

Article

Susceptibility of Fall Armyworm Field Populations to Vip3Aa/Cry Bt Maize in a Tropical Agricultural Region

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Abstract: Fall armyworm (FAW, *Spodoptera frugiperda*) is a polyphagous and migratory lepidopteran pest insect in field crops and is notoriously invasive worldwide. In large portions of the Americas, its populations are managed using transgenic maize or cotton varieties producing insecticidal proteins from *Bacillus thuringiensis* (Bt), primarily Vip3Aa pyramided with Cry Bt proteins. We determined the susceptibility of FAW field populations from locations pressured with such maize hybrids for over five years. We used time–mortality bioassays with F₁ third-instar larvae of six geographically distinct populations collected in maize fields of a tropical agricultural region encompassing four Brazilian states. We maintained the neonate progeny from the field populations on an artificial diet until the third instar, and then determined their survival curves on the foliage of three Vip3Aa/Cry-producing Bt maize hybrids. Death of the mid-size, third-instar FAWs occurred relatively rapidly, with larval mortality rates reaching 98–100% in less than five days regardless of Bt maize hybrid. However, median survival time (ST₅₀) for the larvae differed among the populations, with the lowest and highest ST₅₀ values occurring for PI-Cr (42 h, 1.75 d) and PI-Ur populations (66–90 h, 2.75–3.75 h), respectively. Therefore, the F₁ third-instar larvae of FAW populations were largely susceptible to Vip3Aa/Cry-producing maize foliage, and the most contrasting susceptibility occurred in the insects from Piauí state, Brazil. These results indicate that progeny of FAWs from areas highly pressured with Vip3Aa/Cry Bt maize hybrids are killed on maize foliage producing Vip3Aa and Cry Bt proteins despite field reports of increased leaf damage by the larvae in some locations. This research informs decision making for Bt-crop resistance management by producers, technicians, and researchers in local, regional, and world agriculture.

Keywords: *Spodoptera frugiperda*; *Bacillus thuringiensis*; transgenic Bt crops; resistance management



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1. Introduction

Spodoptera frugiperda (J. E. Smith), the fall armyworm (FAW), is one of the most problematic pest insects in maize (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.) fields of South America [1]. By 2016, the species had expanded its range to Africa, Asia, and Oceania, where it has caused much concern [2]. Fall armyworm traits such as polyphagy, high reproductive capacity, and adult movement in the landscape favor large population sizes in maize and cotton throughout the year in tropical and subtropical regions [3,4]. The insect can attain high populations where warm climates and intensive cropping systems prevail, as occurs primarily in Brazil. The armyworm thrives even more in a new tropical agricultural frontier that has been extended in the country, encompassing the states of

Maranhão, Tocantins, Piauí, and western Bahia (Figure 1). This region is informally named ‘Matopiba’ and is anticipated to produce c. 50 million tons of grains in 11 million ha in the next decade [5]. Such a growth trend will likely surpass the country’s 24% expected increase in grain production [6].

