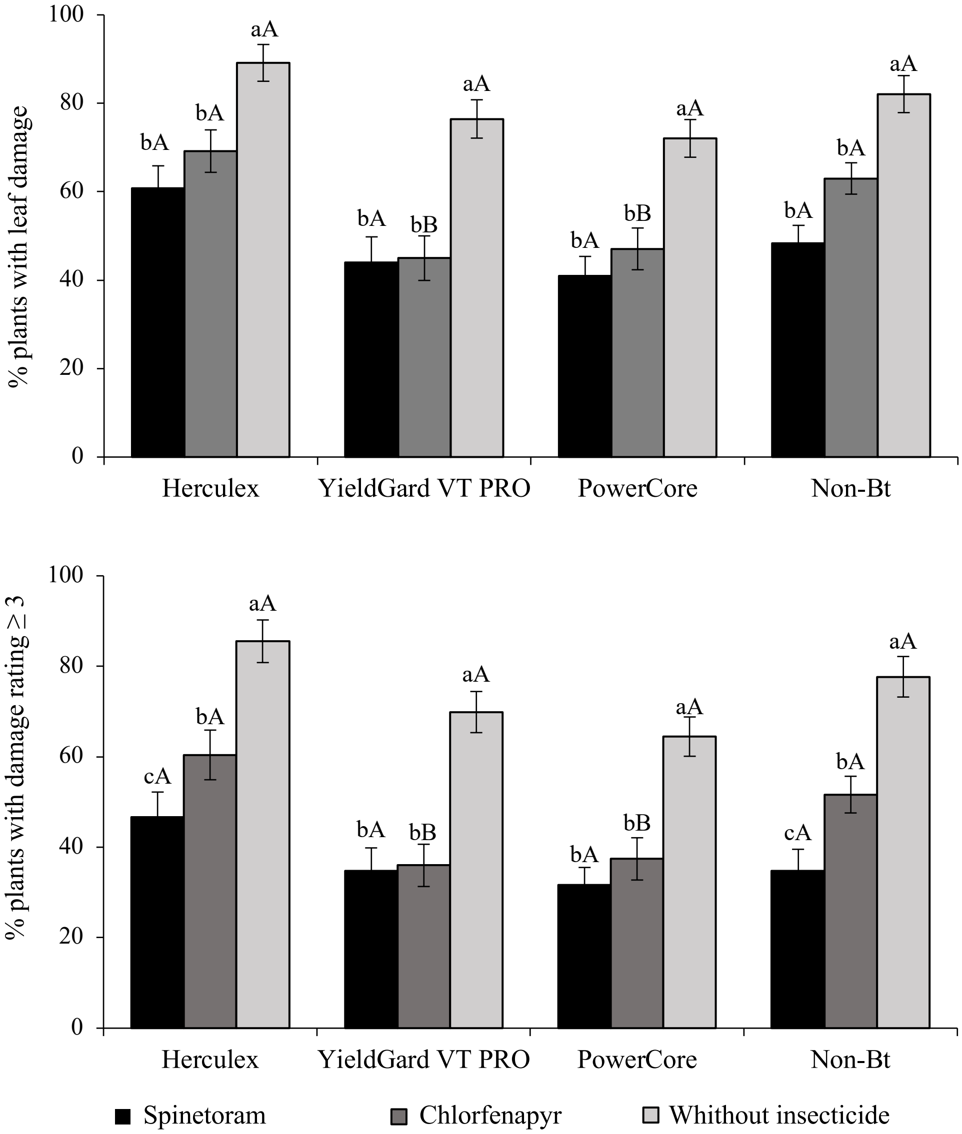

Figure 1. Percentage of plants with leaf damage and plants with damage rating ≥ 3 (Davis scale) caused by FAW on Bt and non-Bt maize in field trial. Bars (± SE – Standard Error) with same lowercase letters in each maize and uppercase letters in distinct maize but same insecticide are not significant different as determined by Scott-Knott test (*P* > 0.05).

4 DISCUSSÃO

A sustentabilidade das principais táticas de controle de *S. frugiperda* é um dos grandes desafios da agricultura brasileira. Dentre as táticas de controle, o tratamento de sementes com clorantraniliprole e imidacloprido + tiodicarbe em sementes de milho Bt e não-Bt aos 7 e 14 dias após a emergência (DAE) reduziu a sobrevivência de linhagens de *S. frugiperda* com resistência a milho Bt em bioensaios em laboratório. Entretanto, aos 21 DAE não foram observadas reduções significativas na sobrevivência. Em condições de campo, as tecnologias de milho Bt com expressão das proteínas Cry1A.105 + Cry2Ab2 e Cry1A.105 + Cry1F + Cry2Ab2 contendo tratamento de sementes com clorantraniliprole ou imidacloprido + tiodicarbe apresentaram menor porcentagem de plantas danificadas por *S. frugiperda* até os 7 DAE. Após esse período observou-se um aumento nos danos causados pela infestação natural de *S. frugiperda*. Embora houve redução na porcentagem de plantas danificadas, quando foi usado o tratamento de semente, constatou-se que essa estratégia de manejo possui baixa eficácia no controle de infestações iniciais dessa espécie. Ainda, pode-se afirmar que o tratamento de semente com os inseticidas testados possui baixo potencial para evitar ou retardar a evolução da resistência de *S. frugiperda* a proteínas Bt expressas em milho.

Quando da avaliação da suscetibilidade a inseticidade em pulverização foliar para o manejo de linhagens de *S. frugiperda* com resistência a Cry1F, Cry1A.105 + Cry2Ab2 e Cry1A.105 + Cry1F + Cry2Ab2 constatou-se que: lagartas resistentes e heterozigotos alimentadas em milho Bt e não-Bt e expostas aos inseticidas espinetoram e clorfenapir tiveram similar suscetibilidade entre si, mas menor suscetibilidade a ambos os inseticidas do que a linhagem suscetível de referência. Essa diferença na suscetibilidade entre as linhagens pode ser atribuida a variação natural na resposta aos inseticidas. Além disso, essa variação na suscetibilidade também pode ser atribuída a diferença no número de gerações das linhagens em laboratório (resistentes 6-7 gerações e suscetível mais de 40 gerações). Em experimentos em casa-de-vegetação e campo com o uso das doses comerciais de espinetoram e clorfenapir pulverizadas em milho Bt e não-Bt houve similar suscetibilidade entre as linhagens de *S. frugiperda* testadas, reforçando as hipóteses acima de que a variação na suscetibilidade não está diretamente vinculada a característica de resistência a proteínas de Bt. Sendo assim, o uso da dose recomendada dos inseticidas é primordial para se obter uma similar eficácia no controle de linhagens de *S. frugiperda* que estejam sobrevivendo sobre milho Bt e não-Bt no campo.

5 CONCLUSÕES

O tratamento de sementes de milho com clorantraniliprole ou imidacloprido + tiodicarbe apresenta baixa eficácia no controle de infestações de *S. frugiperda*.

As linhagens de *S. frugiperda* (resistentes, heterozigotos e suscetível) que sobrevivem em milho Bt e não Bt, num mesmo estágio de desenvolvimento larval, têm similar suscetibilidade aos inseticidas espinetoram e clorfenapir.

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