Survival and Development of *Spodoptera frugiperda* and *Chrysodeixis includens* (Lepidoptera: Noctuidae) on Bt Cotton and Implications for Resistance Management Strategies in Brazil

RODRIGO J. SORGATTO,[1] ODERLEI BERNARDI,[1] AND CELSO OMOTO [1,2]

Environ. Entomol. 44(1): 186–192 (2015); DOI: 10.1093/ee/nvu018

ABSTRACT In Brazil, *Spodoptera frugiperda* (J. E. Smith) and *Chrysodeixis includens* (Walker) are important cotton pests and target of control of Bollgard II (Cry1Ac/Cry2Ab2) and WideStrike (Cry1Ac/Cry1F) cotton technologies. To subsidize an insect resistance management program, we conducted laboratory studies to evaluate the toxicity of these Bt cotton plants throughout larval development of *S. frugiperda* and *C. includens*. In bioassays with leaf disc, the efficacy of both Bt cotton plants against neonates was >80% for *S. frugiperda* and 100% for *C. includens*. However, *S. frugiperda* larvae that survived on Bt cotton had >76% of growth inhibition and stunting. In bioassays with *S. frugiperda* and *C. includens* larvae fed on non-Bt near-isoline during different time period (from 3 to 18 d) and then transferred to Bollgard II or WideStrike leaves showed that larval susceptibility decreased as larval age increased. For Bollgard II cotton, in all *S. frugiperda* instars, there were larvae that reached the pupal and adult stages. In contrast, on WideStrike cotton, a few larvae in fifth and sixth instar completed the biological cycle. For *C. includens*, some larvae in sixth instar originated adults in both Bt cotton plants. In conclusion, Bollgard II and WideStrike cotton technologies showed high efficacy against neonates of *S. frugiperda* and *C. includens*. However, the mortality of these species decreases as larval age increase, allowing insect survival in a possible seed mixture environment and favoring the resistance evolution.

KEY WORDS fall armyworm, soybean looper, transgenic cotton, insect resistance management

Introduction

Spodoptera frugiperda (J. E. Smith, 1797) and *Chrysodeixis includens* (Walker, 1857) (Lepidoptera: Noctuidae) are important defoliating pests of cotton (*Gossypium hirsutum* L.) (Luttrell and Mink 1999, Jost and Pitre 2002, Santos 2011). In Brazil, these pests occur in crops throughout the year due to favorable weather conditions and the intensification of crop production system. On cotton, *S. frugiperda* causes defoliation and also attacks the reproductive structures (Ali and Luttrell 1990, Luttrell and Mink 1999). *C. includens* is a defoliating larva of crescent importance in cotton because this crop is usually cultivated after soybean, from where moths disperse to cause high infestations (Santos 2011).

S. frugiperda and *C. includens* are currently controlled with synthetic insecticides (Diez-Rodríguez and Omoto 2001, Oliveira et al. 2010). However, the control with insecticides is hard because larvae is less exposed to the spray owing to its habit of remaining sheltered under the cotton canopy, especially after the closed canopy (Santos 2011). Another control strategy is the use of transgenic cotton that express insecticidal proteins from *Bacillus thuringiensis* Berliner (Bt), which shows broad-spectrum control of lepidopteran pests, especially plants that express two Bt proteins (Greenberg et al. 2010, Akin et al. 2011). Among transgenic cotton plants, Bollgard II (Cry1Ac/Cry2Ab2) and WideStrike (Cry1Ac/Cry1F) technologies demonstrate effective control of *S. frugiperda* and *C. includens* in several studies in the United States (Stewart et al. 2001, Adamczyk et al. 2008, Siebert et al. 2008, Sivasupramaniam et al. 2008, Tindall et al. 2009, Greenberg et al. 2010, Akin et al. 2011). However, in Brazil the susceptibility of these target pests to these cotton plants is unknown.

To understand the susceptibility of these target pests to Bollgard II and WideStrike cotton is necessary to subsidize an insect resistance management (IRM) programs. In this context, the main strategies include the cultivation of refuge areas with non-Bt plants (Gould 1998, Tabashnik et al. 2009). In Brazil, there is a tendency to use a seed mixed of Bt and non-Bt plants for spatially arrangement of refuge. A positive aspect of seed mix is that appropriate amount of a suitable refuge variety is planted in each Bt field and also distributes refuge plants relatively uniformly within Bt fields (Carroll 2012). However, a difficulty in adopting the seed mix is the need to assume that inheritance of resistance

---

[1] Department of Entomology and Acarology, "Luiz de Queiroz" College of Agriculture (ESALQ), University of São Paulo (USP), Av. Pádua dias 11, Piracicaba, São Paulo 13418-900, Brazil.

[2] Corresponding author, e-mail: celso.omoto@usp.br.

is recessive and that larval mobility of target insects is negligible (Mallet and Porter 1992, Tabashnik 1994, Andow 2008). A recessive inheritance of resistance was recently reported for *S. frugiperda* to Cry1F maize in Brazil (Farias et al. 2014). There is no report of resistance of *C. includens* to Bt proteins.

