SUSCEPTIBILITY OF FALL ARMYWORM (*SPODOPTERA FRUGIPERDA)* GENOTYPES CARRYING VIP3A RESISTANT ALLELES, TO BT PLANTS AND PURIFIED BT PROTEINS

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Abstract

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (FAW), is one of the major target pests of Bt corn and cotton in the U.S. Current control strategies for FAW rely heavily on transgenic plants. Negative side effects of extensive use have resulted in field-evolved resistance (Storer et al 2010, Farias et al. 2014, Huang et al. 2014). Gene pyramiding has been used to delay these resistance issues; however, the durability of this technique can be greatly reduced by cross-resistance. In this study, we investigated the susceptibility of different genotypes of fall armyworm carrying Vip3A resistant alleles to whole Bt corn plants and Bt cotton squares. To determine the cross-resistance of the Vip3A resistant population to other corn Bt technologies whole plant corn bioassays were used. During this bioassay RR were found to survive well on non-Bt and Vip3111 (Vip3a, Cry1Ab) corn. Resistant larvae had moderate survivorship on Herculex (Cry1F) corn, however survivorship diminished on all other technologies. The susceptible population showed high survivorship on non-Bt but no survivorship on any other technology. Cotton square bioassays were utilized to determine the cross-crop resistance of Vip3A resistant FAW. Resistant larvae showed high survivorship across all varieties, regardless of technology. The heterozygote and susceptible larvae showed similar results with high survivorship on non-Bt, Bollgard II, and Widestrike, however very little or no survivorship on Bollgard III, and Widestrike 3. Results generated from this study provided important information for insect pest management and resistance management of Bt crops.

Introduction

Currently, 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, which begins from warmer climates in the southern U.S. moving northward (Hardke et al. 2015). FAW is a highly polyphagous insect and can colonize over 80 host species including corn and cotton (Pashley et al. 1986). This pest can cause up to 15% 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).

Controlling FAW can become difficult due to the behavior of this pest. Cultural practices such as suppressing overwintering habitats may be futile due to the lack of a diapause mechanism. There are several insecticides labeled for the control of FAW. However, with the heavy reliance and usage, resistance in FAW has been developed against these insecticides (Hardke et al. 2015). With difficulty controlling FAW using cultural and insecticidal approaches, heavy reliance has been shifted towards the use of Bt technology.

Transgenic crops that express the entomopathogenic bacteria *Bacillus thuringiensis* (Bt), have been used in production agriculture since 1996 (James 2016). Many benefits are associated with the use of this technology such as, reduced use of chemical insecticides, reduced pest populations, and increased yields and profits for growers (Hutchison et al 2010). Global use of this technology has risen from 1.1 million hectare to 98.5 million hectares in roughly one decade (Tabashnik 2017). 81.8 million acres of corn was planted in 2018, and 94 percent of those acres planted were into a biotech variety (NASS 2018). Upland cotton accounted for 13.3 million acres, of which 94 percent was planted to a biotech variety (NASS 2018). Originally, the Cry1F and Cry2 proteins showed great efficacy for control of FAW. However, with the rapid adoption and extensive use, field evolved resistance to Cry proteins are beginning to surface (Huang et al. 2014, Chen et al. 2018).

Field evolved resistance to Bt technologies is the main threat to the sustainability and future use of this technology (Yang et al. 2017). However, the newest technology, Vip3A is of different mode of actions relative to Cry proteins. It not only has efficacy against FAW but also shows great control of the cotton bollworm *Helicoverpa zea* (Burkness et al. 2010). 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, Sena et al. 2009). With Cry proteins

facing a great risk, preservation of Vip3A is even more critical because Vip3A will be the last effective Bt technology currently available. The objectives of the study are to determine the susceptibility of different genotypes of fall armyworm carrying Vip3A resistant alleles to purified Bt proteins, whole corn plants and Bt cotton squares.

Methods and Methods

A resistant FAW population (RR) was collected out of Bollgard ll cotton from Rapides Parish, Louisiana, and derived from an F_2 screen described by Yang et al. in 2017. RR has been documented highly resistant to purified Vip3A protein as well as Bt plants expressing Vip3A protein (Yang et al., 2017). The susceptible population (SS) was collected from non-Bt corn in Winnsboro, Louisiana. SS has been documented to be susceptible to Cry1F, Cry1A.105, Cry2Ab2, Cry2Ae, and Vip3A. A heterozygote population (RS) was established by making reciprocal crosses between the RR and SS (SS × RR , RR × SS). Since the reciprocal crosses showed no difference in inheritance, progeny were pooled and randomly used throughout the bioassays.

