CROSS-CROP RESISTANCE TO CORN AND COTTON IN A VIP3A RESISTANT STRAIN OF FALL ARMYWORM S*PODOPTERA FRUGIPERDA*

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Abstract

Transgenic crops producing *Bacillus thuringiensis* (Bt) have become a major tool for control of insect pests worldwide. Evolution of insect resistance to Bt proteins has become a serious threat to the sustainability of this technology. Gene-pyramiding, combining two or more dissimilar Bt proteins in a crop has been used to delay insect resistance. However, the durability of gene-pyramiding can be reduced by cross-resistance. Resistance to Cry1F in the fall armyworm (FAW), *Spodoptera fruigiperda* (J.E. Smith), has occurred in the Southern U.S. Vip3A is a relatively new Bt protein with a different mode of action and has been introduced into almost all recently released Bt corn and cotton products. In this study, we provided the first documentation of cross-crop resistance in FAW selected with Viptera 3111 corn (Vip3A+Cry1Ab) to multiple Bt corn and cotton products. Corn varieties used included SMT (Cry1F+Cry1A.105+Cry2Ab2), VT2P (Cry1A.105+Cry2Ab2), Viptera 3111 (Vip3A+Cry1Ab), HX (Cry1F), and Leptra (Cry1F+Vip3A). Cotton varieties include TL (Cry1Ab+Cry2Ae), TL+ (Cry1Ab+Cry2Ae+Vip3A), WS (Cry1Ac+Cry1F), WS3 (Cry1Ac+Cry1F+Vip3A), BG2 (Cry1Ac+Cry2Ab2), and BG3 (Cry1Ac+Cry2Ab2+Vip3A). Results generated from this study provided important information for insect pest management and resistance management of Bt crops.

Introduction

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (FAW) is one of the major target pests of Bt corn and cotton. FAW has been classified as a sporadic pest due to its migratory behavior (Hardke et al. 2015). They do not enter diapause, so annual migration northward begins from warm climates zones such as southern Florida, southern Texas, southern Georgia, Alabama, Louisiana, and other southern coastal areas across the U.S. (Hardke et al. 2015). FAW has a wide variety of host plants ranging from corn, sorghum, forage grasses, turf grasses, rice, cotton, peanuts, and has been reported on over 80 different species in 23 families (Pashley 1988) (Hardke et al. 2015). Invertebrate pest causes up to 15 percent of damage of agricultural production, costing the U.S. approximately 8 billion dollars, 17.7 billion U.S. dollars in brazil, and 359.8 million U.S. dollars in Australia (Zhou et al.2017).

There are several control methods for FAW in corn and cotton. Cultural methods include host plant resistance such as antibiosis in corn. Also suppressing overwintering habitats could be useful, however, since the FAW does not possess a diapause mechanism suppression could be futile. There are many labeled insecticides for this species such as chlorantraniliprole, emamectin benzoate, methoxyfenozide, and several others. However, fall armyworms have been demonstrated to have developed resistance to several classes of insecticides including pyrethroids, organophosphates, and carbamates (Hardke et al. 2015).

Genetically engineered plants that express *Bacillus thuringiensis* (Bt) have become a major tool to control insect pest in corn, cotton and soybeans (James 2015). Global use of these genetically engineered plants has risen from 1.1 million hectares in 1996 to 98.5 million in 2016 (Tabashnik 2017). In 2016, 94 million acres of corn was planted in the U.S. and produced 14.3 billion bushels, that profited over 51 billion U.S dollars (NASS 2017). Ten million acres of cotton was planted in 2016 that produced 16.5 million 480-pound bales bringing a net value of 5.67 billion U.S. dollars (NASS 2017). Of the 94 million acres of corn planted 92 percent contained Bt, and 93 percent of the 10 million acres of cotton contained Bt (NASS 2017). With the extensive use of Bt crops field resistance has occurred in several target species in several different countries (Yang et al. 2017). The evolution of resistance to Bt proteins in insects, is becoming the main threat to suitable use of this technology (Yang et al. 2017).

