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CROSS-RESISTANCE LEVELS BETWEEN THE BACILLUS THURINGIENSIS PROTEINS CRY1AC AND VIP3A IN HELIOTHIS VIRESCENS

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Abstract

Laboratory experiments were conducted with four strains of tobacco budworm, *Heliothis virescens* (F.), to determine the level of cross-resistance between the *Bacillus thuringiensis* toxins cry1Ac and vip3A using concentration-mortality experiments. *H. virescens* strains consisted of three cry1Ac-resistant lines (YHD2, KCBhyb, and CXC) and one susceptible line (YDK). Concentration-mortality data demonstrated that the three Cry1Ac-resistant *H. virescens* strains were at least 52- to 424-fold resistant to cry1Ac compared to the susceptible strain. Concentration-mortality tests with vip3A demonstrated that mortality was similar among all four *H. virescens* strains. Relative larval growth on cry1Ac reflected concentration-mortality test results. Relative larval growth on vip3A was variable at lower doses but was more consistent on concentrations above 25 µg/ml. These results demonstrate that cross-resistance did not exist between cry1Ac and vip3A in *H. virescens*. Therefore, the introduction of VipCot lines could delay cry1Ac-resistance evolution in *H. virescens*.

Introduction

Bollgard® (Monsanto Company, St. Louis, MO) cotton varieties that produce a single *Bacillus thuringiensis* endotoxin, cry1Ac, revolutionized the cotton industry after their introduction in 1996. More recently, two other transgenic cottons were commercialized under the trade names of Bollgard II® (Monsanto Company, St. Louis, MO) and WideStrike® (Dow AgroSciences, Indianapolis, IN). Bollgard II contains Bt genes that produce the cry1Ac and cry2Ab endotoxins, both of which are active against caterpillar pests (Greenplate et al. 2003). WideStrike also produces two Bt endotoxins, cry1Ac and cry1F, which are also active against lepidopteran pests (Pellow et al. 2002). Both of these technologies have demonstrated improved control of bollworm, *Helicoverpa zea* (Boddie) in field tests (Jackson et al. 2003; Jackson et al. 2005). Thus, superior management tools for caterpillar pests of cotton are now available. However, because both pyramided-gene technologies utilize the cry1Ac endotoxin, resistance evolution in *H. zea* and *H. virescens* remains a concern.

Transgenic cottons that produce another Bt protein, vip3A, have recently received a non-regulated status for planting in the United States. This protein is active in the vegetative phase and the sporulation phase, whereas cry1 and cry2 protein activities are restricted to the sporulation phase (Estruch et al. 1996, Schnepf et al. 1998). Field data suggest that vip3A-producing lines 202 and 203 exhibit levels of *H. zea* control similar to that reported for Bollgard II and WideStrike varieties (Bradley et al. 2004), but *H. virescens* control is less than that observed in cry1Ac-expressing cottons. The vip3A protein not only provides a new control measure for caterpillar pests, but may also serve as an excellent resistance management tool for *H. zea* and *H. virescens* (Lee et al. 2003).

Here we report on the impact of vip3A toxin on mortality and growth of *H. virescens* strains that have different mechanisms and levels of resistance to cry1Ac, as well as other toxins.

Materials and Methods

Insects. Four strains of *H. virescens* were used in these experiments: a control strain, YDK, and three cry1Ac-resistant strains, YHD2, CXC, and KCBhyb. YHD2 is highly resistant to cry1Ac, whereas CXC and KCBhyb have moderate levels of resistance to the toxin.

Toxins and diet. The vip3A toxin was obtained as a lyophilized powder (0.66796 µg ai/µg powder) provided by Syngenta Crop Protection. After the first of three runs, additional lyophilized powder containing the Vip3A toxin (0.892857 µg ai/µg powder) was supplied for use in runs two and three. The toxin-containing powder was reconstituted in 200mM ammonium carbonate buffer (ph 9.5) before use in each bioassay. The cry1Ac toxin was obtained from a commercial Bt formulation, MVP II® (Mycogen Corp., San Diego, CA).

Assessing Cross-Resistance. Artificial diet was prepared as described by Burton (1970), and multiple concentration, serial dilution tests were performed for each toxin. A 2-fold increment serial dilution was used to obtain concentrations of 3.125 µg/ml to 400 µg/ml of vip3A toxin. For cry1Ac, concentrations of 0.32 µg/ml to 1000 µg/ml were obtained from a 5-fold increment serial dilution. 0.2 ml of either toxic or non-toxic artificial diet was placed into each well of a 128-well bioassay tray.

