FITNESS COSTS AND CROSS RESISTANCE ASSOCIATED WITH VIP3AA RESISTANCE IN *HELICOVERPA ZEA*

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Abstract

*Bacillus thurginesis (*Bt) proteins play an important role of pest control in major Lepidopteran species such as *Helicoverpa zea.* The Bt proteins used for managing *H.zea* include Cry1, Cry2, and the most recent Vip3Aa proteins. The heavy reliance on Bt proteins has led to the evolution of Bt resistance, and currently there is field-evolved resistance to the Cry1 and Cry2 proteins in the United States. The failures of the Cry1 and Cry2 proteins has put tremendous selection pressure on the Vip3Aa protein for *H.zea* management. Therefore, understanding the risks associated with Vip3Aa resistance is important for evaluating the durability of the Vip3Aa protein. The objectives of this research are 1) to determine the fitness costs associated with Vip3Aa resistance of Vip3Aa-homozygous and heterozygous resistant *H.zea* (2) to evaluate the cross-resistance of Vip3Aa-homozygous and heterozygous resistant *H.zea.* Four insect genotypes were used: a Vip3Aa resistant (RR), a susceptible (SS), and two F1 heterozygous Vip3Aa-resistant strains that were produced from reciprocal crosses between the RR and SS, which are R S and R S . Fitness costs were evaluated on non-Bt meridic diet, and the survivorship and developmental data were used to estimate lifetime parameters. The data suggests evidence of recessive fitness cost of survivability and slight dominant fitness cost of developmental time. Cross resistance was evaluated on cotton leaf tissue and data suggests no evidence of cross resistance between Vip3Aa and Cry proteins. The data suggests that the evidence of fitness costs and no cross resistance can help delay the evolution of Vip3Aa resistance.

Introduction

The utilization of *Bacillus thuringiensis* (Bt) proteins plays a significant role of managing *H.zea.* Currently the Bt proteins used in cotton or corn products for *H.zea* include: Cry1Ab, Cry1Ac, Cry1F, Cry2Ab, Cry2Ae, and Vip3Aa. Vip3Aa is the most recent Bt protein and is found in all new corn and cotton products on the U.S. market.

The heavy reliance on Bt crops to manage *H.zea* has subsequently led to the evolution of Bt resistance. To date, field-evolved resistance to the Cry 1 and Cry2 proteins has been widely reported in the United States. Consequently, the widespread failures of the Cry1 and Cry2 proteins has put tremendous selection pressure on the Vip3Aa protein for *H.zea* management. Although no practical resistance has been reported to the Vip3Aa protein, there are early warning signs of resistance.

In order to delay Bt resistance development, insect resistant management (IRM) strategies are imperative, and currently the two IRM strategies are the high-dose/refuge strategy in corn and gene-pyramiding. The high-dose/refuge strategy in corn involves first using a high-dose Bt crop to kill the susceptible and resistant heterozygotes as well as planting a sufficient nearby source of non-Bt corn to serve as a refuge for hosting susceptible moths to mate with the rare resistant moths. For this strategy to be effective, there are key assumptions that must be met: resistance is recessive, there is rare initial resistance allele frequency, the Bt crop must eliminate the heterozygotes, and there must be a sufficient supply of homozygous susceptible insects in the area to randomly mate with the rare homozygous resistant individuals.

Gene-pyramiding is another IRM strategy utilized to delay the evolution of Bt resistance. Gene-pyramiding is when Bt crops express two or more dissimilar Bt proteins that are targeting the same insect pest and therefore can reduce or possibly eliminate the resistant genotypes. Pyramided Bt crops are expected to be effective in delaying evolution of resistance because when one Bt protein in the pyramids is ineffective, the remaining Bt proteins can kill the insects. However, gene-pyramiding assumes there is no cross resistance among Bt proteins, which occurs when insects are resistant to one Bt protein, they exhibit genetically based reduced susceptibility to the other Bt proteins. Cross resistance can reduce the durability of gene-pyramiding and thus expedite Bt resistance development.

Numerous factors such as fitness costs can affect Bt resistance management and the speed of resistance

development. Fitness costs associated with Bt resistance occur in the absence of Bt proteins, the fitness might be lower for homozygous and/or heterozygous resistant insects compared to susceptible insects. Furthermore, the resistant genotypes may be less competitive and less likely to survive and persist if there are substantive fitness costs.