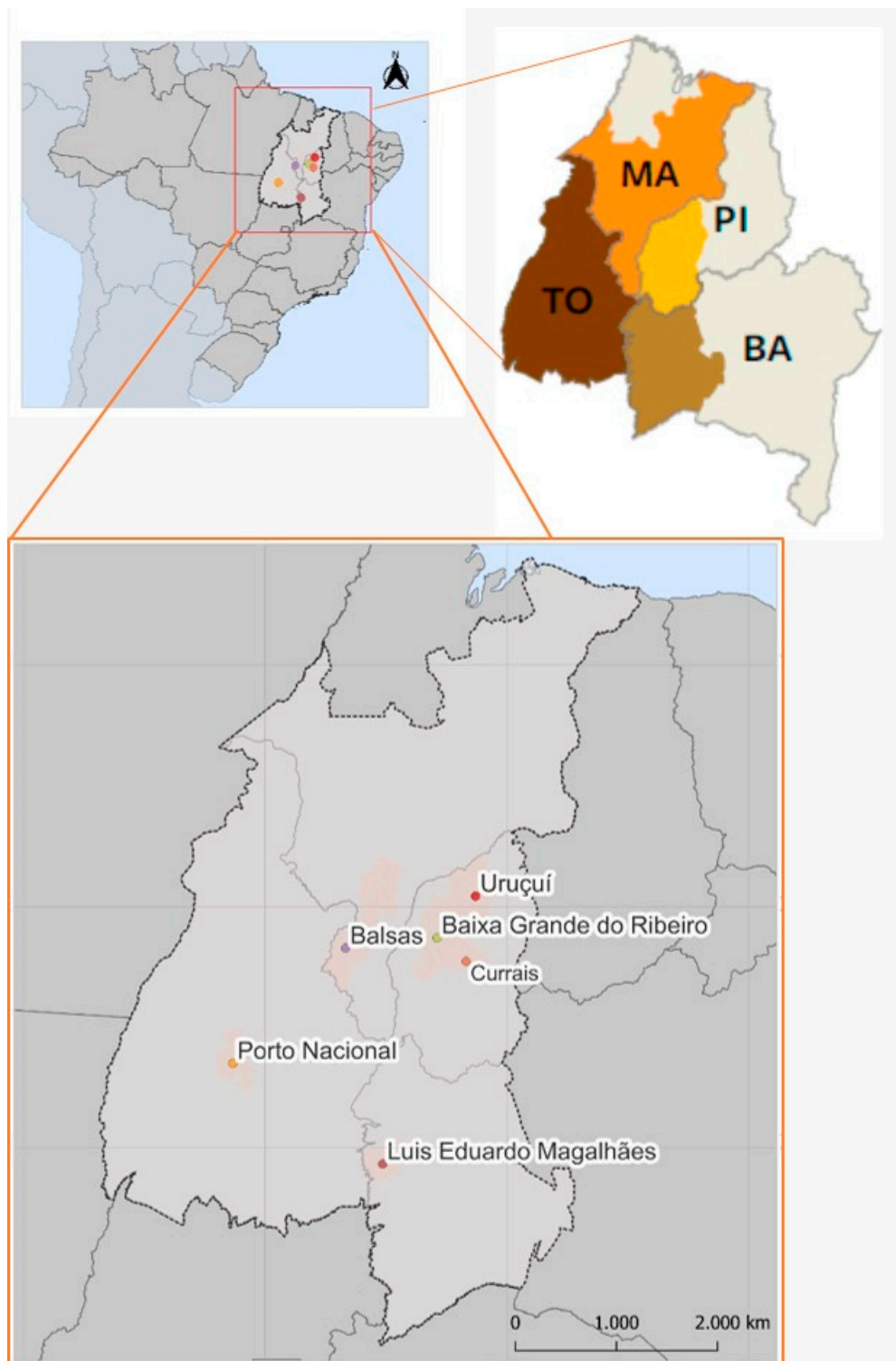


Figure 1. Sampling sites of the field populations of fall armyworm (*Spodoptera frugiperda*). Shown is a graphical representation of the ‘Matopiba’ region, formed by the Brazilian states of Maranhão (MA), Tocantins (TO), Piauí (PI), and Bahia (BA), where the insects were collected.

Transgenic maize hybrids producing insecticidal proteins (or toxins) from *Bacillus thuringiensis* Berliner (Bt) are the paramount control strategy against the FAW in Brazil's intensive cropping systems [7–9]. Bt insecticidal proteins Cry1Ab, Cry1F, Cry1A.105, Cry2Ab2, and Vip3Aa20 have been bio-engineered into maize plants (Bt maize) to protect against a broad range of lepidopteran pests [10] that feed on aerial plant parts, including the stalk, foliage, and ear. The Cry1Ab Bt protein, in maize events MON810 or Bt11, was the first to be deployed in the Americas against the fall armyworm [3]. Subsequently, the TC1507 Bt maize event, producing the Cry1F insecticidal protein from *B. thuringiensis aizawai*, was introduced into the market [3,11]. Almost concurrently, Cry2Ab2, derived from *B. thuringiensis kurstaki*, and Cry1A.105, a synthetic chimera of Cry1Ab, Cry1Ac, and Cry1F, were also introduced in transgenic event MON89034 [12,13]. Lastly, the Vip3Aa20 Bt protein, derived from *B. thuringiensis* strain AB88 and produced in maize transgenic event MIR162, was launched in the market [3] and has become increasingly adopted in Brazil since 2018, after its incorporation in the elite maize hybrids [7].

Bt insecticidal proteins, be they used in biopesticides or transgenic crop cultivars, represent a safe pest management tool because of their specific toxicity against target organisms [14,15]. After ingestion by a susceptible insect, Cry or Vip Bt proteins bind to specific midgut receptors and induce enterocyte lysis, leading to disruption of the intestinal epithelia and ultimately insect death by septicemia or dysfunctional nutrient absorption [14–16]. However, the evolution of resistance in pest populations is the main threat to the sustainability of Bt technologies for pest management, especially the fall armyworm, which is inherently less susceptible to many Bt toxins [3,13,15,16]. The efficacy of Bt maize hybrids producing Cry Bt proteins had already decreased against FAW, and the structural homology of these proteins is conducive to cross-resistance among them [13,16,17]. That contrasts with Vip and Cry, which may bind to distinct sites in protein receptors in the insect gut [13,16–22].

As of 2015, and primarily since 2020, the *vip3Aa20* gene has been introduced into transgenic maize hybrids of the core germplasm families in Brazil [7]. The increase in the adoption of Vip3-producing Bt maize can expose FAW populations to high selection pressure for resistance to the Vip3Aa20 Bt protein. These Bt maize hybrids are so efficacious against the fall armyworm [18,19] that even the more tolerant, late-instar larvae are killed [23,24]. Additionally, data indicate that cross-resistance between Cry and Vip3Aa proteins is unlikely [16–18], and no published reports have documented field-selected resistance against FAW to Vip3Aa. However, there are eventual complaints of control failure among some local field personnel and concerns about the risk of resistance selection [23–29]. FAW's resistance to MON89034 transgenic Bt maize event (Cry1A.105/Cry2Ab2) comes with cross-resistance to other Cry1 Bt proteins [17,19], despite evidence of strong fitness costs of the resistance [20]. Thus, MON89034 combinations with events TC1507 and MIR162 (Cry1A.105/Cry2Ab2 + Cry1F + Vip3Aa20), or combinations of events Bt11 and MIR162 (Cry1Ab + Vip3Aa20), may leave the Vip3Aa20 protein alone or nearly so against FAW larvae.