With field resistance to many insecticides the use of Bt technology has been heavily relied upon. There are currently three different groups of Bt proteins that are targeted for the control of FAW which are categorized as Cry1, Cry2, and Vip3A (Yang et al. 2017). Field resistance to Cry1F has been reported on corn in multiple locations including Puerto Rico, Brazil, and the southeast areas of the U.S. (Storer et al 2010, Farias et al. 2014, Huang et al. 2014). Cry2

proteins have been commercially used for a number of years and may face many challenges in the future years. Documented cases of Cry1F resistant larvae have been selected for resistance to Cry2 proteins in laboratory settings (Santos-Amaya et al. 2015). Unlike the Cry proteins, which are produced during the reproductive phases of growth of Bt, the Vip3A protein is produced during the vegetative phases, and Vip3A has no shared binding sites and no sequence homology with Cry proteins (Chakroun et al. 2016, Lee et al. 2003, Estruch et al. 1996). With Cry2 proteins facing a great risk preservation of Vip3A is even more critical with Vip3A being the last effective Bt technology currently available.

The objectives of this study was to determine the cross-crop resistance between corn and cotton with a strain of Vip3A resistant fall armyworm *Spodoptera frugiperda.* Corn and cotton leaf bioassays were used to determine the cross-crop resistance of corn Bt technologies to cotton Bt technologies using susceptible, Vip3A resistant, and heterozygote fall armyworms. This study also determined if pyramided Bt technologies containing Cry1 and Cry2 proteins are able to manage Vip3A resistant fall armyworms.

Methods and Materials

Corn and cotton leaf bioassays were used to determine the cross-crop resistance of a Vip3A resistant strain of FAW. Corn hybrids DKC 62-08 (SmartStax), DKC 67-72 (VT Double Pro), M78S-3111 (Agrisure Viptera 3111), 1319 HR (Herculex), 1319VYHR (Leptra), DKC 62-95 (nont-Bt), N78N-GT (non-Bt), 1319 (non-Bt), and cotton varieties PHY 312 Avicta (WideStrike), PHY 490 (WideStrike3), DP 1522 B2XF (Bollgard II), 16R338B3XF (Bollgard 3), ST 4949 (TwinLink), FM 1953GLTP (TwinLink Plus), DP1441 (non-Bt) were planted in a greenhouse. Table 1 shows the variety and hybrids used and the Bt proteins that make up each. A resistant (RR), susceptible (SS), and heterozygote (RS) strain of FAW were assayed. RR was derived from an F_2 screen as described by Yang et al. (2017). SS was collected from a non-Bt corn near Weslaco, TX, which is known to be susceptible to Cry1F, Cry1A.105, Cry2Ab, Cry2Ae, and Vip3A. RS was generated by crossing RR with SS. The crosses were SS♀ × RR♂, RR♀ × SS♂. Because there were no differences in susceptibility of reciprocal crosses, therefore the RS progeny were pooled and used in the assays.

When corn reached the V5-V7 growth stage and cotton reached the 7th-8th growth stages, leaves were excised and prepared in the lab. Leaves were washed and cut into 3×3 in. squares and placed into 100×15 mm petri dishes. The dishes were lined with moistened filter paper. In each dish, 5 neonates (<24hr) were placed on the leaf surface and sealed with a lid. The dishes were then placed into a growth chamber at 27 ± 1° C, 50% RH and a 14:10 (L: D) photoperiod. Leaves were changed and the filter paper was re-moistened as needed. Mortality and larval development was assessed 7 days after infestation.

A randomized complete block design was used with 4 replications by genotype and variety/hybrid. Corn non-Bt hybrids had no difference between all three hybrids, so the data was pooled. Data on insect survival was transformed using an arcsine square-root transformation, while data on larval instar and weight were transformed using a log, ln (x + 1) transformation for normal distributions. Non-transformed data are presented. Transformed data were then analyzed using two-way analysis of variance (ANOVA) with insect strain and varieties as the two main factors. Survivorship was calculated as a percent =100* (number of surviving larvae / number of total larvae assayed). Means were then separated using Tukey's method of difference of least square means, with an α=0.05.

