UTILITY OF GS-OMEGA/KAPPA-HXTX-HV1A PEPTIDE (SPEAR-T) FOR MITIGATING *HELICOVERPA ZEA* BT CRY PROTEINS RESISTANCE IN DUAL-GENE BT COTTON

S. Ross
D. Kerns
F. Yang
Department of Entomology
College Station, TX

Abstract

Cotton bollworm, *Helicoverpa zea*, is a major pest of cotton and has developed field evolved resistance to the Cry1 and Cry2 Bt proteins, and there are early warning signs for resistance to Vip3Aa. We conducted three experiments to evaluate the potential of utilizing Spear-T bioinsecticide for managing Bt resistant bollworms. The experiments included diet-based bioassays, leaf-based bioassays, and a field trial. For lepidopterous pests, Spear-T is normally mixed with a foliar Bt which aids in allowing Spear-T access through the insect mid-gut. However, Bt cotton should not necessarily require the addition of a foliar Bt but was uncertain if resistance to Bt proteins will interfere with this relationship. In the diet-based bioassays in the absence of any Bt proteins, Spear-T alone exhibited marginal activity on susceptible, Cry-resistant and Vip-resistant *H. zea,* but when combined with overlays of 10µg/cm^2 of Cry1Ac, toxicity to Cry-resistant *H. zea* was higher. However, the addition of 5µg/cm^2 of Vip3Aa39 did not enhance Spear-T toxicity to Vip-resistant *H. zea*. This suggest that Vip3Aa resistance probably involves binding site resistance which Cry-resistance appears to probably allow binding and some pore formation. The leaf-based bioassays demonstrated that Spear-T does exhibit activity on *H. zea* from plant expressed Bt, but the results are inconclusive due to high mortality within the check. The field efficacy trial demonstrated that applications of Spear-T do offer bollworm control, but additional data is needed since our bollworm population was low.

Introduction

The bollworm, *Helicoverpa zea,* is a major pest of cotton, causing direct damage to squares, flowers, and bolls. Until 2014, cotton expressing Bt protein have been key in controlling bollworm but due to field-evolved resistance to Cry1 and Cry2 Bt proteins control has been inconsistent in dual-gene Bt cotton. Additionally, there are concerns that there are early-warning signs for resistance to Vip3Aa. Currently, insecticides containing the active ingredient chlorantraniliprole, have been widely used for controlling bollworm in Bt cotton, but there is concern that wide-spread reliance on this insecticide may lead to resistance. Thus, there is need to identify alternative control bollworm control measures.

Spear-T, the first peptide-based bioinsecticide and represents a new insecticide mode of action, IRAC group 32. Because it is a new mode of action, the probability of cross resistance to other insecticides is highly unlikely. The Spear-T peptide binds to nAChr resulting in persistent channel opening and nerve depolarization. The peptide may also block two ion channels in the insect nervous system- a voltage gated calcium channel and a calcium-activated potassium channel. To achieve maximum activity, Spear-T require access through the insect mid-gut which can be facilitated by Bt-proteins. The goal is to evaluate the potential of an IPM compatible bioinsecticide for managing bollworms in Bt cotton.

Materials and Methods

Insect Strains

The susceptible (SS) strain was obtained from Benzon and is susceptible to Cry1Ac, Cry2Ab2, Cry1F and Vip3Aa. The Cry-RR strain is maintained at the Entomology Research Laboratory at Texas A&M University and was developed using an F2 screen procedure in 2018. It is resistant to Cry1Ac, Cry1F and Cry2Ab2, but is susceptible to Vip3Aa. Cry-RR strain exhibits a significant level of resistance to Cry2Ab2 corn-leaf powder protein with a resistance ratio of 409.1-fold, compared to the susceptible (SS) strain. The Cry resistance strain showed that it can survival well on TwinLink (Cry1Ab+Cry2Ae) and Bollgard 2 (CryAc+Cry2Ab2) cotton leaf tissues. Studies has confirmed that Cry1 and Cry2 resistance in the Cry-RR strain is autosomal, non-recessive and polygenic.