Another aspect that should be considered in the seed mixture strategy is the larval mobility. The larval mobility of *S. frugiperda* on cotton occurs on the plant and between plants and increases throughout larval development (Ali et al. 1990). For *C. includens*, the larval mobility on cotton is unknown; however, it is widely accepted that larvae are mobile. The larval mobility can exposed the target pests to subdoses of Bt proteins through initial feeding on the Bt plant and subsequent feeding on the non-Bt plant, not ingesting a sufficient amount of toxin to cause mortality and vice versa (Andow 2008). In addition, several studies report that susceptibility of target pests to Bt proteins decreases throughout larval development (Ashfaq et al. 2001, Li et al. 2006, Armstrong et al. 2011). Based on this, to subsidize an IRM program and understand the value of Bollgard II and WideStrike cotton technologies for IPM in Brazil, we evaluated the toxicity of these Bt cotton plants throughout larval development of *S. frugiperda* and *C. includens*.

Materials and Methods

Populations. *S. frugiperda* population was collected from a non-Bt corn area in Rio Verde, Goiás, Brazil, in 2011 (17° 47′53″ S and 50° 51′41″ W). *C. includens* population was collected from a soybean area in Paulínia, São Paulo, Brazil (22° 45′40″ S and 47° 09′15″ W). Both populations were kept in the laboratory for >4 yr free of selective pressures to insecticides or Bt proteins. Both populations were considered as our susceptible reference in our studies. The species were reared on artificial diet proposed by Greene et al. (1976) at a temperature 25 ± 1 °C, 70 ± 10% relative humidity, and a photoperiod of 14:10 (L:D).

Cotton Plants. The following cotton plants were used: Bollgard II (Event MON 15985, Monsanto do Brazil Ltd.), that express Cry1Ac/Cry2Ab2 proteins, WideStrike (Cultivar MXB-13 event 281-24-236/3006-210-23, Dow AgroSciences Industrial Ltd.) with Cry1Ac/Cry1F proteins; and the respective non-Bt near-isolines DeltaOpal and PhytoGen PSC 355. Cotton plants were cultivated in a greenhouse in 12-liter plastic pots (four plants per pot) with two parts of soil and one part of plant substrate, during the summer of the 2012 season.

Leaf-Disc Bioassays. Bioassays with leaf-disc of Bollgard II and WideStrike cotton were conducted in the following sampling times: squaring, 14 d after squaring, flowering, 14 d after flowering, and 28 d after flowering. For the bioassays, fully expanded leaves from the upper third of the plants were used. In the laboratory, leaf discs (2.4 cm in diameter) were cut using a metallic cuter and placed on a gelled mixture of agar–water at 2.5% (1 ml per well) in 12-well acrylic plates (Corning Incorporated, Corning, NY). The leaf discs were separated from the agar–water layer by a filter paper disc. Later, one *S. frugiperda* or *C. includens* neonate (<24 h old) was infested into each well using a fine brush. Plastic film and the plate cover were used for sealing. The plates were placed in a climatic chamber at temperature 27 ± 1 °C, 60 ± 10% relative humidity, and a photoperiod of 14:10 (L:D). The experimental design was completely randomized with 10 replicates per treatment or species, being each replicate formed by 12 neonates. In bioassays with *S. frugiperda*, the leaf disc was replaced every 3 d, and the final evaluation was performed on the ninth day, when the mortality, weight, and instar of surviving larvae were recorded. For *C. includens*, the mortality was recorded 5 d after leaf-disc infestation. For both species, larvae showing no movement and larvae shorter than 2.0 mm were considered dead. Mortality data on Bollgard II and WideStrike leaf-disc were corrected based on the respective non-Bt near-isoline (Abbott 1925). To determine the growth inhibition, the surviving larvae in each replicate were weighed, and the percentage of growth inhibition calculated from the mean weight of larvae fed on non-Bt near-isoline. Stunting consisted of dead larvae plus survivors that did not reach the third instar. Mortality, growth inhibition, and stunting data on Bollgard II and WideStrike cotton were compared with respective non-Bt near-isoline by Mann–Whitney test ($P \leq 0.05$; PROC NPAR1WAY, SAS Institute 2000, Cary, NC).

Survival and Development of *S. frugiperda* and *C. includens* on Bt Cotton. Bioassays were begun when cotton plants reached flowering, when fully expanded leaves were removed from the upper third of plants. Larvae were fed with tissues of non-Bt near-isoline, and then transferred to Bollgard II or WideStrike cotton at different time points (T): 3, 6, 9, 12, 15, and 18 d (time point T3 to T18) or fed on non-Bt near-isoline (TC) or Bt cotton (T0) for their entire larval period. The larvae was placed on insect-rearing cups (100 ml) containing a 10 ml of a mixture of agar–water (2.5%) and a filter paper. Each treatment was formed by 50 neonate larvae (10 replicates of five larvae). Larvae were transferred to the next treatment (T) and fresh tissue provided every 3 d, when the larval survival was evaluated and head capsule widths for 10 previously identified larvae was measured. This procedure was performed until pupation or complete mortality. After pupation, the pupae were placed in glass tubes (8.5 cm in length by 2.5 cm in diameter) containing filter paper that was moistened with distilled water every 2 d until emergence. The bioassays was conducted in a climatic chamber at temperature 25 ± 1 °C, 60 ± 10% relative humidity, and a photoperiod of 14:10 (L:D) h. The survival rate throughout larval development, duration, and viability of the larval period, pupae weight (at 24 h old) and emergence rate were evaluated. The data were compared by Mann–Whitney test ($P \leq 0.05$) between two treatments and by Kruskal–Wallis test ($P \leq 0.05$) to more than two treatments (PROC NPAR1WAY, SAS Institute 2000).