As a part of our efforts to contribute to resistance management programs, we questioned the evidence of decreased susceptibility of fall armyworm populations from Brazil's Matopiba region to Vip3Aa20-producing Bt maize. In this area, selection pressure for Vip3Aa resistance has occurred in FAW for over five years. Specifically, we tested whether the mid-sized, third-instar FAW larvae, which were F₁ progeny from the populations collected in the field, increased survival on leaf tissues of Bt maize hybrids producing Vip3Aa20 and Cry toxins. If no constitutive Bt resistance had developed in the FAW populations, one would expect to detect no substantial changes in the killing power of Vip3Aa/Cry-producing maize foliage to third-instar F₁ FAWs.

2. Material and Methods

2.1. Insect Populations

Spodoptera frugiperda populations were collected in maize fields in six locations in the Matopiba region (Figure 1 and Table 1). We selected these locations to represent a tropical region that is becoming one of the most important for grain production in Brazil.

We collected a large number of larvae to obtain a representative sample of the genetic variability of the local FAW population (Table 1). In each site (c. 300 ha), we collected larvae in at least five different sampling areas of the maize field located at least 100 m apart (Figure 1) in a maize field. We collected larvae in fields informed by the grower or crop consultant as being planted to Vip3A-producing Bt maize hybrids. Collection periods occurred mainly in the spring–summer maize-growing season. The larvae were gently collected using tweezers to facilitate reaching deep into the leaf whorls. The larvae were transferred to 1000 mL plastic pots containing plenty of maize whorled leaves, placed in thermic boxes, and brought to the laboratory.

Table 1. Geographic locations where fall armyworm populations were collected and the approximate number of larvae taken to the laboratory.

Location, State of Brazil (Population Code)	Geographic Coordinates	Number of Larvae Collected
Balsas, Maranhão (MA-Bs)	8°41′16.8″ S 46°40′9.0″ W	1600
Porto Nacional, Tocantins (TO-PN)	10°35′46.5″ S 48°32′3.5″ W	1350
Baixa Grande, Piauí (PI-BG)	8°31′10.1″ S 45°9′22.4″ W	600
Currais, Piauí (PI-Cs)	8°54′26.0″ S 44°40′35.9″ W	1000
Uruçuí, Piauí (PI-Ur)	7°49′37.8″ S 44°31′11.3″ W	850
Luis Eduardo Magalhães, Bahia (BA-LE)	12°15′46.3″ S 46°3′19.6″ W	1450

Geographic coordinates denote the municipality's headquarters, where the larvae were collected.

The larvae were individualized in 100 mL disposable cups and provided with a standardized artificial diet [30] until pupation. The pupae were sexed and transferred to breeding cages, putting seven male–female pairs per cage to obtain the F₁ generation used in the assays. The egg masses were collected on the third day after the initial oviposition. The F₁ neonates were reared on the artificial diet until the third instar (c. 1 cm in size) when they were transferred to maize foliage according to variety and population.

2.2. Bt Maize Hybrids

We used Bt maize hybrids that are grown in the region under study. They were P3551PWU (producing Cry1F, Cry1A.105, Cry2Ab2, and Vip3Aa20), DKB390PRO4 (Cry1A.105, Cry2Ab2, Vip3Aa20, and Cry3Bb1 and RNAi against rootworms), and 30F53VYH (Cry1F, Cry1Ab, and Vip3Aa20) (Table 2). As controls, we used the respective hybrids (i.e., non-Bt isogenic hybrids) to compare the larval survival in the absence of insecticidal protein and estimate their natural mortality (see below).

Table 2. Details on the Bt maize hybrids used in the study.

Maize Hybrid	Lepidopteran-Active Bt Proteins	Bt Technology or Registered Trademark
DKB390PRO4	Cry1A.105, Cry2Ab2, Vip3Aa20	VTPRO4 [®] / YieldGard [®]
P3551PWU	Cry1F, Cry1A.105, Cry2Ab2, and Vip3Aa20	PowerCore [™] Ultra
30F53VYHR	Cry1F, Cry1Ab, and Vip3Aa20	Leptra [®] / Agrisure Viptera [®]
30F53RR	None	None (Round Ready [®])

The plant cultivation was conducted in 12.9 L pots, each containing approximately 5 kg of a standard potting soil mixture in a greenhouse. Bt protein in the maize plants was tested using strips from the QuickStix kits for Cry1, Cry2, and Vip3A (Envirologix Latin America Headquarters, Jaguariúna, SP, Brazil). Fresh foliage from plant whorls was harvested during the V4–V10 maize growth stages. The foliage was cut into 2–3 cm² sections, placed in Eppendorf tubes with perforated lids, and maintained in the laboratory until use.

2.3. Susceptibility to Vip3Aa/Cry Proteins

Time–mortality bioassays were conducted under controlled conditions in the Plant Protection Laboratory at the Federal University of Piau , Bom Jesus, Piau , Brazil. The bioassays were conducted using third-instar larvae from the F₁ generation. Third instars have intermediate Bt susceptibility between early and late instars, are easier to handle and record mortality than the former, and are less susceptible than the latter, thus increasing our odds of encountering reduced susceptibility, if any, among the populations [20].

