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CROSS RESISTANCE EVALUATIONS OF CRY1AC TOLERANT STRAINS OF TOBACCO BUDWORM HELIOTHIS VIRESCENS TO THE NOVEL INSECTICIDAL PROTEIN VIP3A

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Abstract

Cotton plant tissue and surface treated diet bioassays were performed to determine the extent of cross resistance to the novel insecticidal protein Vip3A, in tobacco budworm (*Heliothis virescens*) strains that are highly tolerant to the delta endotoxin, Cry1Ac. Tobacco budworm strains YHD2, KCBhyb, and CXC which are tolerant of Cry1Ac, were subjected to cotton plant tissue and surface treated diet assays containing the vegetative insecticidal protein, Vip3A or Cry1Ac. Control material did not contain either of the insecticidal proteins. Surface treated diet evaluations indicate the resistant strain, YHD2, had lower survival and lower average larval weight on Vip3A than the control strain, YDK. However, the KCBhyb strain which has been previously found to have cross-resistance had somewhat lower mortality and average higher weight than YDK on the Vip3A surface treated diet. Plant tissue bioassays based on leaf area consumption and weight data showed the Cry1Ac tolerant budworm strains were not significantly different in mortality from the susceptible insect strain when compared on cotton varieties expressing Vip3A protein. However, there was a significant difference in susceptibility among the three insect strains on plant tissue expressing Cry1Ac. Our preliminary results indicate there is no strong cross resistance of the Cry1Ac resistance budworm strains to Vip3A. Based upon its dissimilar mode of action to Cry1Ac and the data herein Vip3A promises to be an effective tool for insect resistance management.

Introduction

This study was initiated to determine the extent of cross resistance to the novel insecticidal protein Vip3A, in tobacco budworm strains that are highly tolerant of the *Bacillus thuringiensis* endotoxin Cry1Ac. This approach was made through cotton plant leaf tissue and surface treated diet bioassays. We focused on two toxins; Cry1Ac, the active protein found in Bollgard® cotton from Monsanto, and Vip3A the active protein found in the new line of transgenic cotton from Syngenta, VipCot™. Bollgard cotton has been commercially available since 1996 and there is concern over the potential of tobacco budworm to develop insecticide resistance to this transgenic crop (Gould et al. 1995).

Vip3A is the exotoxin produced during the vegetative growth phase of the soil bacterium *Bacillus thuringiensis.* The Vip3A protein has a wide spectrum of activity against major economically important lepidopteran pests (Estruch et al. 1996), Cry1Ac is a δ endotoxin and shares no structural or sequence homology with Vip3A. In addition to structural dissimilarities, Vip3A possess a different mode of action in its formation of a unique pore channel in the insect gut wall and a protein activation site not homologous to that of Cry1 (Shotkoski and Chen 2003). Vip3A is relevant to insect resistance management as a potential tool for delaying insect resistance in heliothines to transgenic Bt crops (Bradley et al. 2004).

Materials and Methods

Background Information of Insect Strains

A total of four budworm strains including one control and three Cry1Ac resistant strains were subjected to cross resistance testing to Vip3A. The three budworm strains tested are from Dr. Fred Gould's laboratory and have varying tolerance levels to Cry1Ac. The YDK control strain was established from a collection of tobacco budworm eggs from three adjacent counties in North Carolina in 1988 (Gould et al. 1995) and served as a susceptible control for all three strains. Recent evaluations of YDK indicate an LC_{50} value of 0.73 µg Cry1Ac/ml. The YHD2 strain is a subset of the susceptible control strain YDK. The YHD2 strain has developed a very high level of resistance to Cry1Ac, LC_{50} >2000 µg/ml. The spectrum of cross resistance in the YHD2 strain is very narrow. The CXC and the KCBhyb strains were collected from the field at the same time as the YHD2 strain but have a different history of selection with Bt toxins (Fuentes et al. 2002, 2003). Both strains have lower levels of resistance to Cry1Ac, LC_{50} values of 211.20 µg/ml and 137 µg/ml respectively, but their spectrum of cross resistance is broader.