Whole Plant Corn Bioassay

Whole plant corn bioassays were used to determine the cross-resistance of the different FAW genotypes to corn Bt technologies. Seeds were planted in 18.9-liter pots filled with standard potting mix in a greenhouse at the USDA Southern Plains Agricultural Research Center: College Station, Texas. The hybrids used were 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). Table 1 shows the hybrids used along with the traits associated with that particular hybrid. Two-to-three plants per pot were maintained with regular irrigation and fertilization as described by Niu et al. (2014). Plants were maintained insect free until the V5-V7 leaf stage.

At V5-V7 growth stages, 6 neonates (<24hr) of RR or SS were placed into the whorl of the plant. Each combination of genotype and corn hybrid were replicated 4 times with 3 plants per replication. The bioassay was arranged in a randomized complete block design. After 10 days of infestation, the Davis scale of 1 (no damage or few pinholes) to 9 (most leaves with long lesions) was used to evaluate leaf injury (Davis 1992). In addition, larval development and percentage of plants containing live larvae were assessed immediately after damage ratings.

Leaf injury was transformed using the log(x+1) scale, while percentage of plants containing live larvae was transformed using arcsine of ($X^{0.5}$) to normalize treatment variances. Data was then analyzed using two-way analysis of variance (ANOVA) with insect strain and corn hybrid as the two main factors. Treatments were then separated using Tukey s HSD at ± = 0.05 level.

Cotton Squares Bioassay

Cotton square bioassays were utilized to determine the cross-crop resistance from corn Bt technologies to cotton Bt technologies. To produce a sufficient amount of aged appropriate squares, seeds from 5 different cotton varieties were planted at the USDA Southern Plains Agricultural Research Center: College Station, Texas. Varieties used along with their associated traits are listed in Table 1. Match head to medium size squares with bracts attached were selected, excised and brought to the laboratory for preparation. After washing the squares, one square was placed into a 30 mL Dart clear portion containers (Dart Container Corporation, Mason MI). Two early 2nd instar larvae were then placed on the square. Each combination of genotype and variety was replicated 4 times, and there were 15 squares within each replication. Squares were changed when necessary. After infestation, containers were placed in a growth chamber at 27 ± 1° C, with 50% RH and a 14:10 (L: D) photoperiod. Larval survivorship were then assessed after seven days.

Larval survivorship was calculated as a percent = 100* (number of dead larvae/ total number of insects assayed). Survivorship data was then analyzed using a two-way analysis of variance (ANOVA) with insect strain and variety set as the two main factors. Treatments were then separated using Tukey's HSD with ± = 0.05 level.

Results

Whole Plant Corn Bioassay

Non-Bt corn had 100% of the plants containing live larvae of both RR and SS genotypes after 10 days (Figure 1). Vip3111, which contains Cry1Ab and Vip3A proteins, had approximately 90% of the plants containing live RR larvae and no plants containing any SS live larvae. Herculex, which contains the Cry1F protein, had approximatley 50% of the plants containing live RR larvae. Leptra, which contains Cry1Ab, Cry1F, and Vip3A had 0% of the

plants containing any live RR or SS larvae. No genotypes survived on any of the other Bt technologies. Damage ratings showed similar results as the percentage of plants containing live larvae with damage scores around 7 in the non-Bt (Figure 2) for both RR and SS genotypes. Vip3111 was statistically different from the non-Bt damage ratings for RR genotype. Herculex, which had 50% of the plants contain live larvae of RR, had a damage rating of 2. While 50% of the plants contained live larvae, if the parameters were to exclude 1st and 2nd instar larvae the percentage of plants with live larvae would decrease. All other Bt technologies had very low damage ratings.

Cotton Squares Bioassay

RR genotype showed good survivorship on non-Bt squares at roughly 70% (Table 3). RR showed no differences in survivorship on all other varieties regardless of technology, when compared to the non-Bt. RS had 70% survivorship and SS had 73% survivorship on non-Bt. Both genotypes showed good survivorship on Bollgard II, and Widestrike and had no differences when compared to non-Bt. However, survivorship was diminished on Bollgard III and Widestrike 3, which contains the Vip3A protein.

