TRANSGENIC COTTON AGE EFFECTS ON LEPIDOPTERAN LARVAE MORTALITY

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Abstract

Leaves from plants contained various transgenic traits (Non-Bt, Bollgard, Bollgard II, and WideStrike) were assayed for bioactivity in the laboratory against bollworm, *Helicoverpa zea* (Boddie), beet armyworm, *Spodoptera exigua* (Hübner), fall armyworm, *Spodoptera frugiperda* (J. E. Smith), and cabbage looper, *Trichoplusia ni* (Hübner). About 50 leaves from the middle of the plants were collected every 20 d, starting at 40d after cotton was planted (DAP) and until 120 DAP. Leaves from each collection were used for feeding of about 15 neonate or first instar larvae of each insect described above until they died (or pupated). The highest larval mortality showed on Bollgard II and WideStrike Bt traits. Mortality was not significantly different between both dual Bt traits during the growing season. At the end of the season (120 DAP) the mortality of all insects used was lower on WideStrike than on Bollgard II trait. We did not observe cotton age effects on the mortality tested insects. Survival durations depended from consumption of the endotoxin levels that provoke the larvae mortality. Mean biological characteristics of survived lepidopteran, (pupal weight, emergence, and development time) were significantly higher on non-Bt cotton.

Introduction

In the USA, bollworm [*Helicoverpa zea* (Boddie)] and tobacco budworm [*Heliothis virescens* (Fabricius)] continue to be in 2007 the top cotton pests, and the cotton losses from heliothine complex in the USA were 229,186 bales, among them in Texas -78,826 bales, and in LRGV of Texas – 39,063bales (Williams 2007). Over the last two decades, the beet armyworm [*Spodoptera exigua* (Hübner)] has become an increasingly destructive secondary pest of United States cotton. Epidemic outbreaks in cotton have cost as much as $371.0/ac, and the cost of insecticides targeted at this pest as much as $44.0 per application/ac. The fall armyworm [*Spodoptera frugiperda* (J. E. Smith)] also is a destructive migratory pest of many crops in the Western Hemisphere, where it appears to be more common and widespread (Sparks 1979, Young 1979). The cabbage looper [*Trichoplusia ni* (Fabricius)] is also a secondary pest of cotton, and it can cause cotton losses up to 70% without control (Schwartz 1985). The pest status of beet armyworm, fall armyworm, and cabbage looper in the LRGV of Texas cotton agroecosystem may soon change with the initiation of the boll weevil eradication program due to the adverse affects on natural enemies from areawide malathion sprays. Growers are aware that the risk of secondary pest outbreaks will increase during boll weevil eradication program and some Bt cottons are known to reduce the risk from certain lepidopteran pests.

Since it was first introduced in 1996, commercialization of transgenic cotton (Bollgard®, Monsanto Co., St. Louis, MO), that contains the gene from *Bacillus thuringiensis* var. *kurstaki* (Berliner) encoding the Cry 1 Ac insect toxic protein has proven to be highly effective against tobacco budworm, and less effective against bollworm, as well as loopers and armyworms (Mahaffey et al. 1995, Layton 1997, Jackson et al. 2003). In 2002, Bollgard II® (Monsanto Co., St. Louis, MO) was registered for commercial use, which produced the Cry 1 Ac and Cry 2 Ab endotoxins. Bollgard II cotton has shown excellent control of bollworm (Ridge et al. 2001). Private companies continue to improve technology of Bt cotton production. Dow AgroSciences, LLC (Indianapolis, IN) introduced its pyramided-gene technology onto the market in 2004 as WideStrike™. This cotton also produce two Bt endotoxins, Cry 1 Ac and Cry 1 Fa, which are both active against lepidopterans (Adamczyk and Gore 2004). Bt cotton has proven itself to be useful in an eradication of the boll weevil. In non-eradicated zones, Bt cotton can be a useful tool in minimizing risk of outbreaks of lepidopteran, secondary pest problems, and will augment activity of beneficial insects. Approximately 66.1% of Bt cotton were planted in the USA in 2007, with 47.5% utilized in Texas, and 18.5% planted in the LRGV of Texas (Williams 2007). The utilization of transgenic Bt technology is in the beginning stage for cotton production in the LRGV of Texas. The importance of utilizing transgenic technology that included insecticidal proteins should increase as an IPM option because boll weevil eradication was approved for the LRGV of Texas in 2005. Assessing the efficacy of Bt cotton under new environment and management regimes is of prime importance to the growers. The objectives of this study were to evaluate (1) mortality of bollworm, beet armyworm, fall armyworm, and cabbage looper larvae; (2) mean survival durations of lepidopteran larvae, d; and (3) biological characteristics of survived individuals (mean pupae weight, emergence, and development time) after feeding on different age BT cottons.