Assessing the risks associated with Vip3Aa resistance in *Helicoverpa zea* is critical for evaluating the durability of the Vip3Aa protein to manage *H.zea*. The objectives of this research are 1) to determine the fitness costs associated with Vip3A resistance of Vip3Aa-homozygous and heterozygous resistant *H.zea,* 2) to evaluate the cross-resistance of Vip3Aa-homozygous and heterozygous resistant *H.zea.*

Materials and Methods

Insect Sources

The insect sources include a Benzon susceptible (SS) and a Vip3Aa resistant strain (RR) of *H.zea.* The Vip-RR resistant strain was established using an F2 screening method with populations collected from Texas in 2019. The Vip-RR strain has demonstrated significant levels of resistance to Vip3Aa protein in diet-overlay bioassays, with a resistance ratio of >588.0-fold relative to the susceptible colony.

Fitness Costs

To assess fitness costs on non-Bt meridic diet, four insect genotypes were used: a Vip3Aa resistant (RR), a susceptible (SS), and two F1 heterozygous Vip3Aa-resistant strains that were produced from reciprocal crosses between the RR and SS, which are R S and R S .

One neonate (<24 h) was placed in each cell of bioassay tray containing approximately 1 gram of non-Bt diet in each cell of 128-cell bioassay trays. The bioassay trays were held in an environmental chamber maintained at 26 ± 1 °C, ~50% relative humidity (RH), and a photoperiod of 16:8 h (L:D). There were four replications for each insect genotype with 32 larvae in each replication. After 6 days, the live larvae were transferred into 30-mL cups (1 larva/cup) containing approximately 8 grams of the same diet and allowed to develop to the adult stage. Pupal weight, sex ratio, neonate-to-adult survival, and neonate-to-adult developmental time were recorded.

Pupal weight, sex ratio, and neonate-to-adult developmental time were transformed using log (x+1) scale for normal distribution. Neonate-to-adult survival was transformed using arcsine ($Ç^{0.5}$) for normal distribution. The transformed data for each parameter was analyzed with SAS v. 9.4 using a one-way analysis of variance (ANOVA) with insect genotype as the main factor. Treatment means were separated using Tukey's HSD test at $\alpha = 0.05$ level.

Cross Resistance

Cross resistance was assessed on cotton leaf tissue in the laboratory by evaluating larval survivorship. Three insect genotypes were used: a susceptible (SS), Vip3Aa resistant (RR), and heterozygote resistant (RS). Since the inheritance of Vip3Aa resistance in TX-RR is autosomal, the two F1 heterozygous genotypes (R S and R S) were pooled as RS for this study. The leaf tissues were collected from 7 different cotton varieties, including one non-Bt and six Bt varieties (Table 1). The six Bt varieties include: WideStrike (WS) cotton expressing Cry1Ac and Cry1F; Bollgard II (BG2) containing Cry1Ac and Cry2Ab2; TwinLink (TL) containing Cry1Ab and Cry2Ae; WideStrike 3 (WS3) containing Cry1Ac, Cry1F and Vip3Aa; Bollgard 3 (BG3) containing Cry1Ac, Cry2Ab2 and Vip3Aa; and TwinLink Plus (TL+) expressing Cry1Ab, Cry2Ae and Vip3Aa.

The seven cotton varieties were planted at the Texas A&M University Farm in Snook, Texas. Prior to first bloom, fully expanded leaves were collected from the plants and used in the laboratory bioassays. Two to three pieces (H3 cm long) of the leaf tissues were placed into a sterile petri dish lined with moistened Whatman 90 mm (#1) filter paper. Five neonates were placed on the leaf tissue in each petri dish (5 larvae/dish). After 5 days, surviving larvae were individually transferred into new petri dishes along with the new leaf tissues (1 larva/dish) in case of cannibalism. The petri dishes were placed in a growth chamber maintained at 26 ± 1°C, ~50% relative humidity (RH), and a photoperiod of 16:8 h (L:D). Leaf tissues were changed every 1-2 days, while filter paper was wetted daily and changed as needed. There were four replications for each insect strain and each cotton variety, and each replication contained 30 larvae. Larval survival and instar were recorded on the 7th day after inoculation. Larval survival was transformed using arcsine ($Ç^{0.5}$) for normal distribution. The transformed data was analyzed

with SAS v. 9.4 using two-way ANOVA with insect and cotton product as the two main factors. Treatment means were separated using Tukey s HSD at ± = 0.05 level.