Results

Leaf-Disc Bioassays. Neonates of *S. frugiperda* exposed to Bollgard II leaves that express

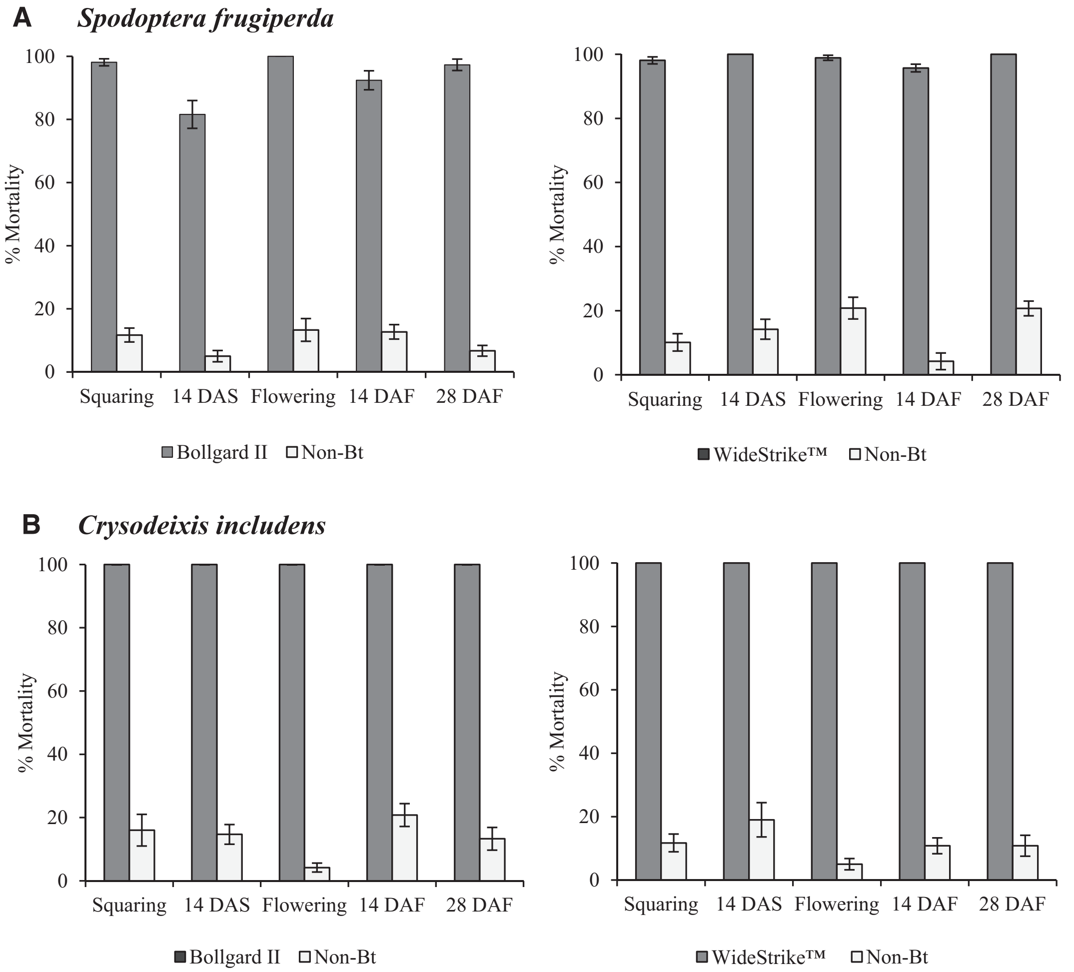

Fig. 1. Mortality of *S. frugiperda* and *C. includens* neonates on Bollgard II and WideStrike cotton technologies at different sampling times. There were significant differences between Bt cotton and respective non-Bt near-isoline in all sampling times (Mann–Whitney test, $P \leq 0.05$). DAS, days after squaring; DAF, days after flowering.

Cry1Ac/Cry2Ab2 insecticidal proteins had a significant high mortality, ranging from 81 to 100% (in all sampling times, $P < 0.0001$; Fig. 1A). In contrast, on non-Bt near-isoline, the mortality was <14%. Although, some *S. frugiperda* larvae survived on Bollgard II cotton, they had a significant growth inhibition, ranging from 82 to 93%. Only one sampling time of Bollgard II cotton (14 d after squaring), ~7% of larvae reached the third instar. At the same time, >69% of larvae fed on non-Bt near-isoline reached the third instar. For *C. includens*, complete mortality was detected on Bollgard II cotton in all sampling times. On non-Bt near-isoline, the mortality was <21% (Fig. 1B).

On WideStrike cotton that expresses Cry1Ac/Cry1F insecticidal proteins, there were a high mortality of *S. frugiperda* neonates, ranging from 96 to 100% (in all sampling times, $P < 0.0001$; Fig. 1A). On non-Bt near-isoline, the mortality was <21%. The *S. frugiperda* larvae survivors on WideStrike cotton showed higher growth inhibition (>76%), and <2% reach the third instar. On non-Bt near-isoline, >70% of larvae reached the third instar. In addition, WideStrike cotton showed complete mortality of *C. includens* neonates in all sampling times (Fig. 1B). In contrast, on non-Bt near-isoline the mortality was <19%.