Materials and Methods

Host plants. Field plots were planted with cotton on March 10, 2005; March 14, 2006 and March 7 2007, at the Farms of Subtropical Agricultural Research Center ARS-USDA, Weslaco Texas. Individual plots were 4 rows (on 30 inch centers) by 240 feet. Each variety was replicated three times in a randomized complete block design. Bt types, traits and varieties used in the experiments described in Table 1.
Insects. Bollworm (BW), beet armyworm (BAW), and fall armyworm (FAW) neonate (or 1st instar) larvae were obtained from a laboratory colony maintained at the KSARC, while those of cabbage looper (CL) from Vegetable IPM Laboratory Texas AgriLife Research.

Design of experiments. There were 60 treatments (Table 2). The cotton leaves were randomly collected every 20 d from the middle of the plants of each variety, starting at 40 d after cotton was planted (DAP) and until 120 DAP. In the laboratory ~7/8 inch leave disks were cut from the leaves of each variety or age and placed in a refrigerator in plastic bags (or leaves were excised and each leaf petiole was placed in a floral aquapic with hydroponic solution). Leaf disks were combined by variety and plant age. Individual leaf disks were placed in 5.5 cm x 1.2 cm Tight-Fit Lid sealing petri dishes containing moistened filter paper. After this a single 1st instar larval was placed in the center leaf disk. Each variety/plant age treatment was replicated 15 times (individual larval per replication). Larvae were checked for mortality every day. The larvae were considered dead if they did not move when touched with a camel-hair brush. Surviving larvae were supplied with a freshly collected leaf disk (or disks for older instars larvae) corresponding to the variety and plant age of the original treatment if disk was consumed or wilted, and the filter paper was moistened if needed. Larvae were held in environmental chambers at 27ºC, 65% RH, and a photoperiod of 13:11 (L:D) h. Pupal weight, emergence, and development time of survived individuals were recorded. Survival durations was estimated as lifetime of each larval from the beginning experiment to it died in the first 15 d after infestation (mean duration of larvae stage on conventional cotton, Greenberg et al. 2001). Data were analyzed using analysis of variance ANOVA (SAS Institute 2001).

Table 1. Cottons used in experiments.

Bt type	Bt trait	Variety	Bt endotoxins	Owner of Bt trait	Owner of Variety
None	Non-Bt	DPL 5415 RR	None	None	Delta & Pineland (Monsanto)
None	Non-BT	AMX 262R	None	None	Americot
Single	Bollgard	NuCOTN 33B	Cry1Ac	Monsanto	Delta & Pineland (Monsanto)
Single	Bollgard	DPL 444 BRR	Cry1Ac	Monsanto	Delta & Pineland (Monsanto)
Dual	Bollgard II	DPL424 BGII/RR	Cry1Ac+Cry2Ab	Monsanto	Delta & Pineland (Monsanto)
Dual	Bollgard II	AMX 1532BRII RR	Cry1Ac+Cry2Ab	Monsanto	Americot
Dual	WideStrike	Phy 485WRF	Cry1Ac+Cry2Fa	DowAgro-science	DowAgroscience

Table 2. Design of experiments.