Vip-RR in maintained at the same locations at the Cry-RR strain. This strain was developed using an F2 screen procedure in 2019. It is resistant to Vip3Aa, but is susceptible to Cry1Ac, Cry1F, and Cry2Ab2. The Vip-RR strain

showed a significant level of resistance to Vip3Aa39 purified protein with a resistance ratio of >588-fold, compared to the susceptible (SS) strain Studies has confirmed that Vip3Aa resistance in the Vip-RR strain is autosomal, recessive, and monogenic.

Experiment 1: Diet-Based Bioassays with Spear-T

The diet-based bioassay treatments included 1) Untreated, 2) Spear-T at 5 µg-ai/ml, 3) Spear-T at 10 µg-ai/ml, 4) Spear-T at 20 µg-ai/ml, 5) Spear-T at 40 µg-ai/ml and 6) Spear-T at 60 µg-ai/ml. All three insect strains, SS, Cry-RR and Vip-RR were evaluated against these dosages. The SS was also evaluated with the above dosages along with overlays of Cry 1Ac at 10 µg/cm^2 and Vip3Aa39 at 5 µg/cm^2. The Cry-RR strain was also evaluated with the above Spear-T dosages along with was used to evaluate efficacy toward the Benzon-SS and the Cry-RR and Vip-RR strains were also evaluated with the above Spear-T dosages along with overlays of Cry1Ac at 10 µg/cm^2 and Vip3Aa-39 at 5 µg/cm^2, respectively.

Bioassay are carried out by using trays that consisted of a total of 128-well within a bioassay tray. A single treatment was assigned to one 16-well section of the tray. For each of the 16-well section (treatment) 25mL of artificial diet was prepared. To incorporate the Spear-T into the diet, 5 mL of 5x concentrated treatment solution was mixed into 20 mL of liquid artificial diet. The Bt proteins, where overlayed with 40µl Bt protein solution onto the surface of each cell after the diet had cooled and set.

When test wells were dry, each cell was inoculated with one *H. zea* neonate (<24 h) and covered with plastic vented lids. Each combination of insect genotype and Spear-T/Bt protein treatment were replicated four times with 16 neonates per replication. After infestation, the insects were maintained under the conditions of 50% RH, 26 ± 1 ˚C, and a photoperiod of 16h (L):8h (D). Larval instar and mortality were recorded on the 7th day after inoculation.

Data Analysis

Mortality was corrected using Abbott's formula. Bioassay data were analyzed using PROC GLIMIMIX and the SLICEDIFF option ($\alpha = 0.05$) was used to compare treatments with and without the Bt proteins.

Experiment 2: Leaf-Based Bioassays

The susceptible (SS) and Cry-RR strains used were the same as used in experiment 1 with the addition of heterozygotes, Cry-RS (RS) that represent reciprocal cross of the Cry-RR strain with the (SS) Benzon strain. Bollgard 2 (BG2) cotton, DP 1646B2XF (Cry1Ac + Cry2Ab), and a non-Bt (NBT) cotton DP 1822XF were grown in the greenhouse at Texas A&M University until first bloom. Plants were removed from the greenhouse and aligned in single columns for insecticide treatment. Insecticide treatments included: on Bollgard 2 plants 1) Untreated, 2) Spear-T at 1 pt./ac, 3) Spear-T at 2 pt./ac, 4) Spear-T at 4 pt./ac, and 5) Spear-T at 1 pt./ac, and on non-Bt plants 5) Spear-T at 4 pt./ac. For each treatment 4 plants were treated. Treatments were applied with a CO_2 pressurized hand boom TX-6 hollow cone nozzles calibrated to deliver 10 GPA. Treatments were allowed to air dry and were then returned to the greenhouse.

On the same day as the application, two leaves were removed from each treated plant and returned to the laboratory. Two to three leaf pieces (≈3 cm long) were placed into a sterile petri dish (100 ×15mm) lined with moistened Whatman 90 mm (#1) filter paper. For each genotype, five neonates (<24-hr old) were placed on the leaf tissue in each petri dish (5 larvae/dish). Petri dishes were placed in an insect rearing room under the conditions of 26 ± 1 ◦C, 60% RH, and a 16:8 (L:D) h photoperiod. Leaf tissues were replaced every 2 days, while filter paper was wetted daily and replaced as needed. There were four replications for each combination of treatment and insect genotype, and each replication consisted of four petri dishes each with 5 insects (n=4x5=20). Larval survival and instar were recorded on the 7th day after infestation.