Discussion

Vip3A protein is crucial to the sustainability of Bt technologies and has been incorporated into second-generation Bt corn and third-generation Bt cotton products for the control of several insect species. The results of this study showed pyramided corn products that contain Cry1 and Cry2 proteins kept survivorship to a minimum. Herculex corn had roughly 50% of plants containing live larvae, which showed that the RR population may have some moderate resistance to Cry1F. This is documented by several cases of field-evolved resistance to Cry1F (Huang et al. 2014, Farias et al. 2014). However, when Cry1F is pyramided such as in Leptra (Cry1Ab, Cry1F, and Vip3A) no plants contained live RR larvae. Damage ratings were statistically lower on the Vip3111 than the non-Bt for RR larvae, which suggests that the resistance is incomplete. Cotton varieties showed poor efficacy against RR larvae across all varieties and technologies. Poor control was achieved on RS, and SS larvae in Bt cotton without Vip3A protein. This data would suggest that the level of expression may vary from within the plant and has been documented by Tritikova et al. (2015). Kranthi et al. (2005) documented that Bt expression levels vary from plant tissues within the plant, with the leaf having the highest expression levels followed by the squares and then bolls. However, despite the high survivorship on cotton squares, Yang et al. (2017) documented that purified Cry1 and Cry2 proteins are highly toxic to the FAW. Pyramided corn products containing Cry1 or Cry2 proteins are still capable of managing Vip3A resistant fall armyworms.

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Table 1. Seed selection.

Corn Hybrids (Objective 2)							
Herculex	SmartStax	VT Double Pro	Agrisure Viptera 3111	Leptra	non-Bt 1*	non-Bt 2*	non-Bt 3*
Cry1F	Cry1F, Cry1A.105, Cry2Ab2	Cry1A.105, Cry2Ab2	Cry1Ab, Vip3A	Cry1Ab, Cry1F, Vip3A	non-Bt	non-Bt	non-Bt

Cotton Varieties (Objective 3)						
Widestrike	Widestrike 3	Bollgard II	Bollgard III	TwinLink[1]	TwinLink Plus[1]	DP 1441 RF
Cry1F, Cry1Ac	Cry1F, Cry1Ac, Vip3A	Cry1Ac, Cry2Ab2	Cry1Ac, Cry2Ab2, Vip3A	Cry1Ab, Cry2Ae	Cry1Ab, Cry2Ae, Vip3A	non-Bt