Treat-ments	Bt trait*	Insects**	Cotton age, DAP***
1-16	Non-Bt, Bt, Bt2, WideStrike*	BW, BAW, FAW, CL	40
17-32	Non-Bt, Bt, Bt2, WideStrike*	BW, BAW, FAW, CL	60
33-48	Non-Bt, Bt, Bt2, WideStrike*	BW, BAW, FAW, CL	80
49-64	Non-Bt, Bt, Bt2, WideStrike*	BW, BAW, FAW, CL	100
64-80	Non-Bt, Bt, Bt2, WideStrike*	BW, BAW, FAW, CL	120

*Varieties used belongs to Bt trait: Non-Bt - DPL 5415 RR, Bt - NuCOTN 33B, Bt2 - DPL424 BGII/RR, WideStrike - Phy 485WRF;
**BW-Bollworm, BAW-beet armyworm, CL-cabbage looper;
***DAP – days after planting

Results and Discussion

In all treatments, the mortality of first instars was significantly higher when they fed on Bt-cottons compared with non-Bt (Fig. 1). The dual Bt types were highly effective against beet armyworm, fall armyworm, cabbage looper, and bollworm than single Bt type. More susceptible to Bollgard was bollworm (mortality ranged from 65.0 to 75.0%) while mortality of beet armyworm (27.8-45.0%), cabbage looper (44.4-50.0%), and fall armyworm (46.5-51.7%) was significantly lower. Mortality of beet armyworm (60.0-75.0%) after feeding on Bollgard II and WideStrike was significantly lower than those of cabbage looper and bollworm (90.0-100%). The fall armyworm mortality of those was intermediate (75.4-90.0%). We did not observe cotton age effects on the mortality tested insects. The statement described above confirm with data about survival durations (before larvae of tested lepidopteran were dead) (Fig. 2). When the larvae fed on less effective Bt type cotton leaves, they needed to consume more leaf material in order to reach the level of endotoxins that provoke the larvae mortality. Feeding on transgenic cottons significantly reduced pupae weight of survived larvae, emergence and development time (Table 3).

This information can be useful for companies producing Bt cottons, cotton growers, cooperative extension, especially in the LRGV under Boll Weevil Eradication Program, providing management options that have positive environmental, social, and economic outcomes. Transgenic cotton age did not affect the mortality of tested insects. Survival duration by feeding insects on different aged transgenic cottons can be used as a criterion for bioassay test methods.