Data Analysis

Larval survival was calculated as 100 * number of larvae that were second instar and above / total number of insects assayed. The data were analyzed using two-way ANOVA with insect strain and treatment as the two main factors. Treatment means were separated using Tukey's HSD at $\alpha = 0.05$ level.

Experiment 3: Field Evaluation

TwinLink (Cry1Ab + Cry2Ae), FM 1830 GLT, was grown at Texas A&M Field Laboratory in a randomized complete block design with four replicates. Plots were 4 row wide x 40 ft long. Treatments consisted of five foliar treatments, an untreated check, Spear-T at 1pt/ac, 2pt/ac, 4pt/ac, and Spear-T at 4pt/ac plus Leprotec (Btk) at 0.5pt/ac. All treatments included the non-ionic surfactant Dyne-Amic at 0.125% v/v. Treatments were applied with a CO_2 pressurized hand boom TX-6 hollow cone nozzles calibrated to deliver 10 GPA. Applications were made on 21 July 2021 prior to infestation with Cry-RR *H. zea*.

This experiment was originally going to rely on natural Cry-RR *H. zea* infestation, but due to low *H. zea* pressure, we artificially infested the plots using egg sheets infested with the laboratory Cry-RR strain. Within the middle two rows, two 1.5-meter areas were marked with flagging tape and infested with egg sheets that contained approximately 200 near enclosing Cry-RR eggs, per sheet. The egg sheets were attached to plant terminals by wrapping the sheet around the terminal stem and stapling the ends of the sheets together. After five days fruit damage and larva counts were performed. The infestation was performed on 17 July. The field site received heavy precipitation on 19 July which likely resulted in high natural mortality.

Damage and infestation assessments were conducted on 26 July within the flagged areas of each plot. Within each plot, 25 plant terminals, 100 squares, 50 flowers and 50 bolls were sampled for *H. zea* damage and the presence of small (< 2nd instar) and large *H. zea* larvae. Field-collected data were analyzed using ANOVA and means were separated using an F-protected LSD ($P < 0.05$).

Results and Discussion

Experiment 1: Diet-Based Bioassays

Cry1Ac bioassays. Percent mortality of the SS was 0.00, 25.39, 11.11, 33.33, 26.99 and 50.79% for the untreated and Spear-T at 5, 10, 20, 40 and 60 µg-ai/ml, respectively (Figure 1a). The addition of the Cry1Ac overlay at 10 µg/cm^2 resulted in 100% mortality for all treatments. The mortality for the Cry-RR strain was 0.00, 8.33, 0.00, 21.67, 26.67 and 40.00 for the untreated and Spear-T at 5, 10, 20, 40 and 60 µg-ai/ml, respectively (**Figure 1**). These values are similar to the SS strain. The addition of the Cry1Ac overlay at 10 µg/cm^2 resulted in 0.00 6.67, 21.67, 51.67, 75.00 and 88.33% mortality, for the untreated and Spear-T at 5, 10, 20, 40 and 60 µg-ai/ml, respectively (Figure 1b). Cry1Ac alone exhibited no mortality to the Cry-RR strain, but the addition of Spear-T at 10, 20, 40 or 60 µg-ai/ml resulted in significantly greater mortality over the Spear-T alone (**Figure 2**). These data suggest that despite the Cry-RR strain being resistant to Cry1Ac, that there is still some Bt protein binding and pore formation allowing the Spear-T access through the insect mid-gut and thus increasing mortality.