Survival and Development of *S. frugiperda* and *C. includens* on Bt Cotton. There were significant differences in the survival of *S. frugiperda* ($\chi^2 = 54.06$; df = 7; $P < 0.0001$) and *C. includens* (U = 155.00; $z = 3.99$; $P < 0.0001$) throughout larval development on leaves of Bollgard II cotton. At 30 d, when the final evaluation was performed, from 8 to 22% of larvae up to fourth instar survived when fed only on Bollgard II cotton (T0) or when transferred to Bt cotton at 3, 6, and 9 d (T3–T9; Table 1). The surviving larvae on Bollgard II cotton in these time points showed larval period ranged from 32 to 35 d, which represent a significant increase of 5 to 10 d when compared with larvae, fed only on non-Bt near-isoline (TC). In addition, larval viability and emergence on Bollgard II cotton

Table 1. Percentage of *S. frugiperda* survival on Bollgard II cotton throughout larval development

Time point[a]	Feeding time on non-Bt (d)	% larval survival every 3 d											% pupae[b]	% adults[b]
		0	3	6	9	12	15	18	21	24	27	30		
TC	All the time	100	92	86	84	82	82	80	76	74	72	72	66ab	64a
T0	0	*100*	44	18	12	12	10	10	10	10	10	8	4d	4d
T3	3	100	*92*	64	44	36	34	32	22	20	14	12	6d	6d
T6	6	100	90	*88*	78	62	60	54	46	40	28	22	10d	10d
T9	9	100	98	94	*92*	84	70	46	34	30	26	18	4d	4d
T12	12	100	91	89	89	*87*	83	70	61	53	47	42	34c	34b
T15	15	100	94	94	90	90	*88*	75	63	61	53	53	41bc	39ab
T18	18	100	100	96	94	92	92	*92*	89	86	78	75	73a	67a

[a] Time point: TC, larvae fed only on non-Bt near-isoline; T0, larvae fed only on Bt cotton; and T3, T6, T9, T12, T15, T18, larvae fed during 3, 6, 9, 12, 15, and 18 d on non-Bt near-isoline and then transferred to Bt cotton. The italicized number shows the time and number of larvae that were transferred to Bt cotton.

[b] Means followed by the same letter in a column are not significantly different by Kruskal–Wallis test ($P \leq 0.05$).

Table 2. Percentage of *C. includens* survival on Bollgard II cotton throughout larval development

Time point[a]	Feeding time on non-Bt (d)	% survival every 3 d							% pupae[b]	% adults[b]
		0	3	6	9	12	15	18		
TC	All the time	100	100	98	96	96	96	96	88a	77a
T0	0	*100*	0	–	–	–	–	–	–	–
T3	3	100	*94*	0	–	–	–	–	–	–
T6	6	100	96	*92*	0	–	–	–	–	–
T9	9	100	96	90	*86*	18	0	–	–	–
T12	12	100	100	96	96	*96*	60	12	8b	8b

[a] Time point: TC, larvae fed only on non-Bt near-isoline; T0, larvae fed only on Bt cotton; and T3, T6, T9, T12, larvae fed during 3, 6, 9, and 12 d on non-Bt near-isoline and then transferred to Bt cotton. The italicized number shows the time and number of larvae that were transferred to Bt cotton.

[b] Means followed by same letter in a column are not significantly different by Mann–Whitney test ($P \leq 0.05$).

ranged from 4 to 10% (Table 1). After 12 and 15 d on non-Bt near-isoline (T12 and T15), there was a predominance of fifth instar, and >42% of larvae survived when fed on Bollgard II cotton (Table 1). The larval period of these survivors' larvae ranged from 29 to 33 d, and <40% of these larvae originated pupae and adults. After 18 d (T18) on non-Bt near-isoline, most of *S. frugiperda* larvae were in sixth instar, and ~75% survived when fed on Bollgard II cotton. In these time point, after 26 d, 73 and 67% of larvae originated pupae and adults, respectively (Table 1). The pupae weight was significantly different among time points, ranging from 111 to 182 mg.

For *C. includens*, there was no survival on Bollgard II cotton from neonates to fifth instar (T0–T9; Table 2). Only larvae in sixth instar fed on non-Bt near-isoline (T12), and then transferred to Bt cotton had 12% of survival. The larval viability and emergence was 8% (Table 2). In contrast, when fed only on non-Bt near-isoline (TC), 88 and 77% of larvae originated pupae and adults, respectively. Pupae weight of *C. includens* on Bollgard II cotton (118 mg) was negatively affected compared with those on non-Bt near-isoline (169 mg).