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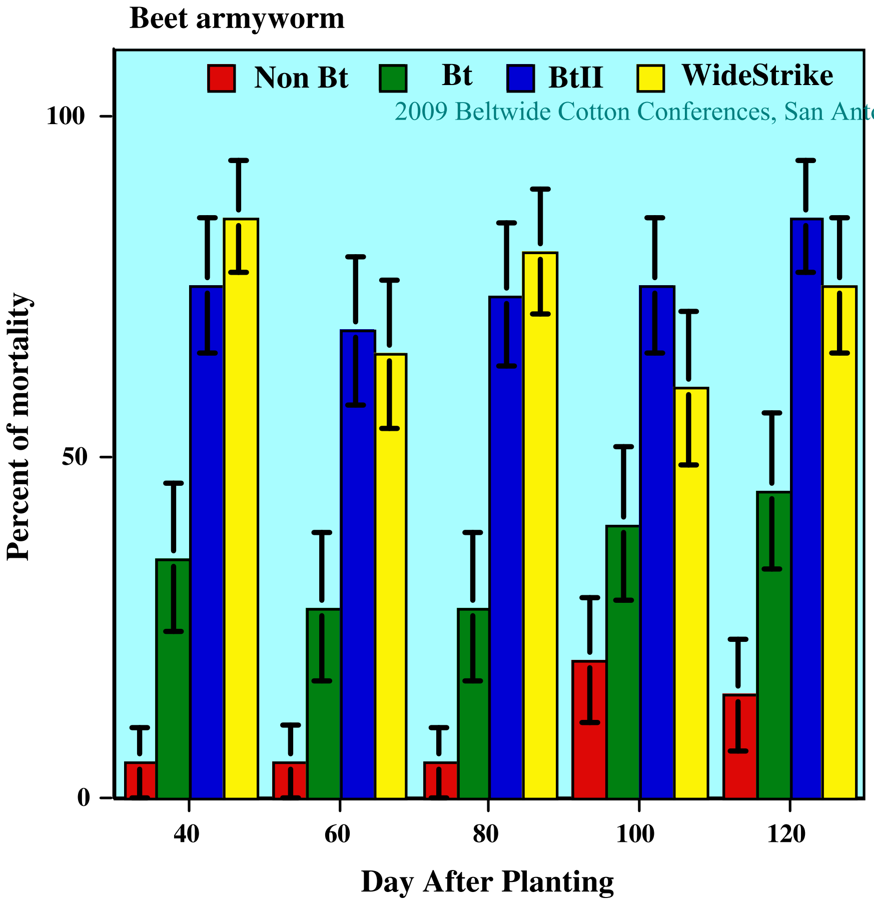
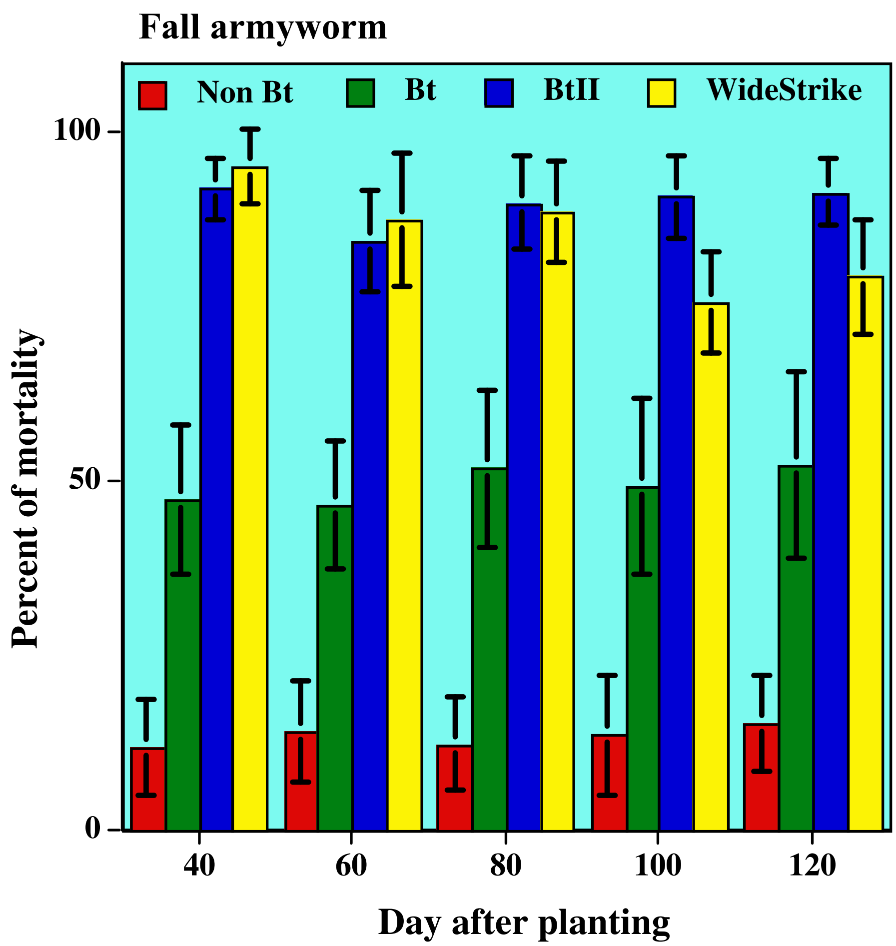
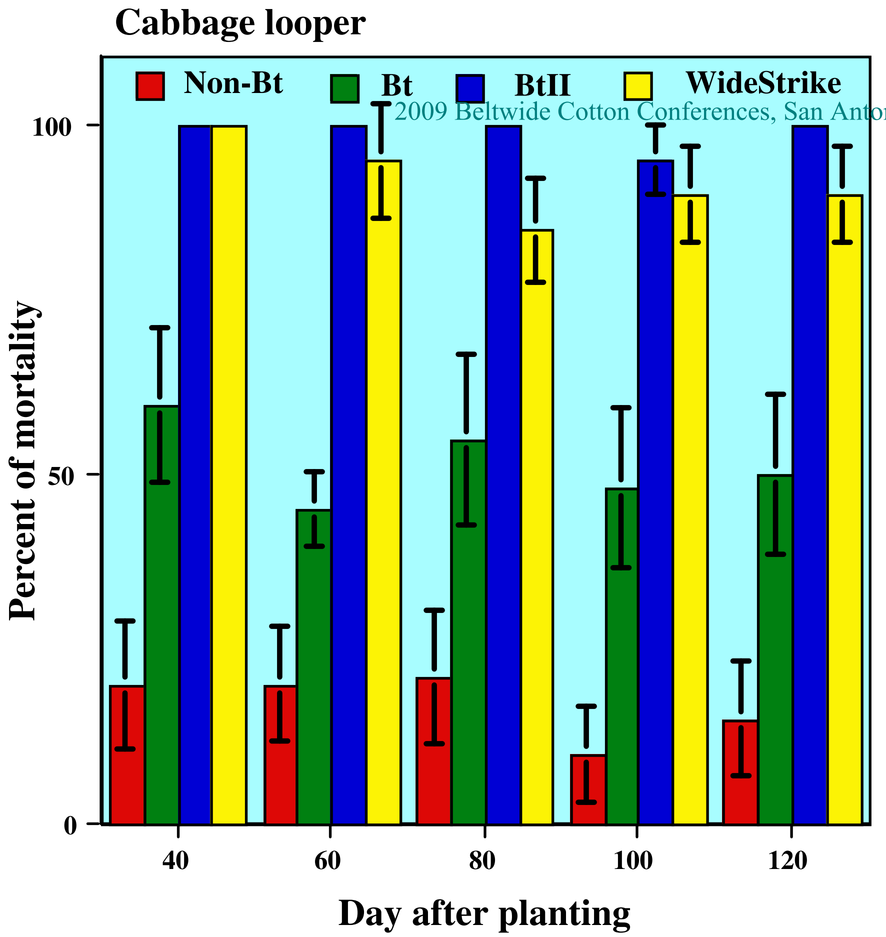
Table 3. Biological characteristics of 3 species lepidopteran after feeding on different aged transgenic cotton