Table 1. Cotton varieties and Bt proteins

Variety	Bt proteins					
	Cry1Ab	Cry1Ac	Cry1F	Cry2Ab2	Cry2Ae	Vip3Aa
Non-Bt						
Widestrike (WS)		X	X			
Bollgard II (BG2)		X		X		
TwinLink (TL)	X				X	
Widestrike 3 (WS3)		X	X			X
Bollgard 3 (BG3)		X		X		X
TwinLink Plus (TL+)	X				X	X

Results and Discussion

Fitness Costs

The susceptible (SS) and the two heterozygous resistant genotypes (R S and R S) neonate-to-adult survival ranged from 97.7-99.2 % with no significant differences among these three genotypes (Figure 1). The Vip3Aa resistant colony (RR) was significantly different with a neonate-to-adult survival of 79.7 %. Since only the RR colony is significantly different, this data suggests a small recessive fitness cost of survival.

Regarding neonate-to-adult developmental time, the susceptible genotype (SS) had the shortest developmental time of 22.5 days (Figure 2) followed by the heterozygote genotypes (R S and R S) (23.8 and 25 days respectively). The RR genotype had the longest developmental time of 26.2 days. There were significant differences among insect genotypes. However, the difference between heterozygote resistant population (R S) and the RR is small (1.2 days), suggesting a slight dominant fitness cost.

In general, pupal weights were consistent across males and females (Figure 3 and 4). Male pupal weight ranged 293.7-312.3 mg and female pupal weight ranged 294.8-313.0 mg. No significant differences among insect genotypes for male and female pupal weight.

With respect to sex ratio, no significant differences among insect genotypes were observed (Figure 5).

Cross Resistance

To assess cross resistance on cotton leaf tissue, larval survivorship was evaluated after seven days. On the non-Bt cotton tissue, survivorship was high for all three insect genotypes (74-80%), with no significant differences among the genotypes (Figure 6). Regarding the second generation Bt technologies, including Widestrike, Bollgard II, and TwinLink, all three insect genotypes exhibited low survivorship compared to survival on the non-Bt cotton tissue. Regarding the 3rd generation Bt products, TwinLink Plus and Bollgard 3 had very low survivorship. Widestrike 3 exhibited low survivorship, but higher survival compared to the other 3rd generation Bt technologies (BG3 and TL+). This data suggests no evidence of cross resistance between Vip3Aa and Cry proteins on cotton leaf tissues.