On WideStrike cotton, the survival of *S. frugiperda* ($\chi^2 = 43.29$; df = 7; $P < 0.0001$) and *C. includens* (U = 149.00; $z = 3.36$; $P = 0.0004$) was also significantly affected throughout larval development. When the final evaluation was performed, *S. frugiperda* neonates fed only on WideStrike cotton (T0) and larvae from first to fifth instar transferred to Bt cotton at 3, 6, 9, 12, and 15 d (T3–T15) showed <5% of survival (Table 3). With the exception of 4% of larvae in fifth instar (T15), the remaining surviving larvae in these time points did not reach the pupae stage (Table 3). After 18 d on non-Bt near-isoline (T18), there was a predominance of sixth instar, and when fed on WideStrike cotton, ~15% originated pupae and adults (Table 3). At the same time on the non-Bt near-isoline (TC), 78 and 74% of larvae pupated and originated adults, respectively. Pupae weight was negatively affected only in those larvae fed on WideStrike cotton at 15 d (145 mg). In non-Bt near-isoline, the pupae weight was ~180 mg.

For *C. includens*, the larval survival throughout development on WideStrike cotton was similar to observed on Bollgard II cotton. Only larvae (sixth instar) fed on non-Bt near-isoline for 12 d (T12) and then transferred to WideStrike cotton showed 39% survival (Table 4). The survivor's larvae showed 16 d of larval period, being similar of those fed only on non-Bt near-isoline. However, the larval viability and emergence on WideStrike cotton was <27%. In contrast, 88% of larvae originated to pupae and adults when fed on non-Bt near-isoline (TC; Table 4). Pupae weight of *C. includens* on WideStrike cotton (132 mg) was significantly lightest of those on non-Bt near-isoline (170 mg). In summary, the toxicity of Bollgard II and WideStrike cotton technologies against *S. frugiperda* and *C. includens* decreased as larval age increased.

Table 3. Percentage of *S. frugiperda* survival on WideStrike cotton throughout larval development

Time point[a]	Feeding time on non-Bt (d)	% larval survival every 3 d											% pupae[b]	% adults[b]
		0	3	6	9	12	15	18	21	24	27	30		
TC	All the time	100	96	92	88	88	88	86	86	86	86	84	78a	74a
T0	0	*100*	8	6	4	4	4	4	4	4	4	4	–	–
T3	3	100	*90*	22	14	10	10	8	8	6	6	4	–	–
T6	6	100	91	*91*	39	16	16	14	12	10	6	4	–	–
T9	9	100	95	93	*92*	41	16	12	10	8	6	2	–	–
T12	12	100	98	95	96	*96*	44	16	10	8	8	2	–	–
T15	15	100	95	95	89	89	*85*	51	8	4	4	4	4b	2b
T18	18	100	95	95	92	92	91	*91*	60	21	21	15	15b	13b

[a] Time point: TC, larvae fed only on non-Bt near-isoline; T0, larvae fed only on Bt cotton; and T3, T6, T9, T12, T15, T18, larvae fed during 3, 6, 9, 12, 15, and 18 d on non-Bt near-isoline and then transferred to Bt cotton. The italicized number shows the time and number of larvae that were transferred to Bt cotton.

[b] Means followed by the same letter in a column are not significantly different by Kruskal–Wallis test ($P \leq 0.05$).

Table 4. Percentage of *C. includens* survival on WideStrike cotton throughout larval development

Time point[a]	Feeding time on non-Bt (d)	% survival every 3 d							% pupae[b]	% adults[b]
		0	3	6	9	12	15	18		
TC	All the time	100	98	96	96	96	92	88	88a	88a
T0	0	*100*	0	–	–	–	–	–	–	–
T3	3	100	*98*	0	–	–	–	–	–	–
T6	6	100	96	*94*	0	–	–	–	–	–
T9	9	100	100	98	*98*	58	0	–	–	–
T12	12	100	100	98	94	*92*	77	39	27b	21b

[a] Time point: TC, larvae fed only on non-Bt near-isoline; T0, larvae fed only on Bt cotton; and T3, T6, T9, T12, larvae fed during 3, 6, 9, and 12 d on non-Bt near-isoline and then transferred to Bt cotton. The italicized number shows the time and number of larvae that were transferred to Bt cotton.

[b] Means followed by the same letter in a column are not significantly different by Mann–Whitney test ($P \leq 0.05$).

Discussion

Transgenic cotton plants expressing Cry1Ac/Cry2Ab2 (Bollgard II) and Cry1Ac/Cry1F (WideStrike) exhibited relative high efficacy against neonates of *S. frugiperda* and *C. includens*. The efficacy in our study was similar to those reported in several studies in the United States (Stewart et al. 2001, Adamczyk et al. 2008, Siebert et al. 2008, Tindall et al. 2009, Greenberg et al. 2010, Armstrong et al. 2011, Akin et al. 2011). However, the toxicity of Bollgard II and WideStrike cotton technologies decreased as larval age increased. In particular, *S. frugiperda* showed relative lower susceptibility to these Bt cotton plants than *C. includens*. On Bollgard II, all fall armyworm larval instars originated to pupae and adults, with a significant increase after the fourth instar. On the other hand, on WideStrike few *S. frugiperda* larvae in fifth and sixth instar completed the biological cycle. For *C. includens*, some larvae in sixth instar originated adults when exposed to both Bt cotton plants. Larvae in sixth larval instar have little time to feed before the occurrence of pupation; not ingesting a lethal dose of Bt proteins, and these affected directly the mortality rate. A decrease of susceptibility in later larval instars was reported on Cry1Ac-cotton for *Heliothis virescens* (F.) (Jenkins et al. 1993), *Helicoverpa zea* (Boddie) (Halcomb et al. 1996), *Trichoplusia ni* (Hübner) (Li et al. 2007), and *C. includes* (Ashfaq et al. 2001). According to Adamczyk et al. (2008), WideStrike cotton was more efficacious against fall armyworm than Bollgard II, and no neonate's larvae successfully pupated on Cry1Ac/Cry1F cotton plants.