Plant Tissue Bioassays

Leaves used in this study were from field grown cotton plants taken from a field test plot located near Jamesville, North Carolina. Plant varieties with insecticidal properties used were Bollgard (Monsanto), Cot 203 and Cot 102 (Syngenta). A Non-Bt cotton variety, Coker 312, was used as a control. The control and the two VipCot varieties were planted on May 20, 2004. Bollgard plants were planted one day prior to the VipCot planting. For this study, the youngest leaves were collected from the terminal region of the cotton plant on August 25, 2004. Upon collection, leaves were sealed in plastic ziplock bags and placed into coolers. They were immediately transported to the lab in Raleigh, NC. Upon arrival leaf disks were punched out of the leaves with a cork borer, diameter 1.5 cm (Area= 1.766 cm^2). Four leaf disks were placed into small clear plastic snug fitting Fisher brand Petri dishes (60 mm diameter, 15 mm height). Approximately ½ ml of distilled water was added to a single circle of filter paper placed inside dishes to help retain moisture. Single newly hatched neonates were placed into each Petri dish with a fine camel hair paint brush. Petri dishes were allowed to incubate at 27°C for 5 days. Mortality was assessed after incubation and all surviving larvae were weighed.

Surface Treated Diet Bioassays

Evaluations were conducted in the lab on September 1, 2004. Approximately 0.2 ml of corn-soy blend insect diet was injected into 2 ml conical bottom plastic vials. The surface area of each was vial was treated with 15 µl of test solution and permitted to dry for one hour. Single newly hatched neonates were transferred to the treated vials with a fine camel hair paintbrush. Four small holes were made into cup lids 24 hours after set up to ensure proper gas exchange. Each concentration was evaluated with 50 individuals and the replicate was incubated under conditions of 27±2°C light:dark photoperiod of 14:10 hours. Overall mortality was assessed six days after treatment and a subset of 30 individuals were weighed at this time. Test dilutions were generated from a lyophilized Vip3A powder reconstituted in 200mM ammonium carbonate buffer pH 9.5. Larval weight data were converted to log weight and analyzed with SAS two-way ANOVA with strain and concentration as fixed variables. The Cry1Ac tolerant tobacco budworm strains, YHD2 and KCBhyb were tested, as well as the susceptible control strain YDK. A two-fold serial dilution, concentration range of 0-400 µg/ml Vip3A, was used for the diet surface treatment study. As a diagnostic control, strains were concurrently tested with a two-fold serial dilution diet surface treated assay incorporating Cry1Ac (MVPII Mycogen) with a concentration range of 0-100 µg/ml Cry1Ac.

Results

Surface Treated Diet Bioassays

Percent mortalities given for Cry1Ac and Vip3A (Figures 1 and 2, respectively) show YDK to be highly susceptible to Cry1Ac with YHD2 being the least affected. Comparing the mean larval log weights for Vip3A diet bioassay (Figure 3), KCBhyb generally had the highest weights, and YDK had higher weights than YHD2. There was not a significant difference among the three strains for concentrations 50 and 100 µg/ml. Only the KCBhyb and YDK strains had survivors at the higher concentrations 200 and 400 µg/ml, with an observed difference in log weights between KCBhyb and YDK at the 400 µg/ml Vip3A concentration.

Plant Tissue Bioassays

In the cotton leaf tissue assays (Figure 4), the CXC, KCBhyb, and YDK strains did not exceed 50% mortality on the Cot 102 or Cot 203 varieties. The YDK strain was very susceptible to the active protein in Bollgard cotton. With regard to the average larval weight of the three strains (Figure 5), YDK and CXC were larger than KCBhyb for the control Non-Bt. However, the KCBhyb strain had the highest mean weight values for the Bollgard and Cot 203 varieties. The three strains did not differ significantly from each other on Cot 102. The leaf consumption (Figure 6) of the susceptible strain YDK on Bollgard cotton leaf was lower than the two Cry1Ac tolerant strains CXC and KCBhyb. YDK consumption was comparable or significantly greater than the two resistant strains for both the Cot 102 and Cot 203 varieties.