Results

Corn

All three genotypes had high survival on non-Bt corn with around 70 percent survivorship (Table 1). RR larvae survived well on Vip3, which contains Cry1Ab and Vip3A, with 72 percent survivorship and showed no statistical difference compared to non-Bt. Moderate survivorship occurred on Herculex corn with the RR genotype. This suggests that there may be some moderate resistance to Cry1F protein. RS and SS had some survivors on Herculex as well though not statistically different from other hybrids containing Cry1 or Cry2 proteins. Pyramided proteins containing Cry1 or Cry2 proteins negated the resistance mechanism of RR which suggests that these technologies are capable of managing the Vip3A resistant strain of fall armyworms. Larval development on non-Bt was normal with larvae reaching on average the 4th instar (Figure 1). RR larvae averaged almost 3rd instar on Vip3 and was statistically

different from non-Bt, this suggest an incomplete resistance. Larval weights mirrored average instars with high weights in all three genotypes on non-Bt and very low weights on all other proteins (Figure2). Cry1 and Cry2 proteins hinder survivorship and development of all three genotypes.

Cotton

All genotypes had high survival on non-Bt cotton, with 80 percent and higher survivorship (Table 2). Cry1 and Cry2 proteins low survivorship across all genotypes. All varieties, other than non-Bt, had low survivorship in all three genotypes with 20 percent survivorship and less. Larval development was hindered by pyramided technologies. All three genotypes developed well on non-Bt averaging over 3rd instar, and the weights are lower compared to non-Bt corn, this can be expected due to the leaf structure of cotton (Figure3). All varieties with surviving larvae averaged 2nd instar, and weighed less than 2mg (Figure 4).

Discussion

Vip3A protein is crucial to the sustainability of Bt technologies and has been incorporated into third generation Bt corn and third generation Bt cotton products for the control of several species of insects and to delay the resistance to other Bt proteins. The results of this study show that current Bt technologies containing Cry1 or Cry2 proteins are still capable of managing Vip3A resistant fall armyworms. Pyramided products containing these proteins kept survivorship to a minimum. Herculex corn had roughly 34 percent survivorship which shows that RR larvae have moderate resistance to Cry1F, this is supported by the already documented cases of resistance in the field. Leptra, which showed less than 10 percent survivorship, contains Cry1Ab, Cry1F, and Vip3A. Cry1Ab is ineffective for the control of fall armyworm, which leaves Cry1F to control RR larvae. With moderate resistance to Cry1F it is understandable to see roughly 10 percent survivorship of RR larvae on Leptra corn. The other 2 hybrids had 0 percent survivorship and showed great control of all three genotypes. Cotton Bt technologies showed great control of all genotypes, with less than 20 percent survivorship was seen across all pyramided technologies. This study showed that the resistant gene is recessive because of the low survivorship of the heterozygote larvae. The RS larvae had 0 survivors on the Vip3 corn (which contains Cry1Ab, and Vip3A). RR did not develop as well on Vip3 when compared to non-Bt which is suggesting the resistance is incomplete. This will be the focus of future studies. Pyramided technologies containing Cry1 or Cry2 proteins, negate the resistance mechanism of resistant larvae, which suggests that these current technologies are still capable of containing Vip3A resistant fall armyworms.

References

Carrière, Y., et al. (2015). "Optimizing pyramided transgenic Bt crops for sustainable pest management." Nature biotechnology 33: 161.

Chakroun M, Banyuls N, Bel Y, Escriche B, Ferré J. 2016. Bacterial vegetative insecticidal proteins (Vip) from entomopathogenic bacteria. Microbiol Mol Biol Rev 80:329–350.

Dhurua, S. and G. T. Gujar (2011). "Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae), from India." Pest Management Science 67(8): 898-903.

Dively, G. P., et al. (2016). "Field-Evolved Resistance in Corn Earworm to Cry Proteins Expressed by Transgenic Sweet Corn." PLOS ONE 11(12): e0169115.

Dong, H. Z. and W. J. Li (2007). "Variability of Endotoxin Expression in Bt Transgenic Cotton." Journal of Agronomy and Crop Science 193(1): 21-29.

Estruch, J. J., et al. (1996). "Vip3A, a novel Bacillus thuringiensis vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects." Proceedings of the National Academy of Sciences of the United States of America 93(11): 5389-5394.

Farias, J. R., et al. (2014). "Field-evolved resistance to Cry1F maize by Spodoptera frugiperda (Lepidoptera: Noctuidae) in Brazil." Crop Protection 64(Supplement C): 150-158.

Gassmann, A. J., et al. (2011). "Field-Evolved Resistance to Bt Maize by Western Corn Rootworm." PLOS ONE 6(7): e22629.
Hardke, J., et al. (2015). Fall Armyworm (Lepidoptera: Noctuidae) Ecology in Southeastern Cotton.

