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Research

Pest Management

Performance of Seed Treatments Applied on Bt and Non-Bt Maize Against Fall Armyworm (Lepidoptera: Noctuidae)

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Abstract

Fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), is the main pest of maize in Brazil, attacking plants from emergence to reproductive stages. Here, we conducted studies to evaluate the efficacy of two seed treatments (chlorantraniliprole alone and imidacloprid combined with thiodicarb) on Bt and non-Bt maize in laboratory bioassays with distinct FAW strains that are susceptible, selected for resistance to Bt-maize single (Cry1F) or pyramided (Cry1A.105 + Cry2Ab2) events and F_1 hybrids of the selected and susceptible strains (heterozygotes), and in the field against a natural infestation. In the laboratory, leaf-discs from seed treated Bt-maize plants at 7 d after emergence (DAE) increased the mortality of FAW resistant, heterozygote, and susceptible strains up to 24.8%, when compared with the respective maize grown without a seed treatment. In the field against natural infestations of FAW, Bt maize with a seed treatment had ~30% less FAW damage than non-Bt maize with the same seed treatment at 7 and 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. Maize seeds treated with chlorantraniliprole alone or imidacloprid and thiodicarb combined presented limited protection against early infestations of FAW strains under laboratory and field studies.

Key words: transgenic maize, Bt protein, insecticide, insect resistance management

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 of up to 57%, if control strategies are not used (Cruz et al. 2012). For its management, maize hybrids expressing *Bacillus thuringiensis* Berliner (Bt) proteins is the main tactic 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 refuge areas of FAW-susceptible maize as part of an Insecticide Resistance Management (IRM) plans exposed FAW populations to selection pressure, resulting in resistance to Bt proteins expressed in maize (Farias et al. 2014, Bernardi et al. 2015, Omoto et al. 2016).

In Brazil, FAW evolved resistance to Cry1F and Cry1Ab in maize (Farias et al. 2014, Omoto et al. 2016). From laboratory assays, FAW has shown resistance to pyramided maize expressing Cry1A.105, Cry2Ab2, and Cry1F (Santos-Amayaet al. 2015, Horikoshi et al. 2016, Bernardi et al. 2017). Resistance to Bt maize by FAW was also reported in Puerto Rico (Storer et al. 2010), United States (Huang et al. 2014), and Argentina (Chandrasena et al. 2018). Since FAW has developed resistance to Bt maize, there has been an increase in insecticide use against this species for maize production in Brazil (Burtet et al. 2017). However, the efficacy of insecticides is directly influenced by the larval behavior of this pest. As FAW larvae grow, they move into the maize whorl, reducing their exposure to foliar applications of insecticides (Ceccon et al. 2004, Malaquias et al. 2017). In Brazil, cases of resistance were documented in laboratory or in field populations of FAW to the following insecticides: lambda-cyhalothrin (Diez-Rodríguez et al. 2001), chlorpyrifos (Carvalho et al. 2013), lufenuron (do Nascimento et al. 2016), spinosad (Okuma et al. 2017), and chlorantraniliprole (Bolzan et al. 2019).

Additional control tactics are needed for the management of FAW on Bt and non-Bt maize. To manage early infestations of FAW, seed-applied insecticides (i.e., a seed treatment) are commonly used (Azevedo et al. 2004, Ceccon et al. 2004, Quintela et al. 2016). Many Brazilian farmers use a seed treatment to prevent damage to maize seeds and seedlings caused by *Euschistus heros* (F.) (Hemiptera: Pentatomidae) (Ceccon et al.

2004), *Liogenys fusca* (Blanchard) (Coleoptera: Melolonthidae) (Santos et al. 2008), *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) (Kullik et al. 2011), *Elasmopalpus lignosellus* (Zeller) (Lepidoptera: Pyralidae) (Viana et al. 2011), *Scaptocoris castanea* (Party) (Hemiptera: Cydnidae) (Silva et al. 2013) and *Dichelops* sp. (Hemiptera: Pentatomidae) (Quintela et al. 2016). 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). Recently, diamide insecticides were used as seed treatment for managing FAW (Jeanguenat 2013, Pes et al. 2020).