Forty-eight neonates from each *H. virescens* strain were tested on each dose of vip3A-containing diet, whereas, only 32 from each strain were tested per concentration of cry1Ac. Larvae were placed onto diet using a fine camel hair paintbrush. Once neonates were in place, bioassay tray lids were used to cover the wells. Trays of insects were maintained in a rearing room at 27±2°C with a 14:10 (L:D) h photoperiod. Mortality assessments were made, and larval weights were obtained 6 d after neonates were placed on diet.

Statistical Analyses. Concentration-mortality data were analyzed using the probit procedure (SAS Institute 1982) to estimate the LC_{50} and fiducial limits for each strain on each toxin. Because of variability among strains in mean larval weights on the control diet in each experiment, raw weight data from sublethal concentrations of each toxin were used to calculate growth ratios and standard errors of larvae on toxic diet relative to larval performance on non-toxic diet.

Results

Multiple concentration-mortality tests with cry1Ac (Table 1) demonstrated that the three cry1Ac-resistant *H. virescens* strains were at least 52- to 424-fold resistant as compared to the YDK strain. LC_{50}s of the CXC and KCBhyb strains ranged from 144.09 µg/ml to 782.32 µg/ml, whereas those of YDK ranged from 0.85 µg/ml to 2.79 µg/ml. No LC_{50}s or fiducial limits were calculated for the YHD2 strain on cry1Ac-containing diet because none of the doses tested (up to 1000 µg/ml) increased the level of mortality above that of the control.

Multiple concentration-mortality tests with vip3A (Table 1) indicated that cry1Ac-resistant and susceptible *H. virescens* strains were equally susceptible to this toxin. The cry1Ac-resistant strains had LC_{50}s ranging from 159.37 µg/ml to 260.52 µg/ml, while those of the susceptible strain ranged from 162.57 µg/ml to 200.96 µg/ml. Within-run concentration-mortality data revealed that 62 to 207 times more vip3A toxin was required to kill 50% of the YDK strain compared to cry1Ac. However, in most instances, less vip3A toxin was required to reach 50% mortality for the cry1Ac-resistant strains compared to cry1Ac.

Relative larval growth on cry1Ac reflected concentration-mortality test results (Figure 1). Most larval growth ratios for YHD2 remained near one, which indicated no difference from larval growth on the control diet. Growth ratios for KCBhyb and CXC suggested a more moderate level of resistance to cry1Ac than that of YHD2.

Growth ratios of larvae on vip3A were highly variable at the lower concentrations (Figure 2). In two of the runs CXC grew larger on vip3A concentrations up to 25 µg/ml than it did on regular diet. The control strain also grew as well on 25 µg/ml as it did on regular diet in one run. In other runs there was a clear pattern of decreasing growth with increasing toxin concentration. Relative growth rates were most consistent on concentrations of vip3A at 50 µg/ml and higher, where growth rates were much lower than on the control diet. In no cases were the differences in growth rates of the strains as pronounced as seen in the cry1Ac experiments.

Discussion

These results demonstrate that *H. virescens* strains with variable levels of resistance to cry1Ac (0- to >20,000-fold) are equally susceptible to the vip3A toxin. The lack of cross-resistance between cry1Ac and vip3A is likely due to the dissimilarity in amino acid sequences (Estruch et al. 1996). Although lysis of midgut epithelium cells is the primary mode of action of vip3A (Yu et al. 1997), this toxin targets different receptors than cry1A toxins

(Nagamatsu et al. 1999; Dorsch et al. 2002; Lee et al. 2003) and forms distinct ion channels compared with the cry1Ab toxin (Lee et al. 2003).

Two of the *H. virescens* strains tested have over 100-fold resistance to cry2A (Gould et al. 1995; Jurat-Fuentes et al. 2003), but show no cross-resistance to vip3A. As with cry1A toxins, the molecular targets of cry2A differ from those of vip3A, and the ion channels differ with regard to voltage dependence and cation selectivity (English et al. 1994; Lee et al. 2003).

The moderate toxicity of vip3A compared to cry1Ac could be caused by a lower saturation of functional binding sites, a difference in assembly of pores, and/or a difference in flux through pores (Lee et al. 2003). However, because field data have demonstrated that vip3A-producing lines are highly efficacious against bollworm, vip3A may be expressed at higher levels in these plants than is cry1Ac in the cry1Ac-expressing plants.

Results of various laboratory studies have demonstrated that COT202 and COT203 express a high dose of the vip3A toxin against both *H. virescens* and *H. zea* (O'Reilly et al. 2005). Assuming that inheritance of resistance to vip3A is recessive, the frequency of resistance alleles should not increase within the next 20 years (McCaffery et al. 2005). These findings, along with those presented here, suggest that the introduction of vip3A-expressing cotton lines to a significant proportion of the area planted to cotton should delay cry1Ac resistance evolution in heliothines, thus increasing the sustainability of commercially available Bt technologies.

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Table 1. LC_{50} PROBIT values for cry1Ac-resistant and susceptible TBW strains on artificial diet containing cry1Ac or vip3A.