Vip3Aa Bioassays

Percent mortality of the SS was 0.00, 25.39, 11.11, 33.33, 26.99 and 50.79% for the untreated and Spear-T at 5, 10, 20, 40 and 60 µg-ai/ml, respectively (**Figure 3**). The addition of the Vip3Aa39 overlay at 5 µg/cm^2 resulted in 100% mortality for all treatments. The mortality for the Vip-RR strain was 0.00, 0.00, 0.00, 12.00, 26.00 and 34.00 for the untreated and Spear-T at 5, 10, 20, 40 and 60 µg-ai/ml, respectively (**Figure 4**). Similar to the Cr-RR strain, these values are similar to the SS strain. The addition of the Vip3Aa39 overlay at 5 µg/cm^2 resulted in 0.00 0.00, 12.20, 10.20, 6.12 and 46.94% mortality, for the untreated and Spear-T at 5, 10, 20, 40 and 60 µg-ai/ml, respectively. The addition of the Vip3Aa did not have any significant effect on mortality relative to the Spear-T concentrations alone. This is most likely due to the mechanism of resistance to Vip3Aa which is hypothesized to involve target site bind affinity.

Experiment 2: Leaf-Based Bioassays

On the untreated BG2 leaves, mortality was 73, 71, and 74% for the SS, RS and RR, respectively (**Figure 5**). The high mortality, particularly for RS and RR, is indictive of a failed bioassay. The reason for the failure is uncertain but we had difficulty with spider mites and whiteflies infesting the test cotton which may have resulted in the leaves becoming unsuitable for the *H. zea* larvae. There were no differences in mortality among the insect genotype within a treatment, but all of the insecticide treatments had significantly higher mortality than the untreated. This test will be repeated in January or February 2022 for more accurate data.

Experiment 3: Field Evaluation

Fruit damage and the number of larvae detected were low; probably due to high natural mortality associated with rainfall the day after infestation (**Figure 6**). Despite low damage, all of the insecticide treatment had significantly less damaged fruit than the untreated, but none of the insecticide treatments differed from each other. There were no detectable differences in live larvae encountered (**Figure 7**).

Conclusion

Overall Spear-T by itself has marginal activity on *Helicovorpa zea*. Spear-T shows greater activity in the Cry resistance bollworm gene meaning pore formation was allowed in the midgut. Spear-T has potential to be a compatible bioinsecticide in Bt cotton. More evaluation is necessary to provide accurate data.

Acknowledgements

I would like to thank Drs. David Kerns and Fei Yang for their guidance, and my lab mates, and student workers for helping with data collecting. Also, would like to thank Vestaron and Cotton Incorporated for project funding.

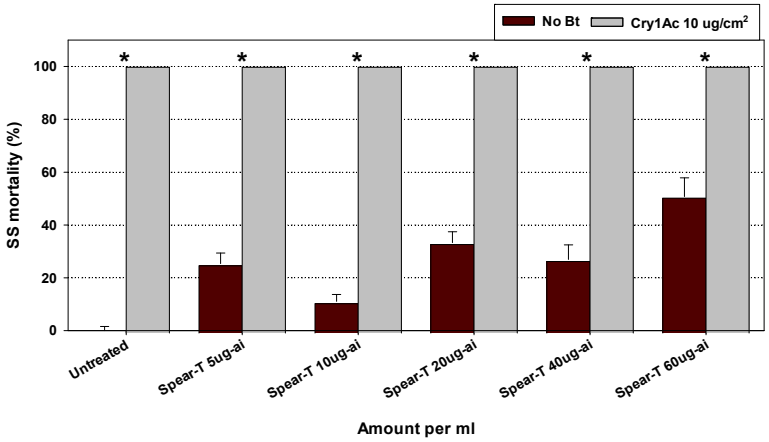

Figure 1. Response of the Benzon-SS strain to an untreated and concentrations of Spear-T with and without an overlay of Cry1Ac Bt protein. * represents a statistically significant response from the addition of the Bt protein.