We used a longitudinal experimental design, assigning fresh whorled leaf sections according to maize variety to 60 microcentrifuge tubes for each of the six FAW populations, as previously described. One FAW larva was placed per tube containing the foliage, totaling 60 individuals exposed for each population and maize hybrid, 240 per population, or 1440 individuals in the study. The insects were maintained under controlled conditions (29 ± 2 °C, 70 ± 15% RH, and 14:10 h L:D photoperiod [20]). We replaced the foliage every 1–3 days or whenever necessary according to the amount consumed by the larva. Mortality assessments occurred every 6 h for 7 days or until the larva died or pupated.

2.4. Data Analysis

The cumulative mortality rates after 7 days of feeding on the foliage of the Bt maize hybrids were analyzed using a generalized linear model with binomial distribution. The survival data were analyzed using Cox proportional hazards regression to test for differences in the survival schedule according to insect population and Bt maize hybrid. We computed survival curves using the log-rank method ($\alpha = 0.05$). We used the “survminer” and “survival” packages in R to compare survival curves and estimate the median survival time (ST₅₀) values and their respective 95% confidence limits (95% CL). ST₅₀ values were considered different when their 95% CL did not overlap their origin, after Tukey’s adjustment for maintaining $\alpha = 0.05$.

3. Results

3.1. Larval Mortality after Seven Days

The cumulative natural mortality of the FAW larvae after 7 days was less than 20% on non-Bt maize foliage and did not vary significantly among the populations ($p > 0.05$, Table 3). The mortality rates on the maize foliage producing Vip3Aa reached 98–100%, no matter the combination of Cry proteins co-produced on the Bt maize hybrid, be they Cry1 or Cry2 Bt proteins (Table 3).

Table 3. Mortality (mean ± standard error) of fall armyworm (*Spodoptera frugiperda*) third-instar larvae from different populations, after five days of feeding on Bt maize foliage.

Population Code	Bt Insecticidal Protein or Trait			Non-Bt
	Cry1A.105 + Cry2Ab + Vip3Aa	Cry1F + Cry1A.105 + Cry2Ab + Vip3Aa	Cry1F + Cry1Ab + Vip3Aa	
MA-Bs	100 ± 0.0 a	100 ± 0.0 a	98.33 ± 1.7 a	18.3 ± 5.4 b
TO-PN	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	12.0 ± 4.1 b
PI-BG	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	5.0 ± 3.0 b
PI-Cs	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	10.0 ± 3.9 b
PI-Ur	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	8.0 ± 3.6 b
BA-LE	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	5.0 ± 3.0 b

Means with the same letters in a column or row are not significantly different according to contrasts generated by the generalized linear model with a binomial distribution ($p \leq 0.05$).

3.2. Survival Schedule as Affected by Bt Proteins and FAW Populations

Considering that the Vip3Aa protein in transgenic Bt maize hybrids is pyramided with other Cry proteins, the time–mortality response of the larvae feeding on leaf tissues producing Vip3Aa and Cry proteins could be conditioned by the Bt maize trait and FAW

population (Figure 2A–F). However, the mortality schedule of the FAWs across the populations was primarily dependent on the presence of Vip3Aa in the maize hybrids and not so much on the number and type of Cry Bt proteins in the maize hybrid (Figure 3A). Indeed, the mean survival time for the larvae differed by only a few hours (<0.5 days) among the different combinations of Cry Bt proteins co-produced in the plant tissues (Figure 3A,B and Table 4).