Horikoshi, R. J., et al. (2016). "Effective dominance of resistance of Spodoptera frugiperda to Bt maize and cotton varieties: implications for resistance management." Scientific Reports 6: 34864.

Huang, F., et al. (2011). "Success of the high-dose/refuge resistance management strategy after 15 years of Bt crop use in North America." Entomologia Experimentalis et Applicata 140(1): 1-16.

Huang, F., et al. (2014). "Cry1F Resistance in Fall Armyworm Spodoptera frugiperda: Single Gene versus Pyramided Bt Maize." PLOS ONE 9(11): e112958.
James, C., Global status of commercialized biotech/GM crops: 2015. Brief No. 52. ISAAA: Ithaca, NY, USA (2016).

Li, G., et al. (2016). "Frequency of Cry1F Non-Recessive Resistance Alleles in North Carolina Field Populations of Spodoptera frugiperda (Lepidoptera: Noctuidae)." PLOS ONE 11(4): e0154492.

Morin, S., et al. (2004). "DNA-based detection of Bt resistance alleles in pink bollworm." Insect Biochemistry and Molecular Biology 34(11): 1225-1233.

(NASS) Nation Agriculture Statistics Service. 2017. United States Department of Agriculture) (accessed November 2017).

Niu, Y., et al. (2013). "SUSCEPTIBILITY OF FIELD POPULATIONS OF THE FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) FROM FLORIDA AND PUERTO RICO TO PURIFIED CRY1F PROTEIN AND CORN LEAF TISSUE CONTAINING SINGLE AND PYRAMIDED BT GENES." The Florida Entomologist 96(3): 701-713.

Omoto, C., et al. (2015). Field-evolved resistance to Cry1Ab maize by Spodoptera frugiperda in Brazil.

Ostlie KR, H. W. a. H. R. (1997). "Bt Corn and European Corn Borer: Long Term Success Through Resistance Management." North Central Region Extension Publication 602.

P Storer, N., et al. (2010). Discovery and Characterization of Field Resistance to Bt Maize: Spodoptera frugiperda (Lepidoptera: Noctuidae) in Puerto Rico.

Pashley, D. P. (1988). "Fall Armyworm Symposium--1988: Current Status of Fall Armyworm Host Strains." Florida Entomologist 71(3).

Santos-Amaya, Oscar F., et al. "Resistance to Dual-Gene Bt Maize in Spodoptera Frugiperda: Selection, Inheritance, and Cross-Resistance to Other Transgenic Events." *Scientific Reports* 5 (2015): 18243. Print.

Sparks, A. N. (1979). "A Review of the Biology of the Fall Armyworm." The Florida Entomologist 62(2): 82-87.

Tabashnik, B. E., (2017). "Surge in insect resistance to transgenic crops and prospects for sustainability " Nature biotechnology 35(10): 926-935.

Tabashnik, B. E., et al. (2013). "Insect resistance to Bt crops: lessons from the first billion acres." Nature biotechnology 31: 510.

van Rensburg, J. B. J. (2007). "First report of field resistance by the stem borer, Busseola fusca (Fuller) to Bt-transgenic maize." South African Journal of Plant and Soil 24(3): 147-151.

Yang, F, Head, G. Huang, F., Gilreath, R. T., Kerns, D., (2017). "F_2 screen, inheritance and cross-resistance of Vip3A resistance in a field-derived population of *Spodptera frugiperda* (Lepidoptera: Noctuidae) in the United States" Pest Management Science.

Yang, F., et al. (2013). Susceptibility of Louisiana and Florida Populations of Spodoptera frugiperda (Lepidoptera: Noctuidae) to Pyramided Bt Corn Containing Genuity®Vt Double Pro ™ and Smartstax ™ Traits.

Yang, F., Kerns, D. L., Head, G. P., Price, P. and Huang, F. (2017), Cross-resistance to purified Bt proteins, Bt corn and Bt cotton in a Cry2Ab2-corn resistant strain of *Spodoptera frugiperda*. Pest Management Science, 73: 2495–2503. doi:10.1002/ps.4644

Zhou, L., et al. (2017)"Combining the high-dose/refuge strategy and self-limiting transgenic insects in resistance management- a test in experimental mesocosms." Evolutionary Applications: n/a-n/a.