Discussion

The evidence gathered from this study does not substantiate a definitive argument for strong cross resistance to the novel insecticidal protein Vip3A for strains of tobacco budworm that are highly tolerant to the δ endotoxin Cry1Ac. In the surface treated diet bioassays for Vip3A we observed definite differences both in mortality and in larval log weight between the highly susceptible strain YDK and the highly Cry1Ac tolerant strain YHD2. Although YHD2 had lower mortality than YDK for Cry1Ac, it was markedly more susceptible to Vip3A than YDK in terms of

mortality. In contrast, the KCBhyb strain performed better on the Vip3A surface treated diet bioassay than the YDK control strain. This is not too surprising since the KCBhyb strain is cross resistant to Cry2Aa. However, the difference between the YDK and KCBhyb was not large. Furthermore, the evidence we gathered from the plant tissue bioassays support the lack of finding definitive cross resistance in the Cry1Ac tolerant strains. With regards to the consumption, mortality, and weight data for the two VipCot varieties Cot 102 and Cot 203, YDK performed comparably or greater than its two resistant counterparts CXC and KCBhyb. Differences in the promoter for the Cot 102 and Cot 203 varieties (Bradley 2004) may explain variations in strain response amongst the two plant varieties. Due to its dissimilar mode of action and the data presented herein Vip3A promises to be an effective tool in insect resistance management. However, further investigation into the response levels of KCBhyb and its potential for cross resistance at higher levels of Vip3A are warranted.

Acknowledgments

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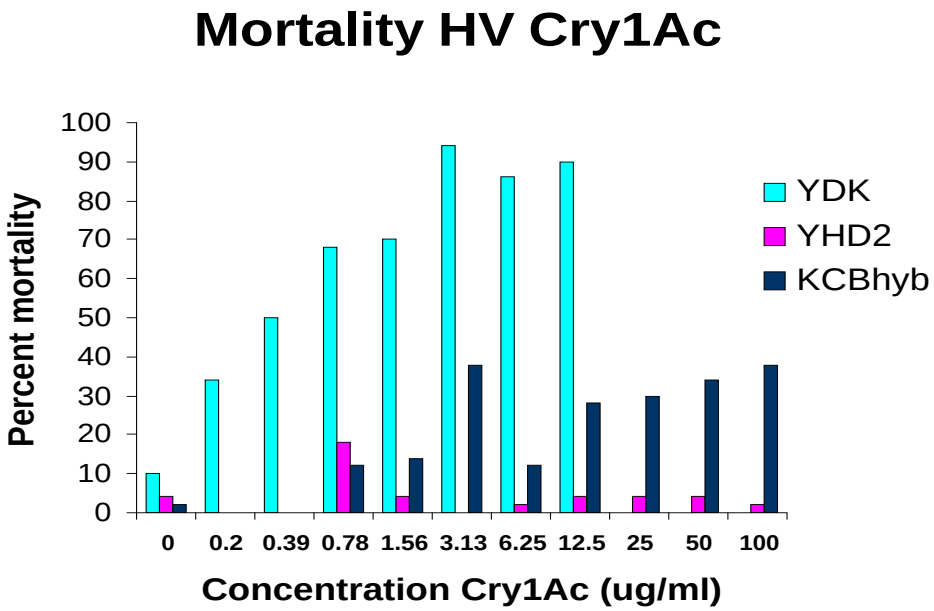

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Figure 1. Mortality of *Heliothis virescens* strains KCBhyb, YHD2, and YDK for Cry1Ac surface treated diet bioassay.