Characteristics	40 DAP						120 DAP					
	BAW		FAW		CL		BAW		FAW		CL	
	Non Bt	Bt	Non Bt	Bt	Non Bt	Bt	Non Bt	Bt	Non Bt	Bt	Non Bt	Bt
Mean pupal wt, mg	71.2± 3.0a	51.8± 5.1b	159.6 ±4.6a	140.3 ±3.8b	158.7 ±3.7a	142.4 ±5.3b	81.1± 4.3a	59.6± 3.1b	149.6 ±3.7a	130.8 ±5.2b	144.5 ±2.7a	128.7 ±6.0b
Emergence, %	95.0± 5.0a	80.0± 9.2b	95.9± 3.8a	83.8± 4.2b	95.5± 1.7a	83.1± 5.2b	90.0± 6.9a	61.2± 11.2b	95.0± 5.0a	82.4± 4.6b	98.6± 1.6a	87.6± 5.0b
Development time, d	22.8± 0.6b	26.4± 1.2a	23.8± 1.4b	28.7± 1.1a	29.9± 0.5b	38.8± 1.2a	22.8± 0.6b	27.8± 0.7a	24.6± 1.9b	28.9± 2.2a	28.2± 0.2b	37.1± 1.1a

Means of one species lepidopteran within a row followed by the different letters are significantly different (Tukey honestly significant difference, $P<0.05$)


Beet armyworm
Non Bt
Bt
BtII
WideStrike
Percent of mortality
100
50
0
40
60
80
100
120
Day After Planting


Fall armyworm
Non Bt
Bt
BtII
WideStrike
Percent of mortality
100
50
0
40
60
80
100
120
Day after planting