In general, survivors' larvae of *S. frugiperda* and *C. includens* on Bollgard II and WideStrike cotton had a significantly increase in the larval period. This aspect may play a role in IPM programs by increasing the exposure on the plant to beneficial arthropods. In addition, *S. frugiperda* and *C. includens* survivors in these Bt cotton plants originated pupae lightest of those on non-Bt near-isoline. Halcomb et al. (1996) also reported reduced pupae weights when bollworm and tobacco budworm were fed on Cry1Ac-cotton.

On Bollgard II cotton, the Cry2Ab2 expression in terminal leaves is ∼40-fold higher than Cry1Ac (Greenplate et al. 2003), while that on WideStrike cotton the expression of Cry1F is up to 29-fold higher than Cry1Ac (Siebert et al. 2009). This relative low expression of Cry1Ac protein on Bollgard II and WideStrike cotton plants is because of the Cry1Ac-cotton genotypes used to get these events, which already showed low toxicity against *S. frugiperda* (Chitkowski et al. 2003, Adamczyk and Gore 2004, Adamczyk et al. 2008, Sivasupramaniam et al. 2008, Akin et al. 2011, Armstrong et al. 2011) and *C. includens* (Ashfaq et al. 2001, Gore and Adamczyk 2004, Akin et al. 2011). In contrast, Cry1Ac protein expressed on Bt soybean (Intacta RR2 Pro) provides high efficacy against soybean looper, but low toxicity for fall armyworm (Bernardi et al. 2012, 2014). Therefore, the addition of

Cry2Ab2 and Cry1F proteins to Cry1Ac-cotton significantly increased the control efficacy of *S. frugiperda* and *C. includens*.

The survival and development of *S. frugiperda* and *C. includens* on Bollgard II and WideStrike cotton should be considered in IRM programs. From the resistance management prespective, these Bt cotton plants do not meet a basic assumption of the "pyramid strategy;" high individual toxicity of each toxin against same pest (Ives et al. 2011). Furthermore, the efficacy of this strategy is reduced if Bt proteins present cross-resistance. For Cry1Ac and Cry2Ab2, cross-resistance was reported in some lepidopteran pests of cotton, such as *H. zea*, *H. virescens*, and *Pectinophora gossypiella* (Saunders) (Brévault et al. 2013). In addition, cross-resistance for Cry1Ac and Cry1F was observed in *H. virescens* and *S. frugiperda* (Gould et al. 1995, Hernández-Rodríguez et al. 2013). These aspects can provide opportunity for heterozygous survival, and consequently increase the probability of resistance evolution.

The Brazilian cropping system is another aspect that increases the potential risk for resistance evolution in populations of *S. frugiperda* and *C. includens*. This agricultural system is characterized for a successive and intensive cultivation of corn, cotton, and soybean, which express different Bt proteins, and exposes these target pests to high selection pressure for resistance. In this cropping system, *S. frugiperda* can complete up to eight generations per year, two of them on cotton (Fitt et al. 2006). For *C. includens*, the number of generations per year is unknown, but it is accepted that it is more than five. Recently, a field-evolved resistance of *S. frugiperda* to Cry1F maize was documented in Brazil (Farias et al 2014). The resistance of *S. frugiperda* to Cry1F maize could impair the efficacy of Bt cotton, especially WideStrike, which expresses Cry1Ac/Cry1F proteins. For IRM, the use of large refuges areas is of the strategies to delay or prevent the pest adaptation to Bt plants (Andow et al. 2008).

In Brazil, because of the low adoption of structured refuges, there the use "refuge in a bag," which consists of a seed mixture of Bt and non-Bt plants has been considered as IRM strategy. However, we concluded that the mortality of *S. frugiperda* and *C. includens* on Bollgard II and WideStrike cotton technologies decreases as larval age increase, allowing insect survival in a possible seed mixture environment and favoring the resistance evolution.

Acknowledgments

We thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting scholarship to R.J.S.

References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–266.

Adamczyk, J. J., Jr., and J. Gore. 2004. Laboratory and field performance of cotton containing Cry1Ac, Cry1F, and both Cry1Ac and Cry1F (WideStrike^{TM}) against beet armyworm and fall armyworm larvae (Lepidoptera: Noctuidae). Fla. Entomol. 87: 427–432.

Adamczyk, J. J., Jr., S. Greenberg, J. S. Armstrong, W. J. Mullins, L. B. Braxton, R. B. Lassiter, and M. W. Siebert. 2008. Evaluations of Bollgard®, Bollgard II®, and WideStrike® technologies against beet and fall armyworm larvae (Lepidoptera: Noctuidae). Fla. Entomol. 91: 531–536.

Akin, D. S., S. D. Stewart, M. B. Layton, and J. A. Mills. 2011. Efficacy of cotton expressing pyramided *Bacillus thuringiensis* insecticidal proteins against lepidopteran pests. Midsouth Entomol. 4: 1–13.

Ali, A., and R. G. Luttrell. 1990. Survival of fall armyworm (Lepidoptera: Noctuidae) immatures on cotton. Fla. Entomol. 73: 459–465.