In this study, a series of laboratory and field experiments were conducted to determine whether the addition of a seed treatment to maize (either with or without Bt protein expression) 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 the two main 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 and Cry1A.105 + Cry2Ab2). In addition, we conducted field trails with these seed treatments and maize varieties against natural infestation of FAW.

Materials and Methods

FAW Strains

The F_2 screen technique proposed by Andow and Alstad (1998) was used to select FAW strains resistant to Bt protein-expressing maize lines. We selected strains with high survival on maize expressing the following Bt proteins: Cry1F (Herculex; P3779H, DuPont Pioneer, Santa Rosa, RS, Brazil) and Cry1A.105 + Cry2Ab2 (YieldGard VT PRO; DKB390PRO, Dekalb, Uberlandia, MG, Brazil). The FAW colonies resistant to Cry1F (hereafter H-R) and to Cry1A.105 + Cry2Ab2 (hereafter Y-R) were selected using leaf tissue bioassays 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). Surviving larvae from F_2 generation was reared on excised leaves of the respective Bt maize on which they were selected until pupal stage. 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 an artificial diet where they remained until the pupa stage. We also used a strain of FAW that was collected in non-Bt maize in Mogi Mirim, São Paulo, Brazil (22°28′31″S and 46°54′21″W) and has been maintained in the laboratory since 2012 without exposure to Bt-proteins and insecticides. We refer to this colony as a susceptible strain (hereafter Sus). To evaluate heterozygous strains, the reciprocal cross between resistant ♀ × susceptible ♂ were performed for the two resistant strains. We only used these heterozygote strains because inheritance of resistance is autosomal inherited (Storer et al. 2010, Farias et al. 2014, Bernardi et al. 2015, Santos-Amaya et al. 2015, Chandrasena et al. 2018).

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 + Cry2Ab2 proteins (DKB390PRO, Dekalb, Uberlândia, MG, Brazil), and a non-Bt maize (30A37, Dow AgroSciences, Jardinópolis, SP, Brazil). Each variety was industrially treated with doses recommended for maize of two seed treatments: chlorantraniliprole (Dermacor, Corteva Agriscience, Marinette, WI) at 30 g active ingredient (a.i.) per 60,000 seeds, and imidacloprid + thiodicarb (CropStar, Bayer CropScience, Belford Roxo, RJ, Brazil), at 52.5 + 157.5 g a.i. per 60,000 seeds. Maize seeds were planted on 19 January 2018 in a field located in Santa Maria, Rio Grande do Sul, Brazil (29° 43′ 3.96″ S and 53° 44′ 2.33″ W) at a density of 90,000 plants per ha with a row spacing of 0.50 m (four rows per maize treatment). Maize leaves were collected for use in the bioassays at 7, 14, and 21 d after emergence (DAE) representing the V_2, V_{3-4}, 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) for Cry2A and Cry1F.

In the laboratory, 1.2 cm diameter leaf-discs were cut from the maize whorl leaf randomly and placed on a 2.5% mixture of water-agar (1 ml 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 (resistant, heterozygote, or susceptible strain) was infested on each leaf-disc. Plates were sealed with self-adhesive plastic sheets and conditioned in a growth chamber (temperature: 25 ± 1°C; relative humidity: 60 ± 10%; photoperiod: 14:10 (L:D) h). The experimental design was completely randomized with eight replicates per treatment (16 neonates per replicate). Survival was evaluated at 5 d, because at this time surviving insects develop beyond second instar. Larvae were considered dead when they showed no apparent movement. Data were 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(SAS Institute 2000). Data were subjected to analysis of variance (ANOVA) using the PROC GLM procedure in SAS 9.1(SAS Institute 2000). 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 2000).