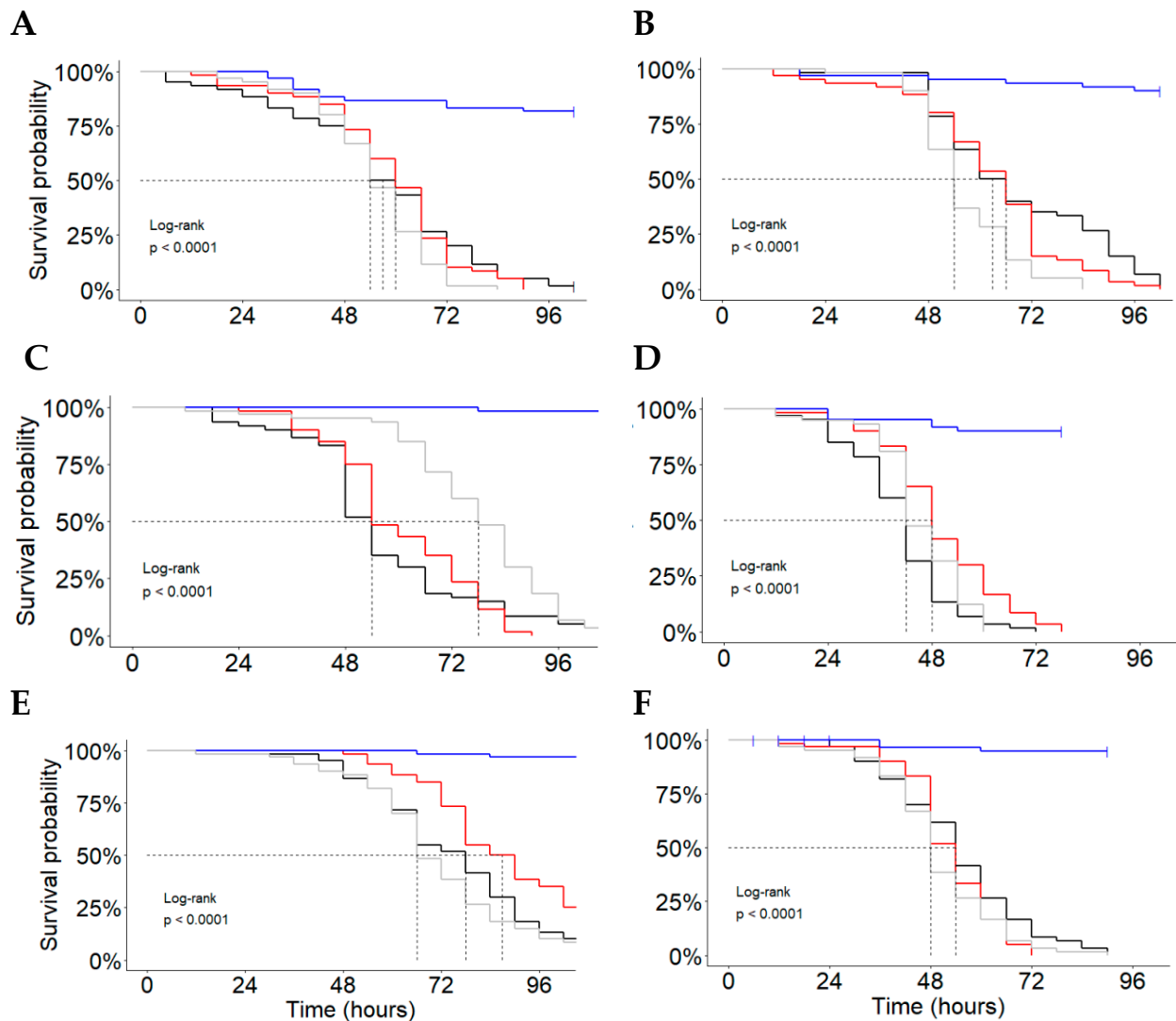


Figure 2. Larval survival curves for fall armyworm populations (*Spodoptera frugiperda*) feeding on maize leaf tissues producing Vip3Aa/Cry Bt proteins. The third-instar larvae exposed are progeny from individuals collected in maize fields of a tropical agricultural frontier region in Brazil. The population codes are as follows: (A) MA-Bs, (B) TO-PN, (C) PI-BG, (D) PI-Cs, (E) PI-Ur, and (F) BA-LE. + Non-Bt; + Cry1F + Cry1Ab + Vip3Aa; + Cry1A.105 + Cry2Ab + Vip3Aa; + Cry1F + Cry1A.105 + Cry2Ab + Vip3Aa.

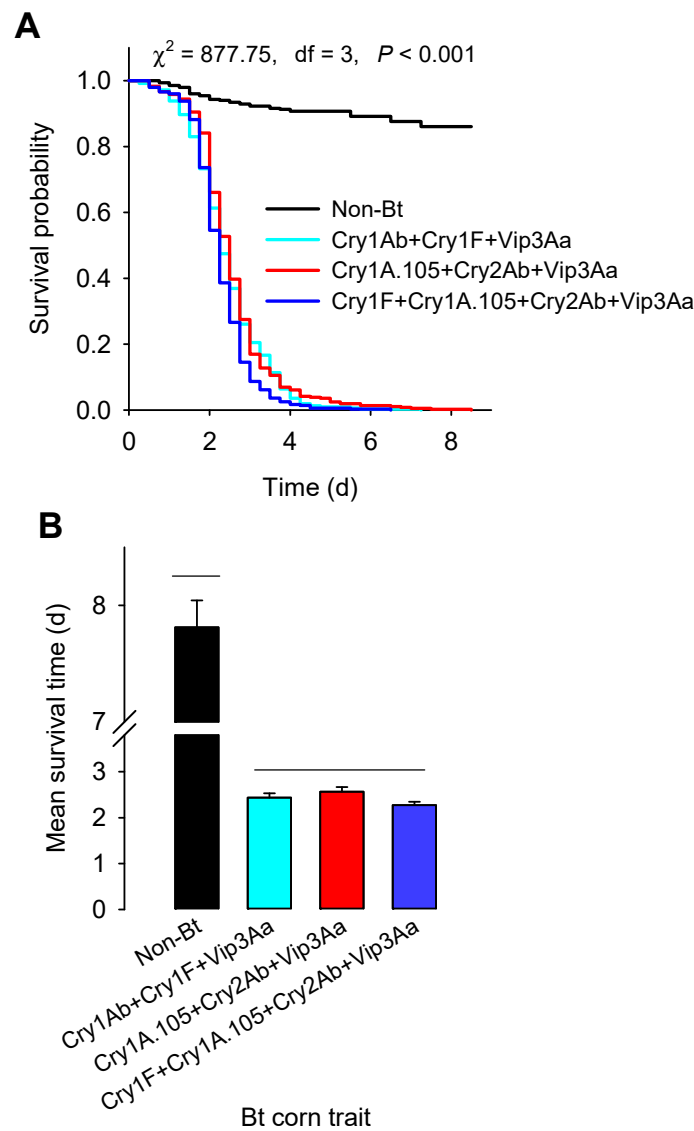


Figure 3. Mortality dynamics of third-instar fall armyworms (*Spodoptera frugiperda*) as affected by Vip3Aa/Cry-producing Bt maize hybrids. The larvae derived from field collections in a region of a tropical agricultural frontier in Brazil. (A) Mortality schedule. (B) Mean survival time. Columns with the same line segments are not significantly different ($p > 0.05$, Tukey’s HSD adjustment).

