TRANSCRIPTIONAL OVEREXPRESSION OF CYTOCHROME P450 GENE(S) *CYP6B8/CYP6B28*, IS ASSOCIATED WITH CYPERMETHRIN SURVIVORSHIP OF FIELD-COLLECTED BOLLWORM MALES (*HELICOVERPA ZEA*)

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Abstract

The bollworm, *Helicoverpa zea*, currently dominates the heliothine complex in the cotton belt. The purpose of this study is to elucidate the mechanisms of pyrethroid resistance present in *H. zea* moths. cDNA synthesis and reverse transcription-polymerase change reaction (RT-PCR) tests were conducted using resistant and susceptible males. Results indicated that the expression of both *CYP* transcripts was significantly higher in *H. zea* males surviving high dosages (30 and 60 µg/vial) of cypermethrin in comparison to the susceptible males. The results of this study will greatly improve the ability to monitor resistance and make better control recommendations.

Introduction

The bollworm, *Helicoverpa zea*, is a major agricultural pest especially in cotton, corn and grain sorghum, and currently dominates the heliothine complex in the cotton belt. Pyrethroid applications, widely used and effective against *H. zea*, exert high selection pressure on bollworm populations, making it important to monitor the frequency and level of resistance. The Texas pyrethroid resistance monitoring program for *H. zea* started in 1999 and is still operational today (Pietrantonio et al., 2007). The Adult Vial Test (AVT) with different concentrations of cypermethrin is used to asses the susceptibility of field-collected male moths (Hopkins and Pietrantonio, 2010a). Susceptibility to pyrethroids varies with the geographical location. Some populations such as those from Nueces and Uvalde Counties exhibited the highest levels of resistance in past years, while populations from Swisher and Hockley Counties in the High Plains have remained susceptible to this date. There are multiple reports of resistance to pyrethroid insecticides in *H. zea*; however, metabolic mechanisms responsible for insect survivorship have yet to be elucidated at the molecular level. In previous studies overexpression of several P450 genes (mono-oxygenases; cytochrome P450 enzymes) was found associated with pyrethroid resistance in different insect species. The purpose of this study is to elucidate the mechanisms of pyrethroid resistance present in *H. zea* moths. This is important because oxidative mechanisms could also be effective in degrading other novel insecticides introduced against *H. zea*. We investigated if the transcriptional overexpression of cytochrome P450 genes, *CYP6B8/CYP6B28* and *CYP6B9*, was associated with survivorship to cypermethrin in field collected males, using semi-quantitative reverse transcription PCR (Semi-Q RT-PCR) and qPCR.

Methods

Resistant and susceptible males were frozen for analyses. cDNA was synthesized from all abdominal tissues of males that were scored as resistant in the adult vial test and from the abdomens of susceptible males from the laboratory colony. Reverse transcription-polymerase change reaction (RT-PCR) tests were performed with the individual cDNAs using cytoplasmic actin as the internal control. The relative levels of expression (relative gene expression presented as the ratio of the band intensities of the *CYP6B8/CYP6B28* gene(s) product(s) over the corresponding actin RT-PCR products) were compared between resistant and susceptible insects. Some males that survived the 5 µg/vial cypermethrin dosage in the adult vial test were collected on June 2006 and stored at -80°C and others collected on July 1-8 in 2010; these resistant insects were analyzed in 2010 by L. Castillo (REU-NSF student). Other results were obtained from resistant insects that had survived concentrations of 30- and 60-µg/vial collected from 2003 to 2009 and kept in storage at -80°C (Hopkins et al., 2010). Other methodological details are as in Hopkins et al., 2010. Due to the high similarity in sequence between the genes that encode *CYP6B8* and *CYP6B28*, the primers that amplify the transcript for *CYP6B8* will most likely amplify *CYP6B28,* therefore we cannot distinguish these two PCR amplification products; for this reason we designate them as *CYP6B8/CYP6B28*.

Results and Discussion

We found that the expression of both *CYP* transcripts was significantly higher in *H. zea* males surviving high dosages (30 and 60 µg/vial) of cypermethrin in comparison to the susceptible males. The level of overexpression in these males ranged from a factor of 3.7 to 34.9 for *CYP6B*8/*CYP6B28* and from 5.6 to 39.6 for *CYP6B9* when transcripts were analyzed by qPCR using primers common to the three forms of cytoplasmic actin as the internal standard (Hopkins et al., 2010). In RT-PCR analyses performed with insects collected in 2010, relative levels of expression of *CYP6B8/6B28* with respect to actin analyzed from males surviving 5 µg cypermethrin per vial ranged from 0.89 to 3.25 and for those collected in 2006 ranged from 1.21 to 1.48. Resistant males collected in 2006 also showed CYP6B9 overexpression as seen in agarose electrophoresis but the band ratio was not quantified. In summary, analysis of individual males collected across ecological regions over years in Texas and 2010 in Burleson County showed that overexpression of cytochrome P450 *CYP6B8/CYP6B28* and *CYP6B9* genes is a mechanism associated with survivorship to pyrethroids in *H. zea.* Our discovery will allow for greater focus on increased metabolism studies at the population level. However, we have previously identified mutations in the sodium channel in cypermethrin resistant males (Hopkins and Pietrantonio, 2010b), indicating that at least two mechanisms for pyrethroid resistance, target site insensitivity and oxidative metabolism are present in field collected insects. Understanding mechanisms responsible for resistance will greatly improve the ability to monitor resistance and make better control recommendations.

Acknowledgements

[§] L. Castillo participated as undergraduate student in the NSF-REU program summer 2010. * Dr. Singh is a visiting fellow from the Punjab Agricultural University, India. # B.W. Hopkins current address: Dow AgroSciences, Westerville, Ohio. Funding was from NSF for support of Liliana Castillo for the summer Research Experiences for Undergraduates, EXCITE program in the Department of Entomology, and from IRAC and Texas AgriLife Research to P.V.P.

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