Efficacy of a Seed Treatment on Field Grown Bt and Non-Bt Maize Under Natural Infestations of FAW

To evaluate the efficacy of seed treatments against infestations of FAW on Bt and non-Bt maize, a field study was performed using the same two seed treatments and three maize varieties described earlier. Each maize variety was planted (90,000 plants per ha) in the same area, which has a history of FAW incidence, and date mentioned earlier. Thus, plants were exposed to the same abiotic factors in the laboratory and field trials. At planting, an application of 225 kg per ha of Nitrogen–Phosphorus–Potassium (NPK; 5–20–20) was performed. 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 3 × 3 factorial arrangement, and each block contained nine plots. The first factor (A) was represented by two Bt maize varieties (Cry1F and Cry1A.105 + Cry2Ab2) and one non-Bt maize. The second factor (B) was represented by two seed treatments (chlorantraniliprole alone and imidacloprid + thiodicarb combined) and an untreated control. Damages caused by natural

Table 1. Survival of FAW neonates (% ± standard error) on Bt and non-Bt maize with and without a seed treatment at 7, 14, and 21 d after emergence (DAE) in laboratory bioassays

Seed treatment	Resistant strain[a,b]		Heterozygous strain[a,b]		Susceptible strain[a]	
7 DAE						
	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 + Cry2Ab2 maize	Non-Bt maize	Cry1A.105 + Cry2Ab2 maize	Non-Bt maize	Cry1A.105 + Cry2Ab2 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 a	n/a[d]
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 a	n/a
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 a	n/a
14 DAE						
	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 + Cry2Ab2 maize	Non-Bt maize	Cry1A.105 + Cry2Ab2 maize	Non-Bt maize	Cry1A.105 + Cry2Ab2 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 a	n/a[d]
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 a	n/a
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 a	n/a
21 DAE						
	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	96.8 ± 1.2 aB	0.0 ± 0.0 aA	89.1 ± 1.0 aB
	Cry1A.105 + Cry2Ab2 maize	Non-Bt maize	Cry1A.105 + Cry2Ab2 maize	Non-Bt maize	Cry1A.105 + Cry2Ab2 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 a	n/a[d]
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 a	n/a
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 a	n/a

[a]Means within a column for each data 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 maize and Y-R strain on Cry1A.105 + Cry2Ab maize).

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

[d]n/a, not applicable.

infestation of FAW was estimated on 20 plants within the two central rows of each plot at 7, 14, and 21 DAE. A damage rating was attributed to each plant according to the Davis scale: 0 = no damage to 9 = severe damage (Davis and Willians 1992). These results were then converted to a percentage of plants damaged by FAW and a 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 (IRAC 2019). If ≥ 20% of the plants within a plot received rating ≥ 3, the plot was considered to be damaged significantly. Data were subjected to two-way ANOVA using the PROC GLM procedure in SAS 9.1 (SAS Institute 2000). Seed treatments, maize technologies, and their interactions were considered fixed factors in the model.

Results

Selection of the FAW Resistant Colonies

Two FAW strains, one resistant to Cry1F maize and other resistant to Cry1A.105 + Cry2Ab2 maize, were selected using the F_2 screen technique (Andow and Alstad 1998). In total, 102 isofamilies were screened (>9,700 larvae tested) on each Bt maize with 27 isofamily lines containing resistance alleles (>2,000 larvae survived per Bt maize). This represents ~80% of larval survival per positive line. During six generations, resistant colonies were reared on the Bt maize on which they were selected, as described in detail in M&M. After this time, laboratory bioassays were started.