Table 4. Median survival time for fall armyworm (*Spodoptera frugiperda*) larvae that ingested Bt maize foliage as third instars. The larvae were derived from individuals collected in different locations in the ‘Matopiba’ region, Brazil.

Population Code	Median Survival Time in Hours, ST ₅₀ (95%CL) *			
	Cry1A.105 + Cry2Ab + Vip3Aa	Cry1F + Cry1A.105 + Cry2Ab + Vip3Aa	Cry1F + Cry1Ab + Vip3Aa	Non-Bt
MA-Bs	60 (54–66) BCa	54 (50–58) Bb	57 (54–60) Bab	NA
TO-PN	66 (60–72) Ba	54 (50–58) Bb	63 (60–66) Ba	NA
PI-BG	54 (48–60) Cb	78 (72–84) Aa	54 (50–58) Cb	NA
PI-Cs	48 (42–54) Ca	42 (40–44) Ca	42 (40–44) Da	NA
PI-Ur	90 (78–102) Aa	66 (60–72) Aa	78 (72–84) Aa	NA
BA-LE	54 (48–60) Ca	48 (42–54) BCa	54 (50–58) Ca	NA

* ST₅₀, the time (h) needed to kill 50% of the test population. 95% CL, 95% confidence limits. Capital letters compare values within the column, while lowercase letters compare values within the row. NA, Not Applicable.

The larval susceptibility of FAW populations varied significantly in all Bt trait combinations producing Vip3Aa ($p < 0.001$) (Figure 4A–D). For all the FAW populations, more than 80% of the larvae survived when feeding on non-Bt leaf tissues during the bioassays, with no significant difference ($p > 0.05$) in natural mortality (Figure 4A). The survival curves showed that the third-instar larvae died rapidly (2–3 days) when feeding on Vip3Aa Bt maize foliage, with mortality rates reaching 80% or more after 5 days, no matter whether Vip3Aa was co-produced with Cry1Ab/Cry1F, Cry1A/Cry2A, or Cry1F/Cry1A/Cry2A (Figure 4B–D). The lowest value of the median survival time (ST_{50} , c. 1.75 days or 42 h) occurred for the PI-Cs population on the foliage of maize hybrids producing Vip3Aa together with two Cry1 proteins or one Cry1 and one Cry2 protein (Figure 4B–D and Table 4). Conversely, the highest ST_{50} values were for the PI-Ur population, ranging from 66 to 90 h (2.75–3.75 days) (Figure 4B–D and Table 4).