Ali, A., R. G. Luttrell, and H. N. Pitre. 1990. Feeding sites and distribution of fall armyworm (Lepidoptera: Noctuidae) larvae on cotton. Environ. Entomol. 19: 1060–1067.

Andow, D. A. 2008. The risk of resistance evolution in insects to transgenic insecticidal crops. Collect. Biosaf. Rev. 4: 142–199.

Armstrong, J. S., J. J. Adamczyk, Jr., and S. M. Greenberg. 2011. Efficacy of single and dual gene cotton *Gossypium hirsutum* events on neonate and third instar fall armyworm, *Spodoptera frugiperda* development based on tissue and meridic diet assays. Fla. Entomol. 94: 262–271.

Ashfaq, M., S. Y. Young, and R. W. McNew. 2001. Larval mortality and development of *Pseudoplusia includens* (Lepidoptera: Noctuidae) reared on a transgenic *Bacillus thuringiensis*-cotton cultivar expressing CryIAc insecticidal protein. J. Econ. Entomol. 94: 1053–1058.

Bernardi, O., G. S. Malvestiti, P. M. Dourado, W. S. Oliveira, S. Martinelli, G. U. Berger, G. P. Head, and C. Omoto. 2012. Assessment of the high-dose concept and level of control provided by MON 87701 × MON 89788 soybean against *Anticarsia gemmatalis* and *Pseudoplusia includens* (Lepidoptera: Noctuidae) in Brazil. Pest Manag. Sci. 68: 1083–1091.

Bernardi, O., R. J. Sorgatto, A. D. Barbosa, F. A. Domingues, P. M. Dourado, R. A. Carvalho, S. Martinelli, G. P. Head, and C. Omoto. 2014. Low susceptibility of *Spodoptera cosmioides*, *Spodoptera eridania* and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to genetically-modified soybean expressing Cry1Ac protein. Crop Prot. 58: 33–40.

Brévault, T., S. Heuberger, M. Zhang, C. Ellers-Kirk, X. Ni, L. Masson, X. Li, B. E. Tabashnik, and Y. Carrière. 2013. Potential shortfall of pyramided transgenic cotton for insect resistance management. Proc. Natl Acad. Sci. USA 110: 5806–5811.

Carroll, M. W., G. P. Head, and M. Caprio. 2012. When and where a seed mix refuge makes sense for managing insect resistance to Bt plants. Crop Prot. 38: 74–79.

Chitkowski, R. L., S. G. Turnipseed, M. J. Sullivan, and W. C. Bridges, Jr. 2003. Field and laboratory evaluations of transgenic cottons expressing one or two *Bacillus thuringiensis var. kurstaki* Berliner proteins for management of Noctuid (Lepidoptera) pests. J. Econ. Entomol. 96: 755–762.

Diez-Rodríguez, G. I., and C. Omoto. 2001. Herança da resistência de *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) a lambda-cialotrina. Neotrop. Entomol. 30: 311–316.

Farias, J. R., D. A. Andow, R. J. Horikoshi, R. J. Sorgatto, P. Fresia, A. C. Santos, and C. Omoto. 2014. Field-evolved resistance to Cry1F maize by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Brazil. Crop Prot. 64: 150–158.

Fitt, G. P., C. Omoto, A. H. Maia, J. M. Waquil, M. Caprio, M. A. Okech, E. Cia, N. H. Huan, and D. A. Andow. 2006. Resistance risks of Bt cotton and their management in Brazil, pp. 300-345. *In* A. Hilbeck, D. A. Andow, and E.M.G.

Fontes (eds.), Environmental risk assessment of genetically modified organisms: Methodologies for assessing Bt cotton in Brazil. CABI Publishing, London, England.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annu. Rev. Entomol. 43: 701–726.

Gould, F., A. Anderson, A. L. Reynolds, L. Bungarner, and W. Moar. 1995. Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. J. Econ. Entomol. 88: 1545–1559.

Gore, J., and J. J. Adamczyk. 2004. Characterization of soybean looper (Lepidoptera: Noctuidae) tolerance to Bollgard® cotton: Implications for resistance management. J. Entomol. Sci. 39: 235–242.

Greenberg, S. M., Y. X. Li, and T. X. Liu. 2010. Effect of age of transgenic cotton on mortality of lepidopteran larvae. Southwest. Entomol. 35: 261–268.

Greene, G. L., N. C. Lepla, and W. A. Dickerson. 1976. Velvetbean caterpillar: a rearing procedure and artificial medium. J. Econ. Entomol. 69: 488–497.

Greenplate, J. T., J. W. Mullins, S. R. Penn, A. Daham, B. J. Reich, J. A. Osborn, P. R. Rahn, L. Ruschke, and Z. W. Shappley. 2003. Partial characterization of cotton plants expressing two toxin proteins from *Bacillus thuringiensis*: relative contribution, toxin interaction, and resistance management. J. Appl. Entomol. 127: 340–347.

Halcomb, J. L., J. H. Benedict, B. Cook, and D. R. Ring. 1996. Survival and growth of bollworm and tobacco budworm on nontransgenic and transgenic cotton expressing a CryIA insecticidal protein (Lepidoptera: Noctuidae). Environ. Entomol. 25: 250–255.