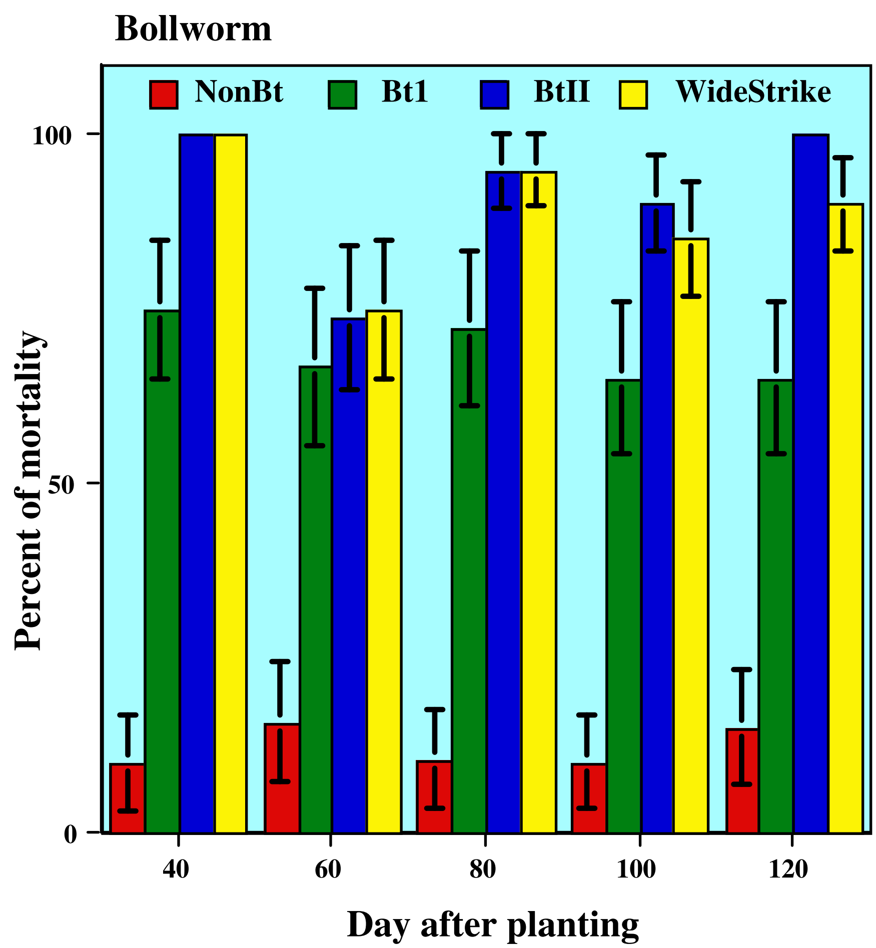
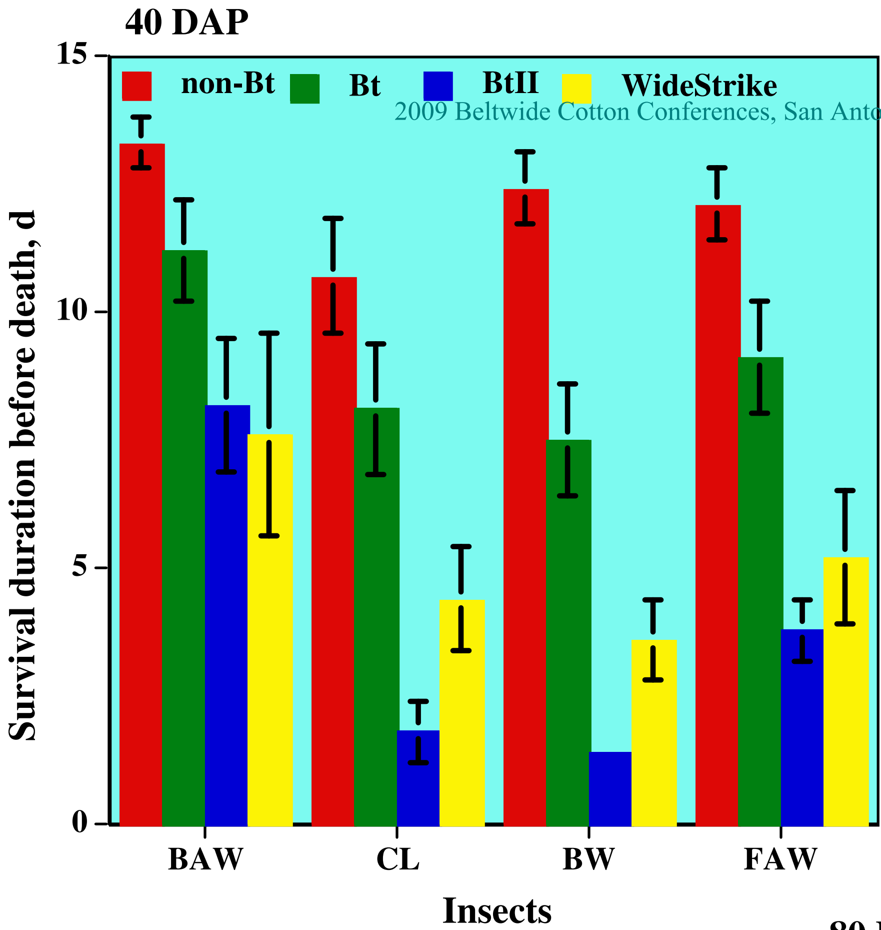