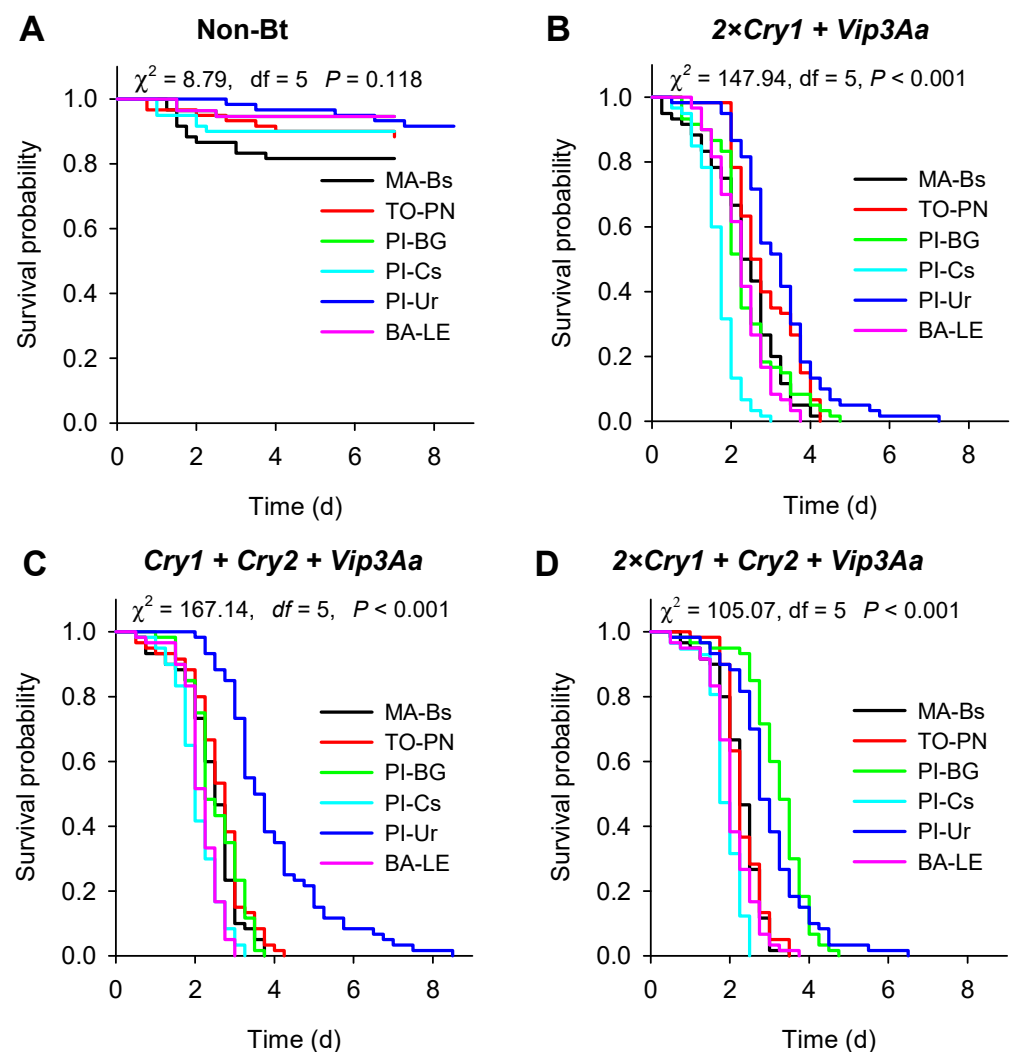


Figure 4. Comparative time–mortality responses of third-instar fall armyworms (*Spodoptera frugiperda*) of six populations collected in a tropical agricultural frontier region. The larvae were assayed using foliage maize hybrids representing following lepidopteran-active Bt traits (maize hybrids): (A) non-Bt (30F53RR), (B) Cry1A + Cry1F + Vip3Aa (30F53VHYR), (C) Cry1A.105 + Cry2Ab + Vip3Aa (DKB390PRO4), and (D) Cry1F + Cry1A.105 + Cry2Ab + Vip3Aa (P3551PWU).

Comparing the FAW populations across the Vip3Aa-producing maize hybrids (Figure 5A), their survival curves showed variations, with the PI-Cs FAWs dying faster than others, and the PI-Ur larvae being the slowest to die on the Bt maize leaf tissues (Figure 5A). A summary of the median survival time values shows the pattern observed more precisely

(Figure 5B). The ST_{50} values overlapped in the range of 48 to 66 h (2–2.75 days) for the BA-LE, TO-PN, and MA-Bs populations, which are from locations in Bahia, Tocantins, and Maranhão states, respectively (Figure 5B).

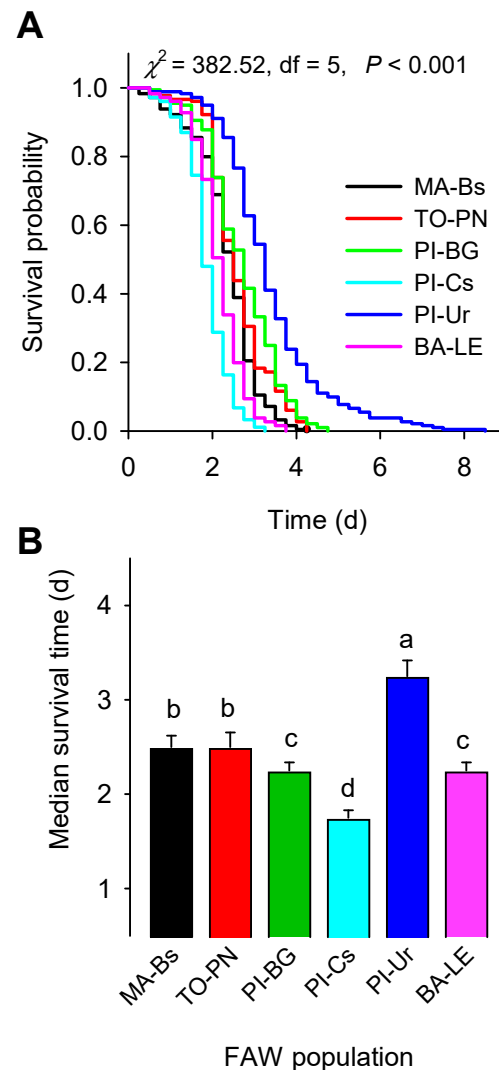


Figure 5. Overall Vip3Aa/Cry susceptibility of the fall armyworm (*Spodoptera frugiperda*) populations. Third-instar larvae were assayed with fresh leaf tissues of three Bt maize hybrids producing the protein. (A) Mortality schedule across the three Bt maize hybrids. (B) Median survival time values as a summary of the results. Error bars are 95% confidence limits. Columns with the same letter do not differ if confidence intervals do not overlap their origins ($p > 0.05$).

4. Discussion

We tested the hypothesis of reduced larval susceptibility to Vip3Aa and Cry Bt proteins in fall armyworms that were F_1 progeny of populations collected from tropically warm regions, where rain-fed intensive cropping systems of maize and soybean prevail in the regional agricultural landscape. Significant differences existed in the larval mortality schedule among the populations, although the third-instar larvae were killed relatively fast by the pyramided Bt maize hybrids producing the toxins. After more than five years of high adoption rates of these Bt maize traits in the focal region, we found no evidence that the Vip3Aa and Cry proteins have lost much of their combined efficacy against F_1 FAW larvae of field populations collected in 2022. Given that Vip3Aa is relatively potent against *Spodoptera* spp. [18–21,23,28,31], its high-dose condition in Bt maize traits and the

low initial frequency of Vip3Aa resistance alleles [25,29] likely helped delay their selection in the FAW field populations.