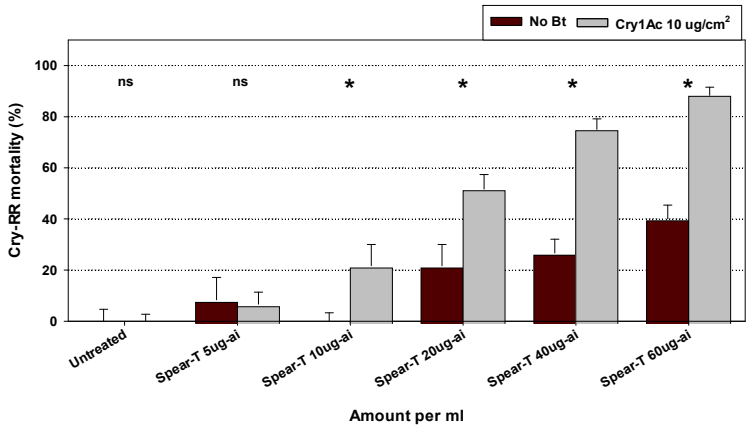

Figure 2. Response of the Cry-RR strain to an untreated and concentrations of Spear-T with and without an overlay of Cry1Ac Bt protein. * represents a statistically significant response from the addition of the Bt protein.

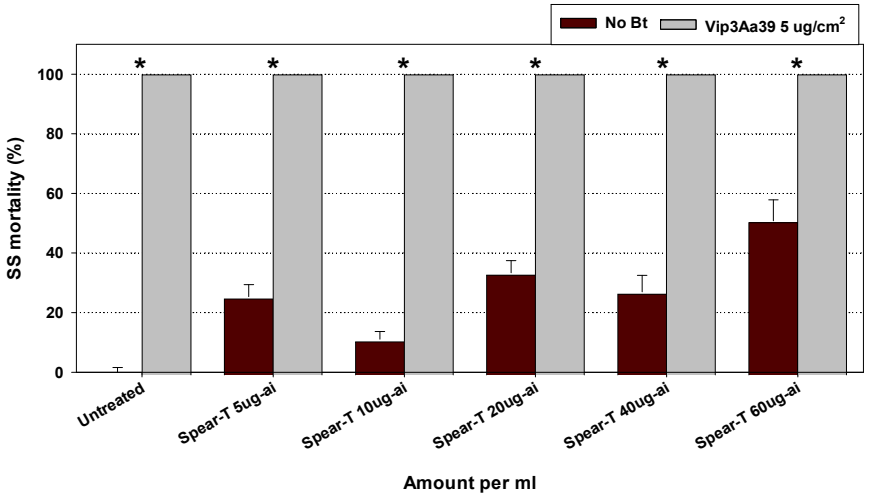

Figure 3. Response of the Benzon-SS strain to an untreated and concentrations of Spear-T with and without an overlay of Vip3Aa39 Bt protein. * represents a statistically significant response from the addition of the Bt protein.

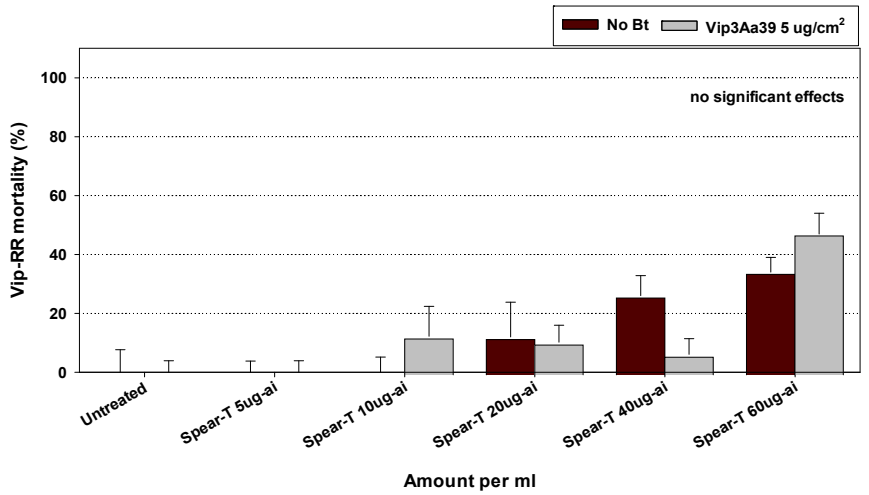

Figure 4. Response of the Vip-RR strain to an untreated and concentrations of Spear-T with and without an overlay of Vip3Aa39 Bt protein.