Table 2. Summary of ANOVA regarding the effect of seed treatment, Bt-maize, and their interactions on plants damaged by FAW under field conditions

Variable[a]	Source of variation	Type III SS	df	Mean square	*F*	*P*
7 DAE						
% plants damaged	Maize × seed treatment	346.66	4	86.66	0.92	0.4655
	Maize	4,328.66	2	2,164.33	23.11	<0.0001
	Seed treatment	7,802.16	2	3,901.08	41.66	<0.0001
	Block	446.30	3	148.76	1.59	0.2181
	Model (total)	12,923.81	11	1,174.89	12.54	<0.0001
	Error	2,246.94	24	93.62		
	Corrected total	15,170.75	35			
% plants with significant damage[b]	Maize × seed treatment	1,281.11	4	320.27	2.73	0.0523
	Maize	3,480.72	2	1,740.36	14.87	<0.0001
	Seed treatment	8,986.05	2	4,493.02	38.40	<0.0001
	Block	68.08	3	22.69	0.19	0.8995
	Model (total)	3,746.31	11	340.57	2.91	<0.0001
	Error	2,807.66	24	116.98		
	Corrected total	6,553.97	35			
14 DAE						
% plants damaged	Maize × seed treatment	511.11	4	127.77	0.62	0.6489
	Maize	1,901.38	2	950.69	4.65	0.0196
	Seed treatment	168.05	2	84.02	0.41	0.6674
	Block	2,897.22	3	965.74	4.72	0.0099
	Model (total)	5,477.78	11	497.98	2.43	0.0021
	Error	4,902.77	24	204.28		
	Corrected total	10,380.55	35			
% plants with significant damage[b]	Maize × seed treatment	345.66	4	86.41	0.30	0.8738
	Maize	2,271.16	2	1,135.58	3.96	0.0325
	Seed treatment	465.16	2	232.58	0.81	0.4556
	Block	795.86	3	265.28	0.92	0.4430
	Model (total)	3,877.87	11	352.53	1.23	<0.0001
	Error	6,870.88	24	286.28		
	Corrected total	10,748.75	35			
21 DAE						
% plants damaged	Maize × seed treatment	163.61	4	40.90	0.26	0.8995
	Maize	362.72	2	181.36	1.16	0.3301
	Seed treatment	481.05	2	240.52	1.54	0.2348
	Block	11,723.63	3	3,907.87	25.02	<0.0001
	Model (total)	12,731.38	11	1,157.39	7.41	<0.0001
	Error	3,748.61	24	156.19		
	Corrected total	16,479.63	35			
% plants with significant damage[b]	Maize × seed treatment	127.11	4	31.77	0.16	0.9551
	Maize	275.72	3	137.86	1.70	0.5032
	Seed treatment	369.38	2	184.69	0.94	0.4020
	Block	6,447.63	3	2,149.21	11.01	0.0001
	Model (total)	7,219.86	11	656.35	3.36	<0.0001
	Error	4,681.11	24	195.04		
	Corrected total	11,900.97	35			

[a]DAE = days after emergence.
[b]Significant damage is based on a plant receiving a score ≥ 3 on the Davis scale.

Efficacy of Seed Treatment Against FAW Strains in Laboratory Bioassays

In general, the impact of a seed treatment was most noticeable at 7 and 14 DAE (Table 1). Except for the heterozygote strain fed the non-Bt maize, the highest survival was observed on untreated maize. The greatest impact of a seed treatment was observed on maize varieties that produced a Bt-toxin. The Y-R strain was the most susceptible to the chlorantraniliprole seed treatment when fed on a treated Bt maize, when compared with the control treatment grown without a seed treatment. Significant reductions in survival of FAW larvae due to the seed treatment 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 DAE (Table 1), seed treatment significantly reduced the survival only for the Y-R strain fed on leaves of Cry1A.105 + Cry2Ab2 maize. After 14 DAE, the susceptible strain did not suffer any mortality from either seed treatment. Neonates from the H-R heterozygote 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 did not provide significantly reduction in mortality when compared with the untreated control.

No significant reduction in FAW survival was observed at 21 DAE (Table 1), regardless of the seed treatment and the FAW strain. However, just one Bt resistant FAW strains had significant lower survival rates when fed on Bt maize than on non-Bt maize (Table 1). On non-Bt maize, the susceptible strain had a similar survival on leaf-discs from plants with or without seed treatment (> 89%) (Table 1). These results indicate that at 21 DAE, the seed treatments were not a source of FAW mortality.