		Run 1		Run 2		Run 3	
TBW Strain	**Toxin**	**LC_{50}**	**Fiducial Limits**	**LC_{50}**	**Fiducial Limits**	**LC_{50}**	**Fiducial Limits**
YDK	cry1Ac	0.97	0.52 – 1.73	2.79	0.78 – 8.89	0.85	0.60 – 1.18
YHD2	cry1Ac	NC	NC	NC	NC	NC	NC
KCBhyb	cry1Ac	410.89	212.06 – 1071.94	144.09	85.04 – 332.08	335.89	NC
CXC	cry1Ac	558.76	NC	208.47	NC	782.32	485.99 – 1771.93
YDK	vip3A	200.96	162.33 – 260.62	173.90	152.78 – 201.49	162.57	139.07 – 192.87
YHD2	vip3A	260.52	231.54 – 296.88	161.33	141.87 – 186.79	214.78	151.34 – 359.52
KCBhyb	vip3A	186.22	163.35 – 217.82	209.44	187.14 – 238.27	159.37	141.21 – 182.87
CXC	vip3A	232.1	182.94 – 322.76	185.61	149.24 – 241.11	208.52	163.98 – 290.82

NC, not calculated by SAS (SAS Institute 1982) probit program because of poor fit to log/probit model.

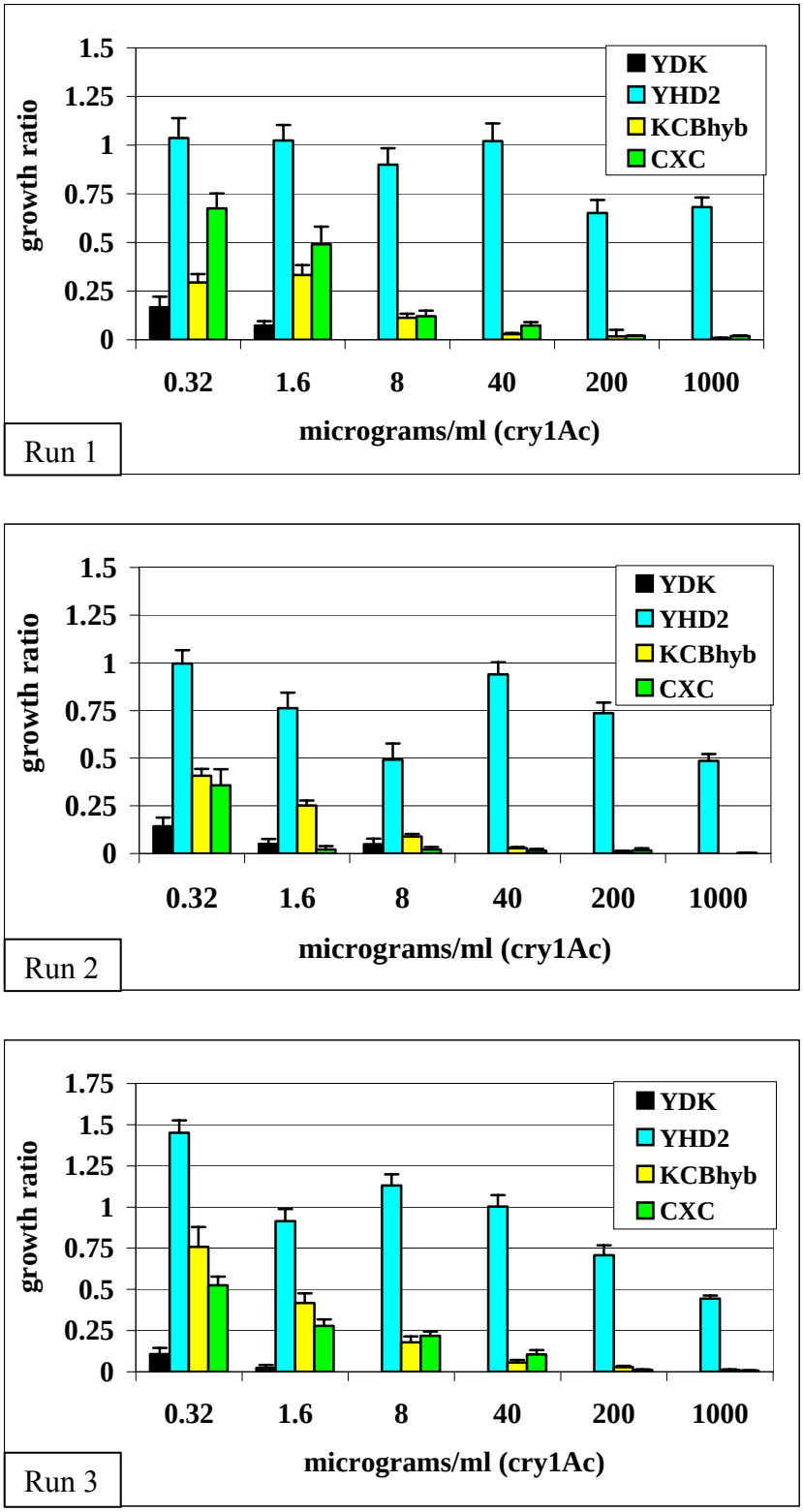

Figure 1. Comparative growth ratios of cry1Ac-resistant and susceptible *Heliothis virescens* strains on cry1Ac serial dilutions within each of three runs.