*No differences; pooled for results
[1]Unable to assay

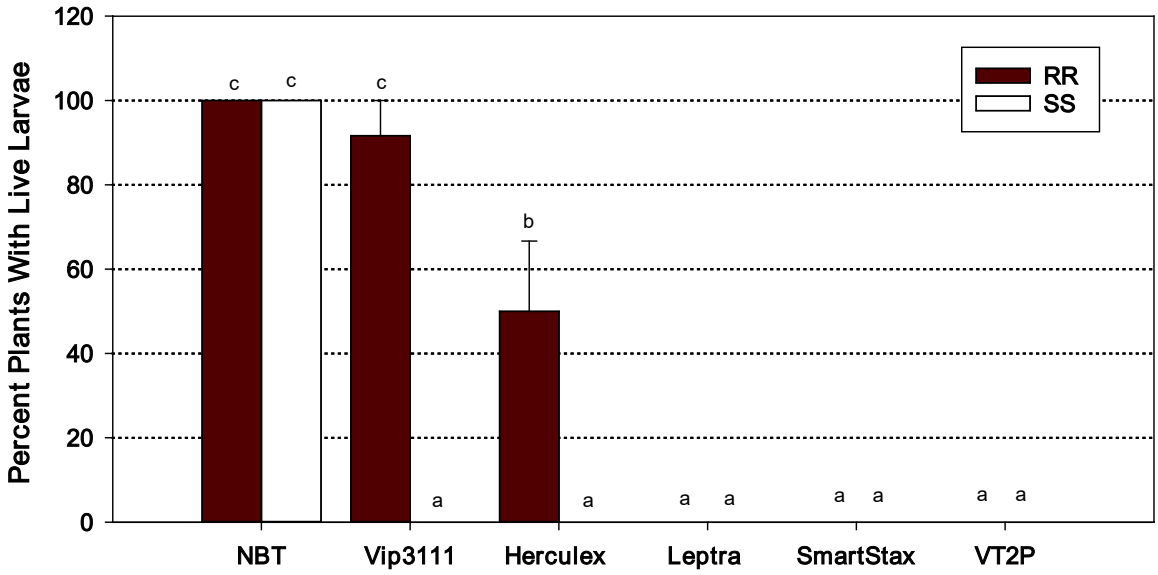

Figure 1. Percentage of plants with live larvae after 10 days. Mean values in figure followed by the same letter are not significantly different based on a two-way ANOVA (Tukey's HSD P> 0.05). RR = Resistant larvae, SS = Susceptible larvae

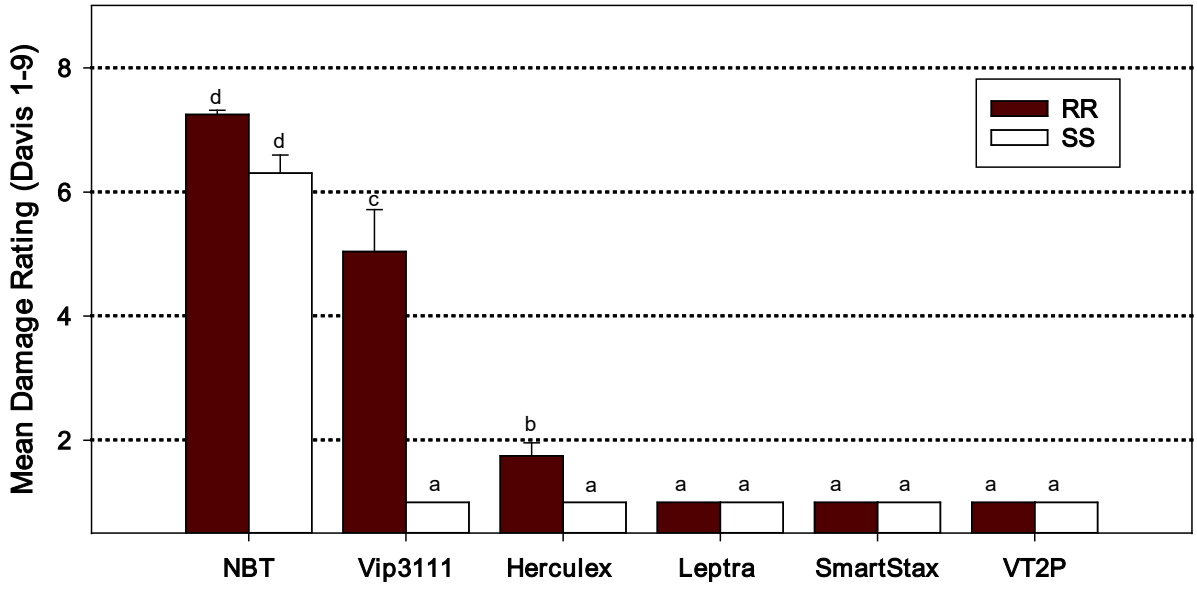

Figure 2. Mean damage ratings after 10 days. Mean values in figure followed by the same letter are not significantly different based on a two-way ANOVA (Tukey's HSD P> 0.05)
RR = Resistant larvae, SS = Susceptible larvae

Table 2. Percent survivorship of *Spodoptera frugiperda* on cotton squares.

Insect Genotype	Survivorship (%)				
	Non-Bt	Bollgard II	Bollgard III	Widestrike	Widstrike 3
RR	67.5 ± 4.98 abc	63.33 ± 3.6 abc	64.17 ± 2.85 abc	36.67 ± 3.04 c	40.83 ± 4.38 bc
RS	70.00 ± 3.33 abc	55.00 ± 9.08 abc	0.83 ± 0.83 d	63.33 ± 2.36 abc	12.5 ± 8.65 d
SS	73.33 ± 5.93 ab	80.00 ± 5.27 a	0.00 ± 0.00 d	38.30 ± 4.19 bc	11.60 ± 8.66 d

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)
RR = Resistant larvae, RS = Heterozygote larvae, SS = Susceptible larvae