Efficacy of Seed Treatment Applied to Bt and Non-Bt Maize Against Natural Infestation of FAW

There was not a maize × seed treatment interaction for the percentage of plants damaged by FAW and plants with significant damage at 7, 14, and 21 DAE (Table 2). However, the percentage 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 effect at 7 DAE for the percentage of plants with damage and plants with significant damage (Fig. 1).

The significant protection from FAW was observed on Cry1A.105 + Cry2Ab2 maize treated imidacloprid + thiodicarb when compared with the non-Bt maize (Table 3). However, this protection was only observed for 14 DAE, by 21 DAE, the % of plants with damage and the % of plants with significant damage was similar to the untreated, non-Bt line across all treatment combinations. The combination of Cry1A.105 + Cry2Ab maize with an imidacloprid + thiodicarb seed treatment had the fewest percentage of plants damaged by FAW (41%) and the fewest with significant damage (13.5%) at 7 DAE (Table 3). However, by 21 DAE (Table 3), this combination did not have fewer damaged plants or fewer with significant damage when compared with any other treatment combination. We did not observe a significant difference in either parameter measured at 21 DAE (Table 3).

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). This heterozygote strain also had limited mortality when exposed to Cry1F maize grown with a seed treatment. In contrast, we observed no survival by heterozygous larvae exposed to leaves of Cry1A.105 + Cry2Ab2 with and without seed treatments.

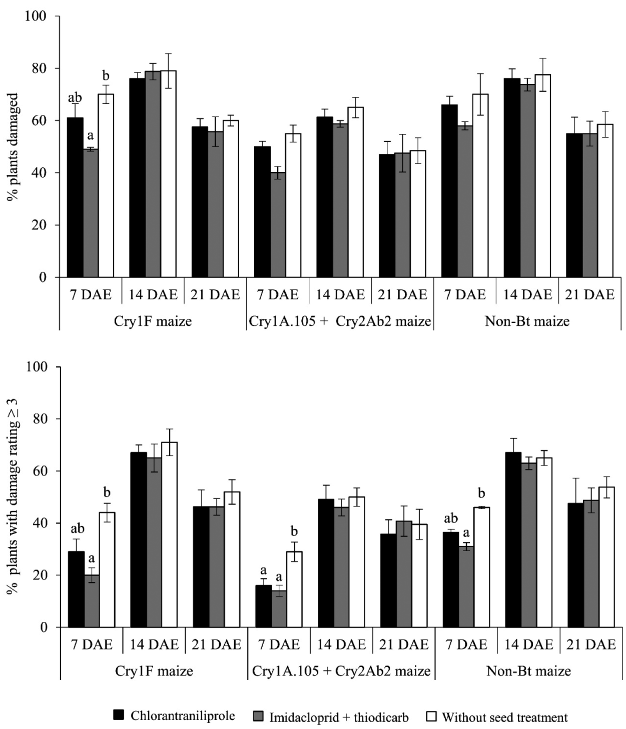

Fig. 1. Effect of seed treatment in percentage of damaged plants and plants with damage rating ≥ 3 (Davis scale) caused by FAW on Bt and non-Bt maize at 7, 14, and 21 d after emergence (DAE) in a field experiment. Group of bars (± SE—Standard Error) without letters for each maize and evaluation time did not differ from each other (LSMEANS with Tukey's adjustment; $P > 0.05$).

In field conditions, at 7 DAE, Bt maize with seed treatments had significant lower damage by FAW than non-Bt maize without a seed treatment. At this time, seed treated Cry1A.105 + 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 an economic threshold level after 7 DAE.