Despite the efficacy of the Vip3Aa20 transgenic trait in Bt maize hybrids against the larvae of FAW populations studied, there was variation in the susceptibility among the populations, as indicated by differences in the median lethal time for the larvae [32]. While one population showed median lethal time values as low as 1.75 days, another had median survival time as high as 2.75–3.75 days. These populations may have genetic differences caused by evolutionary processes conditioned by the environment, including pest management practices in each location, past selection pressure with Vip3Aa, and the FAW adaptive potential [3,27]. F₂ screenings for Vip3Aa resistance alleles conducted in 2014 also found variation among locations and growing seasons [29]. Noteworthy is that the population that showed reduced susceptibility to Vip3Aa (PI-Ur) did not show any fitness costs of that trait to larval survival (see Figure 4A).

We purposely tested third-instar larvae to increase the odds of detecting reduced susceptibility in the populations, because mid-size larvae have higher tolerance to Bt proteins than neonates [23,24,32]. Even so, the median survival time values for the FAWs in the test populations were less than four days, which is a relatively short lethal time for Bt products [23,24,33]. Vip3Aa20 maize leaf tissues seem to produce a concentration of protein sufficiently high for the high-dose condition for FAW [18]. Such a condition was supported by survival data previously obtained [23,24,31] and the relatively fast action of Vip3Aa20 against third-instar FAWs observed here. Some Cry Bt proteins, such as Cry1F, were initially quite potent against armyworm larvae [32,34], but they have lost efficacy against FAW larvae in the Americas [17,35,36]. This situation may place the Vip3Aa Bt traits at high risk of resistance selection in FAW populations.

There was no evidence that the efficacy of the Vip3Aa20 protein against the FAWs studied was affected by the presence of Cry1Ab, and Cry1A.105, Cry1F, and Cry2Ab2 in Bt maize leaf tissues. These results indicate that antagonistic interactions among Cry and Vip3Aa may not be an issue in Bt maize hybrids [37]. As resistance to Cry Bt proteins seems widespread in FAW field populations in the Americas [35,36,38–41], Cry-protein combination with Vip3Aa may not be so helpful for resistance management [13,42], despite broadening the range of target pests that can be controlled. If these combined Bt traits are deployed when the initial frequency of resistance alleles in the field populations is low, they could be more beneficial in preserving pest susceptibility [42,43]. Such a condition may or may not hold for regions of Africa, Asia, and Oceania, where FAW populations have recently expanded their range [43,44].

More studies may be needed to understand the genetic changes in FAW susceptibility to Vip3Aa/Cry insecticidal proteins and help inform decision making for resistance management [32,43,45]. FAW population pressure on host crops is likely influenced by the genetic background of field populations and their environmental factors, including climatic variables, native or transgenic plant resistance traits, and pest and crop management practices [3,20,46,47]. Our field collections theoretically came from Bt maize plants that produce Vip3Aa/Cry insecticidal proteins. However, some Bt maize hybrids may contain up to 5% of off-type plants that do not produce these proteins. Although Vip3Aa is known to be highly effective against FAW [18,23,24], resistance to the protein may not have been fixed in the collected population. Therefore, the F₀ adults that were mass-mated may have produced F₁ offspring that may carry Vip3Aa resistance alleles but are unlikely to survive in the Bt corn hybrids used. This may have contributed to the high Bt susceptibility of FAW larvae in our study. In addition, inducible resistance to Vip3Aa [48] is yet to be tested in field-selected FAW populations. We showed that the Vip3Aa and Cry proteins produced in Bt maize hybrids remain effective at killing third-instar F₁ FAWs descendants from the larvae collected in the fields described here. In field settings, however, the larvae may have the chance to behaviorally reduce exposure time to the toxins, and that may ultimately reduce Bt trait efficacy and plant protection [33,49]. The high toxicity of Vip3Aa to FAW larvae is unique to the protein and worthwhile to preserve, perhaps using effective in-farm

refuges as recommended for Bt crops. However, their adoption by growers is often low, as many do not see the value of the practice, and they need to manage other economic pests like maize leafhoppers and stinkbugs in regions of intensive cropping systems in tropical and subtropical America. Pest managers should continue to be alert for increased injury by FAW larvae in Vip3Aa/Cry Bt maize fields and observe the population density of late instars as indicator reduced susceptibility of *S. frugiperda* to Vip3Aa and Cry.

5. Conclusions

After over five years under field selection pressure, there is no evidence that the Vip3Aa and Cry Bt traits lost much of their efficacy against F₁ larvae derived from tropical native FAW populations. However, there is variation in susceptibility among the FAW populations, indicated by the differences in median lethal time for the larvae. While the values are as low as 1.75 days for one population (Currais, Piauí, Brazil), they were as high as 2.75–3.75 days for another population (Uruçuí, Piauí, Brazil). There is no evidence that Cry1Ab, Cry1A.105, Cry1F, or Cry2Ab2 traits affect the efficacy of the Vip3Aa trait in maize leaf tissues, indicating that their combination in Bt maize hybrids could be effective against fall armyworm and helpful to preserve its susceptibility to Bt if deployed timely when the resistance allele frequencies are low.

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