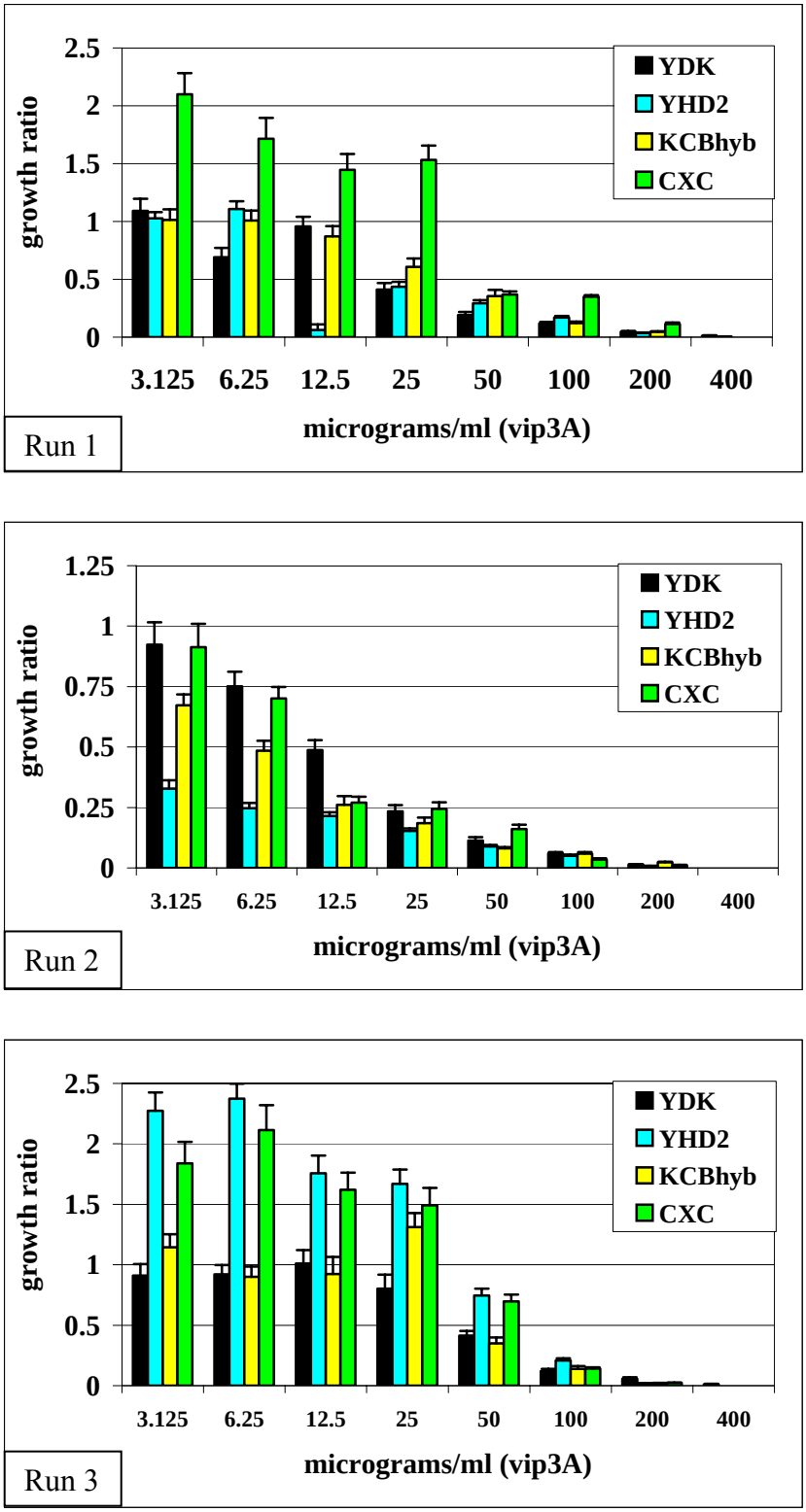

Figure 2. Comparative growth ratios of cry1Ac-resistant and susceptible *Heliothis virescens* strains on vip3A serial dilutions within each of three runs.