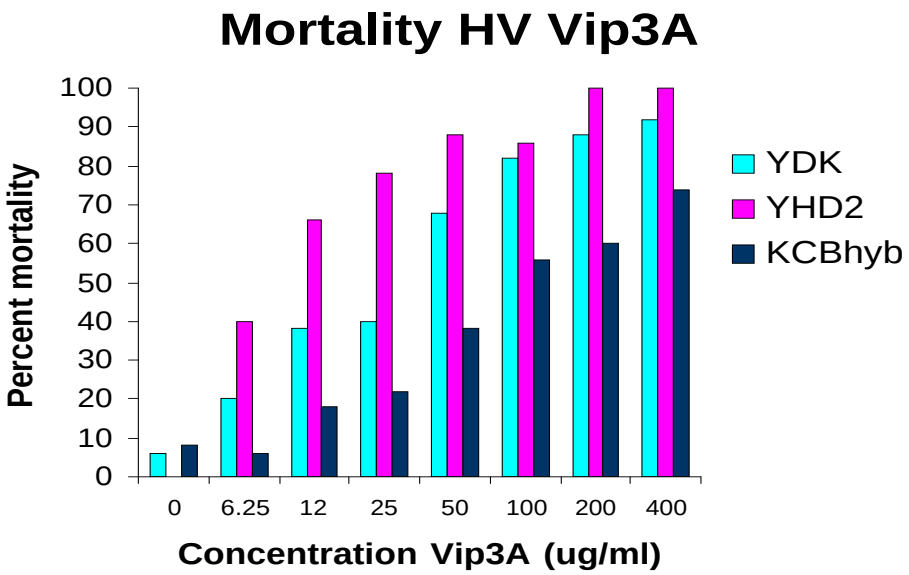

Figure 2. Percent mortality of *Heliothis virescens* strains KCBhyb, YHD2, and YDK for Vip3A surface treated diet bioassay.

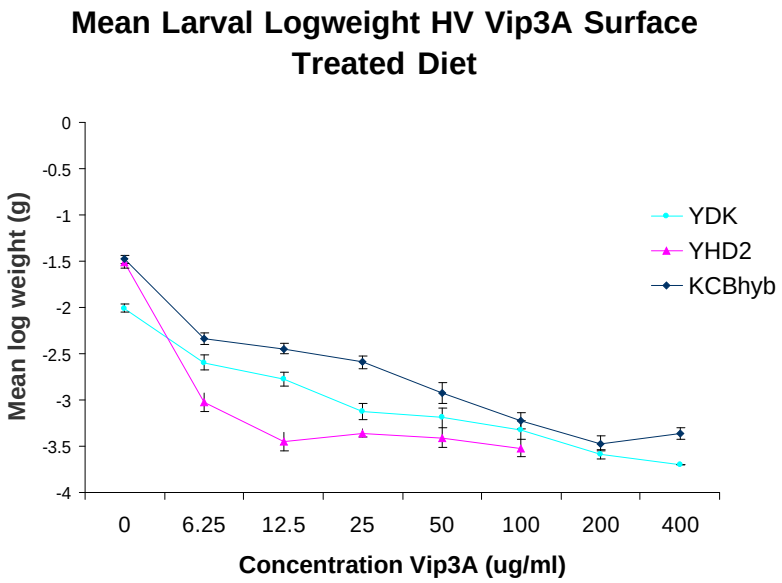

Figure 3. Mean logweight and SEM of *Heliothis virescens* strains KCBhyb, YHD2, and YDK for Vip3A surface treated diet bioassay.

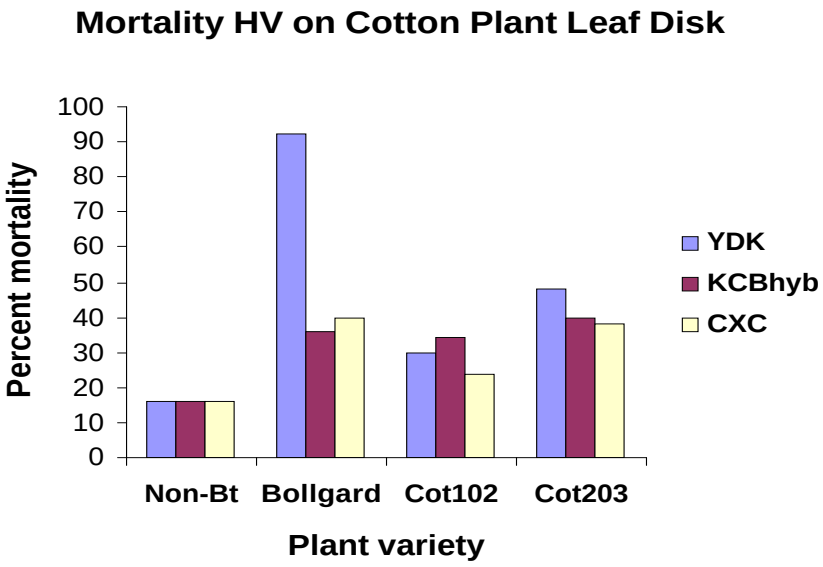

Figure 4. Percent mortality of *Heliothis virescens* strains CXC, KCBhyb, and YDK (5 DAT) for cotton leaf material.