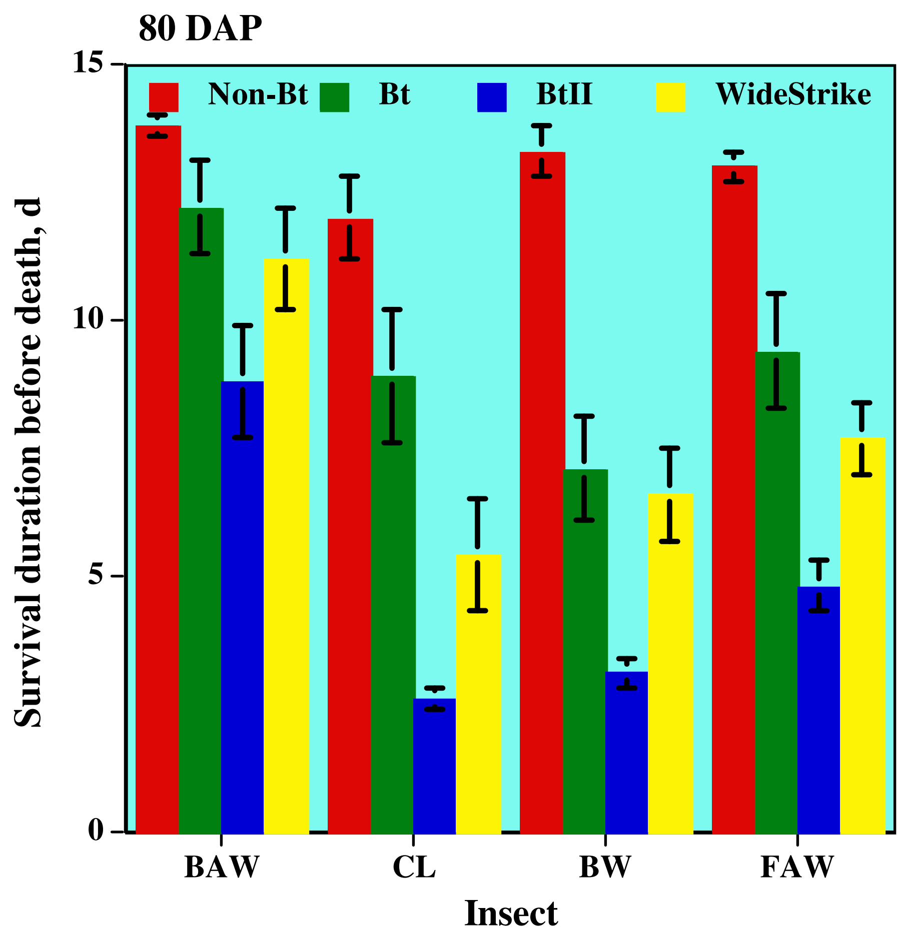