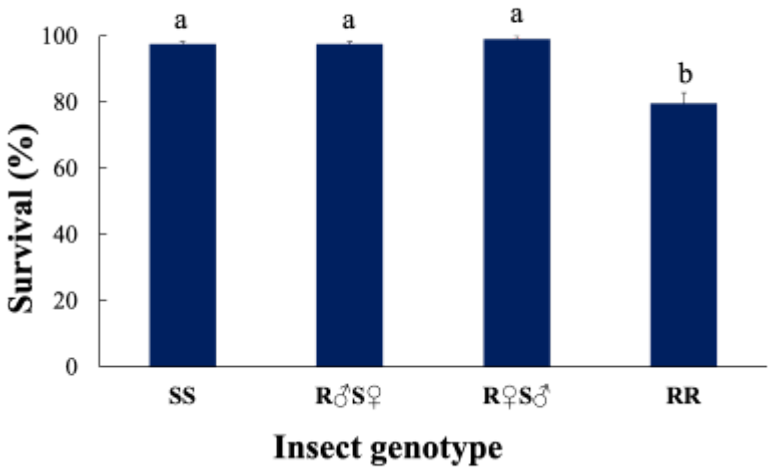

Figure 1 Neonate-to-adult survival

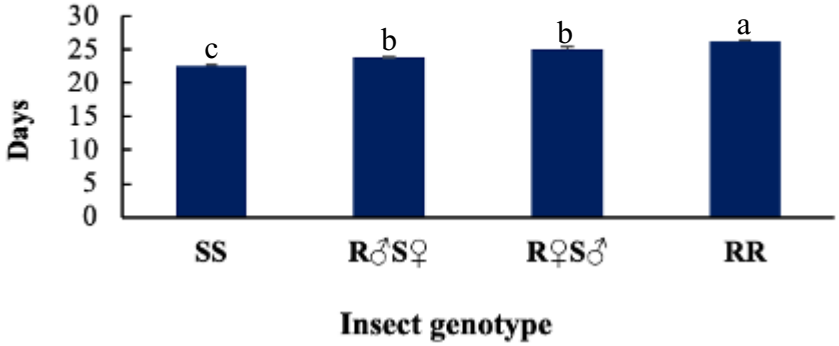

Figure 1 Neonate-to-adult developmental time

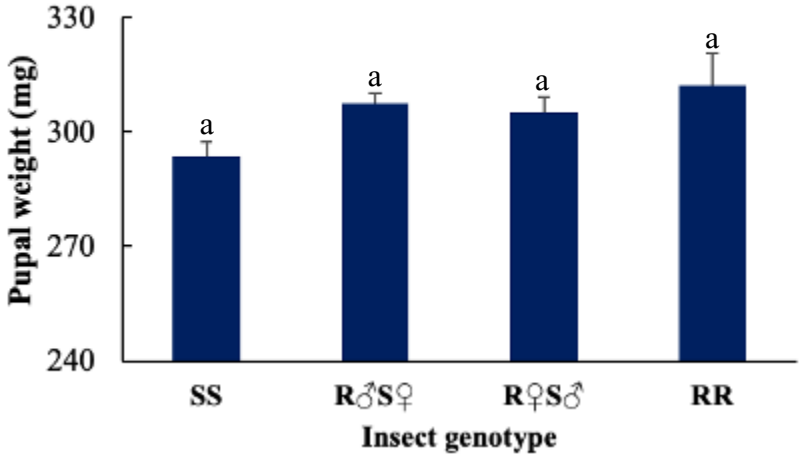

Figure 3 Male pupal weight

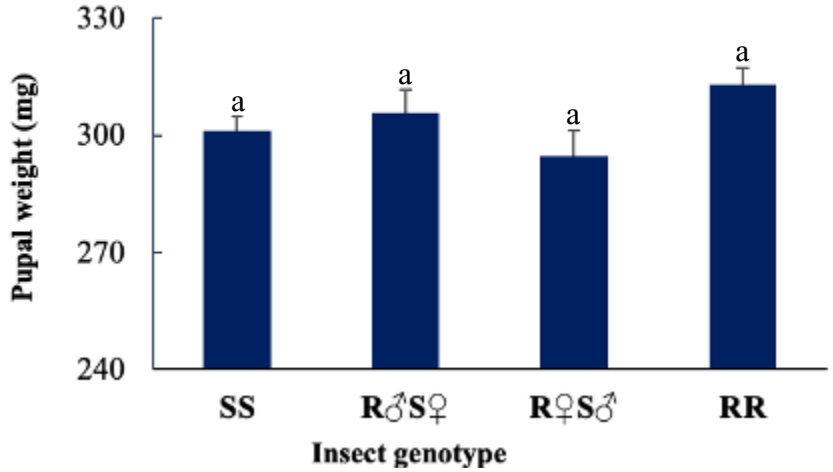

Figure 4 Female pupal weight

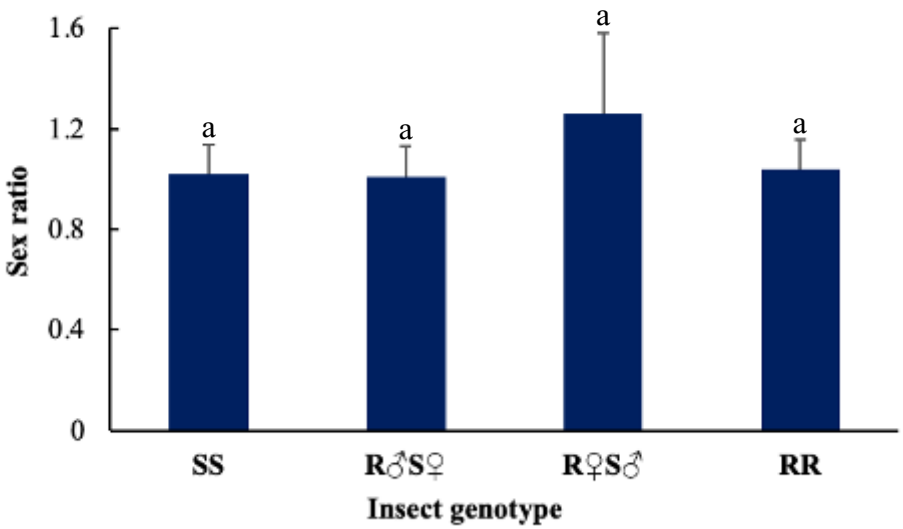

Figure 5 Sex ratio

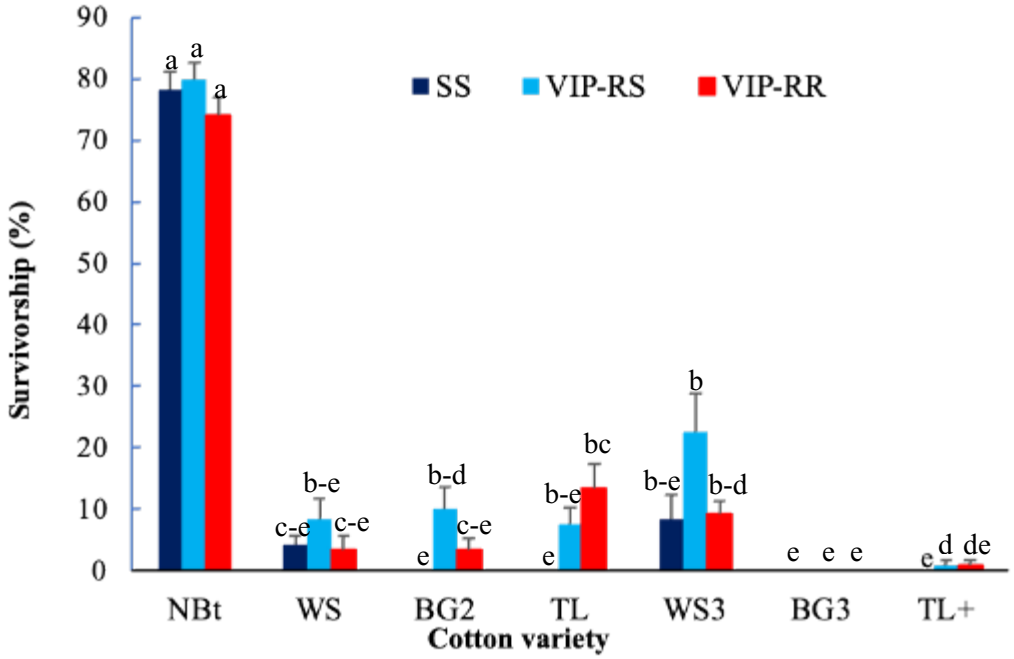

Figure 6 Larval survivorship after 7 days on cotton leaf tissue

Conclusion

In conclusion, the first objective was to determine fitness costs associated with Vip3Aa resistance and the data suggests evidence of fitness costs on non-Bt meridic diet. There is evidence of a recessive fitness cost of survivability and a slight dominant fitness cost of developmental time. Further evaluation of fitness costs will be assessed on non-Bt cotton leaf tissue and non-Bt corn ears. The second objective was to evaluate cross resistance and the data suggests no evidence of cross resistance between Vip3Aa and Cry proteins on cotton leaf tissues. Survivorship was low for all three insect genotypes on the 2nd and 3rd generation Bt cotton products compared to the survival on the non-Bt cotton tissue. Future research objectives include fitness costs on non-Bt corn ears and non-Bt cotton tissue and cross resistance on Bt corn technologies. Overall, the data suggested evidence of fitness costs and lack of cross resistance can help delay evolution of Vip3Aa resistance These results can be useful in determining the durability of Vip3Aa protein to manage *H.zea* and the evolution of Bt resistance.