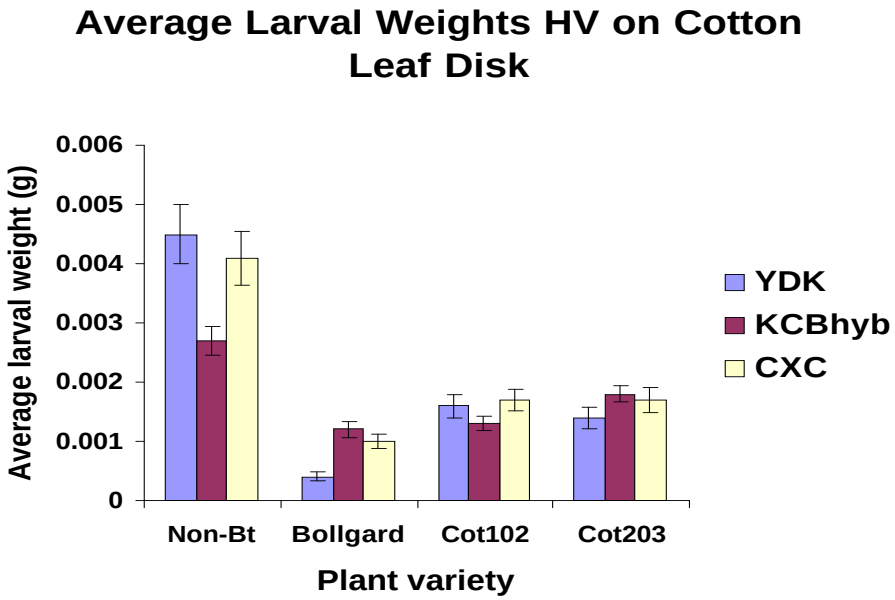

Figure 5. Larval weight averages five days after placement onto leaf material of *Heliothis virescens* strains CXC, KCBhyb, and YDK.

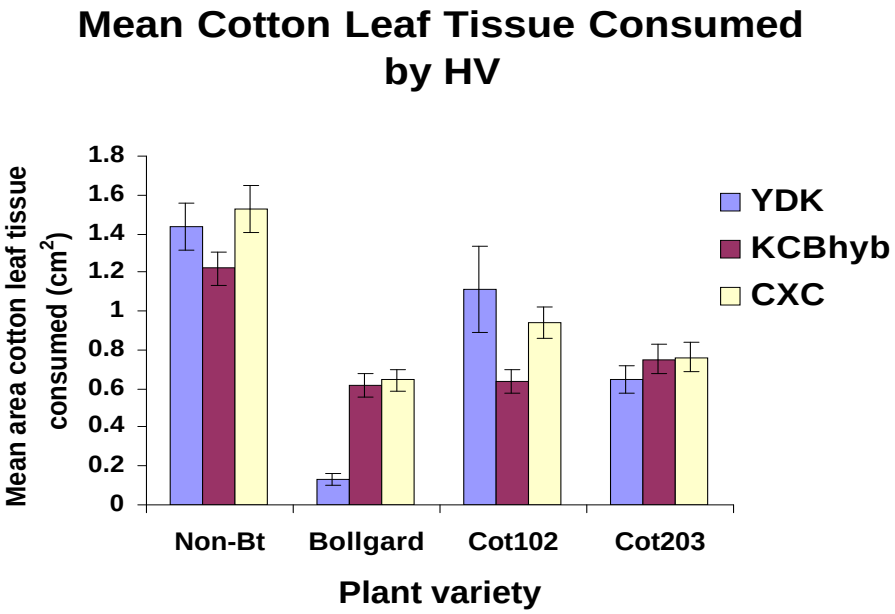

Figure 6. Approximate mean area of leaf tissue (cm^2) consumed by *Heliothis virescens* strains CXC, KCBhyb, and YDK.