Figure 1. Mortality of larvae different lepidopteran when they fed on Bt-cottons


40 DAP
non-Bt
Bt
BtII
WideStrike
Survival duration before death, d
15
10
5
0
BAW
CL
BW
FAW
Insects


80 DAP
Non-Bt
Bt
BtII
WideStrike
Survival duration before death, d
15
10
5
0
BAW
CL
BW
FAW
Insect

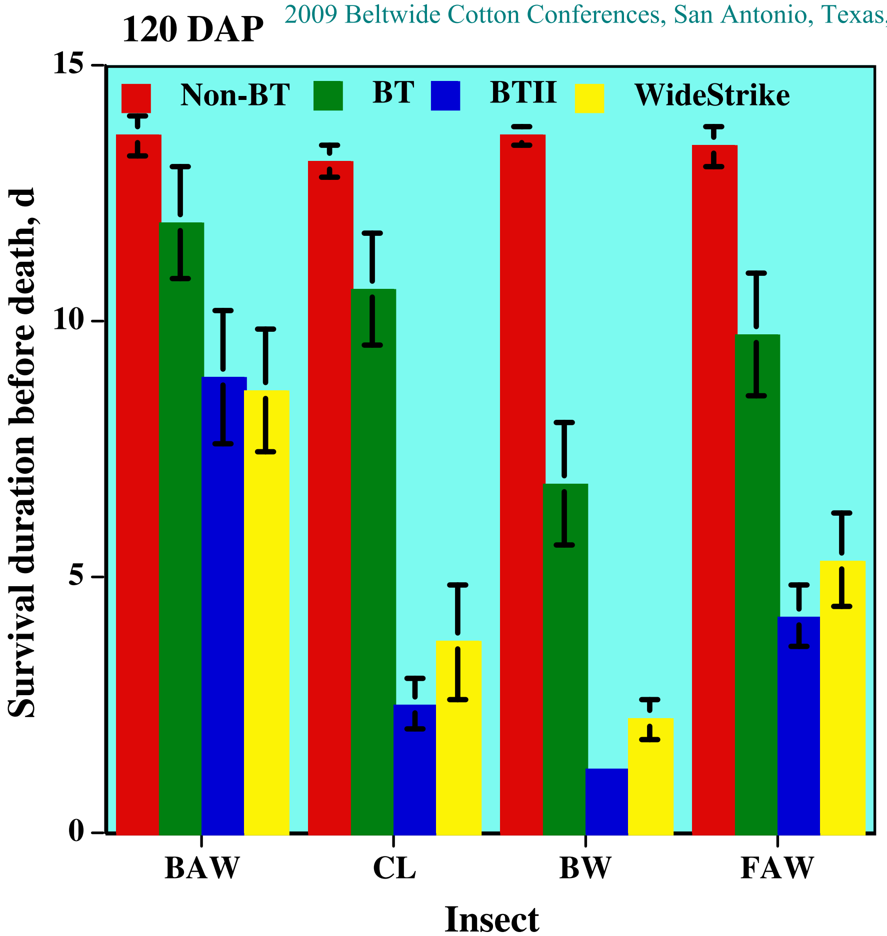


Figure 2. Survival duration of larvae lepidopteran fed on Bt-cottons after planting 40, 80, 120 days