In previous studies, chlorantraniliprole used as a seed treatment on soybean and maize also showed limited efficacy against FAW (Thrash et al. 2013) and *Mythimna unipuncta* Haworth (Lepidoptera: Noctuidae) (Carscallen et al. 2019). A low efficacy of thiamethoxam, carbofuran, imidacloprid, fipronil, and thiodicarb applied on maize seeds against FAW has also been reported (Ceccon et al. 2004). Furthermore, carbofuran and thiamethoxan applied

Table 3. 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 d after emergence (DAE) in a field experiment

Maize	% plants damaged[a]			% plants with significant damage[a,b]		
	7 DAE	14 DAE	21 DAE	7 DAE	14 DAE	21 DAE
Chlorantraniliprole						
Cry1F	61.0 ± 5.4 b	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 + Cry2Ab2	50.0 ± 2.0 a	61.2 ± 3.1 a	47.5 ± 5.0 a	16.0 ± 2.6 a	48.7 ± 5.5 a	35.7 ± 5.5 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 a	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 + Cry2Ab2	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
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 a	60.0 ± 2.0 a	44.2 ± 3.6 b	71.2 ± 5.1 b	52.0 ± 4.6 a
Cry1A.105 + Cry2Ab2	55.0 ± 3.3 a	65.7 ± 3.8 a	48.5 ± 4.9 a	28.7 ± 3.7 a	50.0 ± 3.5 a	42.0 ± 5.8 a
Non-Bt	76.2 ± 3.7 b	77.5 ± 4.7 a	58.5 ± 3.9 a	46.2± 3.7 b	66.5 ± 6.3 b	53.7 ± 5.5 a

[a]Means within a column for Bt and non-Bt maize followed by the same letter are not significantly different (LSMEANS with Tukey's adjustment; $P > 0.05$).
[b]Significant damage is based on a plant receiving a score ≥ 3 (% ± SE) on the Davis scale.

as a 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* (F.) (Lepidoptera: Crambidae) and *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Farias et al. 2013). Although chlorantraniliprole and imidacloprid have been shown to be transported upward throughout the plant via xylem, when applied as seed treatment (Lahm et al. 2007, Stamm et al. 2016, Carscallen et al. 2019) their ability to provide protection from insect herbivory is limited.

The limited protection of seed treatments against early infestations of FAW may be due the poor translocation through a plant (Lanka et al. 2013, Stamm et al. 2016). It is known that 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 and Krupke 2017). In contrast, chlorantraniliprole applied as seed treatment in maize was translocated by xylem and caused 57% mortality of FAW larvae until V_6 stage, when plants were cultivated in small pots with a mixture of 30% substrate and 70% soil in greenhouse conditions (Pes et al. 2020).

According to Santos et al. (2018), the soil in our experimental area is classified as Dystrophic Red Latosol. Soil analysis indicated low levels of clay (25%) and organic matter (2.1%), moderate fertility, and low water storage capacity. From planting to 21 DAE were registered an average temperature of 23.4°C, relative humidity >40% and accumulated precipitation of 144 mm, which are normal environmental conditions for the time of year and region. Previous studies indicated that greatest seed treatment benefits often are seen when soils are cool and wet (Ghate and Phatak 1982, Baxter and Waters, Jr. 1986).

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. Our field experiment was conducted in the late-planting season (January) when there is a greater risk of a FAW infestation after plants emerge, due to rapid population growth after winter (September onwards), which may have influenced our estimate of the efficacy of seed treatments against an early infestation of FAW. Higher infestations of FAW in maize have been observed in late planted maize in southern Brazil and up to four foliar insecticides sprays were necessary to prevent yield loss (Burtet et al. 2017).

According to our results, monitoring both the presence of FAW larvae and damage on Bt and non-Bt maize plants with and without seed treatment 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 sprays against FAW on Bt maize and non-Bt maize is recommended when 20% of the plants show a damage rating ≥3 (IRAC 2019). By 21 DAE in our field experiment, none of the maize varieties were below this threshold whether a seed treatment was used or not. Bt maize could also be integrated with other control tactic such as biological control with baculovirus (Bentivenha et al. 2019). In summary, integrate control tactics with diverse mortality factors would considered to control FAW in maize in Brazil and this also would contribute to the IRM programs.

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