Hernández-Rodríguez, C. S., P. H. Hernández-Rodríguez, J. Van Rie, B. Escriche, and J. Ferré. 2013. Shared midgut binding sites for Cry1A.105, Cry1Aa, Cry1Ab, Cry1Ac and Cry1Fa proteins from *Bacillus thuringiensis* in two important corn pests, *Ostrinia nubilalis* and *Spodoptera frugiperda*. PLoS ONE 8: e68164.

Ives, A. R., P. R. Glaum, N. L. Ziebarth, and D. A. Andow. 2011. The evolution of resistance to two-toxin pyramid transgenic crops. Ecol. Appl. 21: 503–515.

Jenkins, J. N., W. L. Parrott, J. C. McCarty, Jr., F. E. Callahan, S. A. Berberich, and W. R. Deaton. 1993. Growth and survival of *Heliothis virescens* (Lepidoptera: Noctuidae) on transgenic cotton containing a truncated form of the delta endotoxin gene from *Bacillus thuringiensis*. J. Econ. Entomol. 86: 181–185.

Jost, D. J., and H. N. Pitre. 2002. Soybean looper (Lepidoptera: Noctuidae) oviposition on cotton and soybean of different growth stages: influence of olfactory stimuli. J. Econ. Entomol. 95: 286–293.

Li, Y. X., S. M. Greenberg, and T. X. Liu. 2006. Effects of Bt cotton expressing Cry1Ac and Cry2Ab and non-Bt cotton on behavior, survival and development of *Trichoplusia ni* (Lepidoptera: Noctuidae). Crop Prot. 25: 940–948.

Li, Y. X., S. M. Greenberg, and T. X. Liu. 2007. Orientation behavior, development and survival of *Trichoplusia ni* (Lepidoptera: Noctuidae) larvae on cotton expressing Cry1Ac and Cry2Ab and conventional cotton. J. Insect Behav. 20: 473–488.

Luttrell, R. G., and J. S. Mink. 1999. Damage to cotton fruiting structures by the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J. Cotton Sci. 3: 35–44.

Mallet, J., and P. Porter. 1992. Preventing insect adaptation to insect resistant crops: are seed mixtures or refugia the best strategy? Proc. R. Soc. Lond. B Biol. Sci. 250: 165–169.

Oliveira, J. R. G., M. C. Ferreira, and R. A. A. Román. 2010. Diameter of droplets and different equipments for the application of insecticide to control *Pseudoplusia includes*. Eng. Agríc. 30: 92–99.

Santos, W. J. 2011. Manejo de pragas do algodão com ênfase no cerrado, pp. 495–566. *In* E. C. Freire (eds.), Algodão no cerrado do Brasil. Associação Brasileira de Produtores de Algodão. ABRAPA, Aparecida de Goiás, Góias, Brazil.

SAS Institute. 2000. Statistical analysis system: getting started with the SAS learning. SAS Institute, Cary, NC.

Siebert, M. W., J. M. Babock, S. Nolting, A. C. Santos, J. J. Adamczyk, Jr., P. A. Neese, J. E. King, J. N. Jenkins, J. McCarty, G. M. Lorenz, et al. 2008. Efficacy of Cry1F insecticidal protein in maize and cotton for control of fall armyworm (Lepidoptera: Noctuidae). Fla. Entomol. 91: 555–565.

Siebert, M.W., T. G. Patterson, G. J. Gilles, S. Nolting, L. B. Braxton, B. R. Leonard, J. W. Van Duyn, and R. B. Lassiter. 2009. Quantification of Cry1Ac and Cry1F *Bacillus thuringiensis* insecticidal proteins in selected cotton plant tissue types. J. Econ. Entomol. 102: 1301–1308.

Sivasupramaniam, S., W. J. Moar, L. G. Ruschke, J. A. Osborn, C. Jiang, J. L. Sebaugh, G. R. Brown, Z. W. Shappley, M. E. Oppenhuizen, J. W. Mullins, et al. 2008. Toxicity and characterization of cotton expressing *Bacillus thuringiensis* Cry1Ac and Cry2Ab2 proteins for control of lepidopteran pests. J. Econ. Entomol. 101: 546–554.

Stewart, S. D., J. J. Adamczyk, Jr., K. S. Knighten, and F. M. Davis. 2001. Impact of Bt cottons expressing one or two insecticidal proteins of *Bacillus thuringiensis* Berliner on growth and survival on noctuid (Lepidoptera) larvae. J. Econ. Entomol. 94: 752–760.

Tabashnik, B. E. 1994. Delaying insect adaptation to transgenic plants: seed mixtures and refugia reconsidered. Proc. R. Soc. Lond. B Biol. Sci. 255: 7–12.

Tabashnik B. E., J.B.J. Van Rensburg, and Y. Carrière. 2009. Field-evolved insect resistance to Bt crops: Definition, theory and data. J. Econ. Entomol. 102: 2011–2025.

Tindall, K. V., M. W. Siebert, B. R. Leonard, J. All, and F. J. Haile. 2009. Efficacy of Cry1Ac:Cry1F proteins in cotton leaf tissue against fall armyworm, beet armyworm, and soybean looper (Lepidoptera: Noctuidae). J. Econ. Entomol. 102: 1497–1505.

Received 4 May 2014; accepted 4 November 2014.