Table 1. Seed selection

Corn Hybrids							
Cry1F	Cry1F, Cry2Ab2, Cry1A.105	Cry1A.105, Cry2Ab2	Cry1Ab, Vip3A	Cry1Ab, Cry1F, Vip3A	Non-Bt	Non-Bt	Non-Bt
Herculex	SmartStax	VT Double Pro	Agrisure Viptera 3111	Leptra	Non-Bt 1	Non-Bt 2	Non-Bt 3
Cotton Varieties							
Cry1F, Cry1Ac	Cry1F, Cry1Ac, Vip3A	Cry1Ac, Cry2Ab2	Cry1Ac, Cry2Ab2, Vip3A	Cry1Ab, Cry2Ae	Cry1Ab, Cry2Ae, Vip3A	Non-Bt	
WideStrike	WideStrike 3	Bollgard II	Bollgard III	TwinLink	TwinLink Plus	DP 1441RF	

Table 2. Percent survivorship of different genotypes of *Spodoptera frugiperda* on corn leaves.

Insect Genotype[1]	Survivorship (%)					
	Non-Bt	Vip3	Herculex	Leptra	SmartStax	VT Double Pro
RR	75.42 ± 3.66a	71.25 ± 5.54a	33.75 ± 8.00b	8.75 ± 2.39bc	0.00 ± 0.00c	0.00 ± 0.00c
RS	77.08 ± 4.06a	0.00 ± 0.00c	12.50 ± 3.23bc	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c
SS	70.00 ± 5.81a	0.00 ± 0.00c	12.50 ± 9.46bc	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c

Means in a column or row followed by the same letter are not significantly different based on a two-way ANOVA (Tukey's HSD $P > 0.05$)

[1]RR = Resistant larvae, RS = Heterozygote larvae, SS= Susceptible larvae

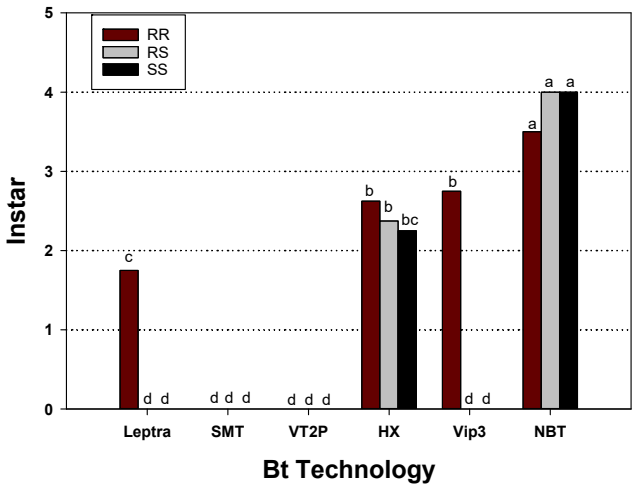

Figure 1. Average instar for surviving larvae on corn leaves. Mean values in figure followed by the same letter are not significantly different based on a two-way ANOVA (Tukey's HSD P > 0.05)
RR = Resistant larvae, RS = Heterozygote larvae, SS= Susceptible larvae

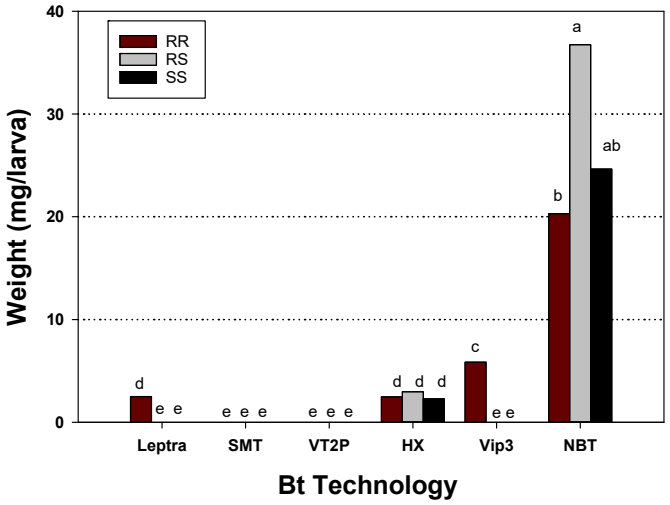

Figure 2. Average weights for surviving larvae on corn leaves. Mean values in figure followed by the same letter are not significantly different based on a two-way ANOVA (Tukey's HSD P > 0.05)
RR = Resistant larvae, RS = Heterozygote larvae, SS= Susceptible larvae