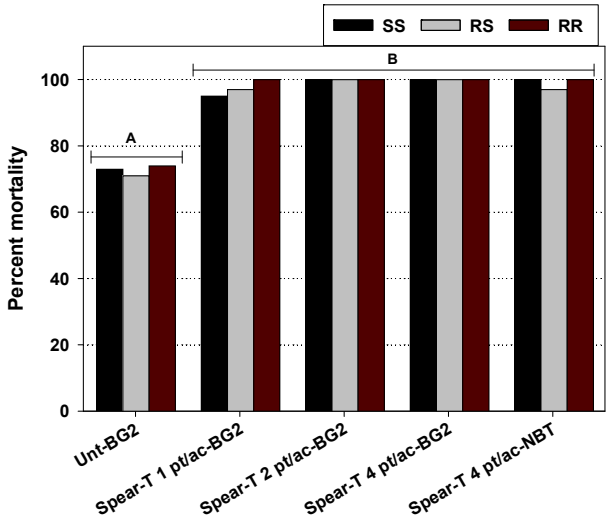

Figure 5. Response of Cry-SS, RS and RR strains of *H. zea* to excised Bt and non-Bt cotton leaves treated with Spear-T 7 days after treatment.

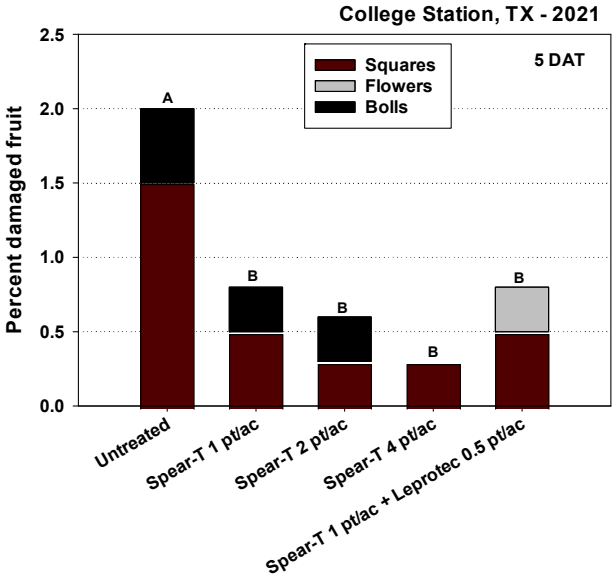

Figure 6. Percent damaged squares, flowers and bolls 5 days post application.

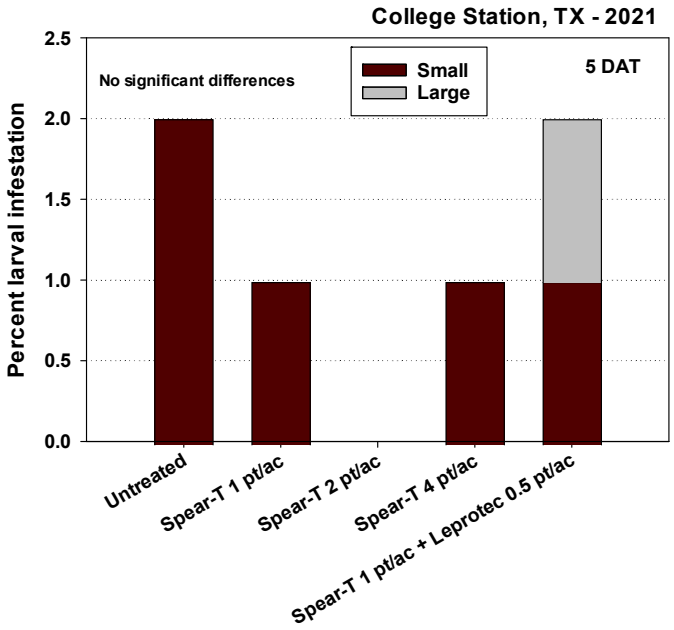

Figure 7. Percent larval infestation 5 days post application.