Table 3. Percent Survivorship of different genotypes of *Spodoptera frugiperda* on cotton leaves

Insect Genotype[1]	Survivorship (%)						
	Non-Bt	WideStrike3	WideStrike	TwinLink+	TwinLink	Bollgard 3	Bollgard 2
RR	83.75 ± 9.21a	12.50 ± 3.23bc	18.75 ± 3.75b	16.25 ± 5.15bc	2.50 ± 1.44c	7.50 ± 3.23bc	11.25 ± 1.50bc
RS	81.25 ± 2.39a	0.00 ± 0.00c	7.5 ± 4.33bc	0.00 ± 0.00c	3.75 ± 1.25c	0.00 ± 0.00c	17.50 ± 2.50bc
SS	90.00 ± 2.04a	0.00 ± 0.00c	3.75 ± 1.25c	0.00 ± 0.00c	10.00 ± 4.08bc	0.00 ± 0.00c	12.50 ± 2.50bc

Means in a column or row followed by the same letter are not significantly different based on a two-way ANOVA (Tukey's HSD P > 0.05)
[1]RR = Resistant larvae, RS = Heterozygote larvae, SS= Susceptible larvae

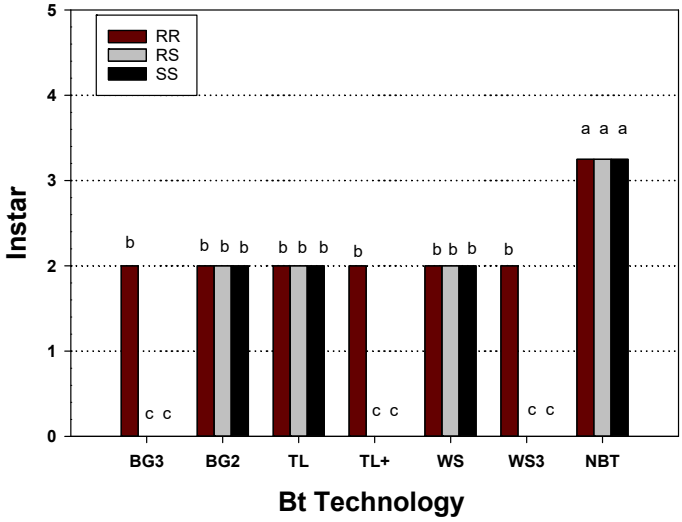

Figure 3. Average instar for surviving larvae on cotton leaves. Mean values in figure followed by the same letter are not significantly different based on a two-way ANOVA (Tukey's HSD P > 0.05)
RR = Resistant larvae, RS = Heterozygote larvae, SS= Susceptible larvae

Figure 4. Average weights for surviving larvae on cotton leaves.

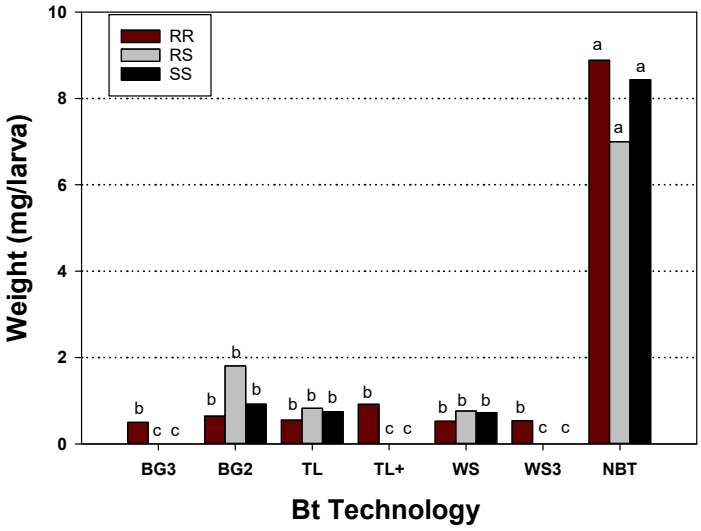

Figure 4. Average weights for surviving larvae on cotton leaves. Mean values in figure followed by the same letter are not significantly different based on a two-way ANOVA (Tukey's HSD P > 0.05)
RR = Resistant larvae, RS = Heterozygote larvae, SS= Susceptible larvae