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VARIATION IN SUSCEPTIBILITY OF ARKANSAS POPULATIONS OF HELICOVERPA ZEA TO CYPERMETHRIN

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Abstract

Eight colonies of *Helicoverpa zea* and seven colonies of *Heliothis virescens* were evaluated for susceptibility to cypermethrin in standard topical assays of larvae and vial assays of adults. The colonies were established from field collections made in Arkansas and the southeastern U.S. for susceptibility assays to Bt toxins. LD50s for *H. virescens* exposed to cypermethrin as larvae varied about 10-fold among field colonies at 48 h posttreatment with a colony from North Carolina being the least susceptible. Survival of *H. virescens* adults exposed to 10 ug/vial doses of cypermethrin ranged from 0% for a laboratory susceptible colony to 63% for a colony from Louisiana. Survival of *H. zea* adults at a 5 ug/vial dose of cypermethrin ranged from 21 to 88%. LD50s for cypermethrin were significantly correlated with survival in adult vial tests across the different *H. zea* colonies. Similar relationships between LD50s of larvae and survival of adults in vial tests were not evident across the different *H. virescens* colonies.

Introduction

Resistance to pyrethroid insecticides has been documented for *Heliothis virescnes* since the mid-1980s (Wolfenbarger et al. 1991), and pyrethroids provide little or no economic control of this pest in US cotton (Bagwell et al. 2001). Conversely, *H. zea* has remained susceptible to pyrethroids for almost three decades. Bt cotton provides exceptional control of *H. virescens* with no known survival in field situations. *H. zea* requires routine suppression on Bt cottons with pyrethroid insecticides providing an important and economical compliment to management of this pest on Bt cotton.

At times, populations of *H. zea* have exhibited increased tolerance or reduced susceptibility to the pyrethroids. Several years ago, pyrethroid resistant *H. zea* were identified in South Carolina (Brown et al. 1998). Payne et al. (2001) reported declining mortality of *H. zea* in adult vial tests across the southeastern US, and Pietrantonio et al. (2005) have annually chronicled a trend for pyrethroid resistance in *H. zea* across Texas. The historical Louisiana dataset of pyrethroid resistance shows a significant decline in mortality of *H. zea* in adult vial tests since 1998 (Cook et al. 2003). The concern for evolving pyrethroid resistance in *H. zea* has recently expanded to the sweet corn growing regions of the Atlantic Coast (Payne et al. 2005), and entomologists in the Midwest have initiated studies to understand declining field control of *H. zea* in sweet corn.

We have been assaying field populations of *H. virescens* and *H. zea* for susceptibility to the Cry1Ac and Cry2Ab insecticidal proteins expressed in Bt cotton for several years. During 2005, we supplemented these assays with topical and vial assays of cypermethrin to compare responses to historical data and information being generated in other states. We were also interested in the potential impact of Bt susceptibility on pyrethroid susceptibility.

Materials and Methods

Seven colonies of *H. virescens* were studied from those available for Bt assays: (1) UAVIR, a laboratory susceptible colony maintained at the University of Arkansas for ~20 years, (2) F10705, collected in Tillar, Arkansas from paulownia in August, (3) F11805, collected in Manila, Arkansas from velvetleaf in September, (4) F15305, collected in Fayetteville, Arkansas from garbanzo bean in October, (5) GA, a mixture of five different colonies collected on tobacco in Georgia in May and June, (6) LA, a mixture of three colonies from garbanzo and velvetleaf collections in Louisiana during June and July, and (7) NC, a mixture of two colonies from tobacco in North Carolina during June.

Eight colonies of *H. zea* were obtained from those available for Bt assays: (1) ZA, a laboratory susceptible colony maintained at the University of Arkansas, (2) F10805, collected from corn in Tillar, Arkansas during August, (3) F11005, collected from a light trap in Foreman, Arkansas during August, (4) F12005, collected from BGII cotton in Georgia during September, (5) F12105, collected from BG cotton in Georgia during September and suspected to be

partially resistant to pyrethroids, (6) F7205, collected from a light trap in Tillar, Arkansas during July, (7) FORGBZA collected from garbanzo bean in Foreman, Arkansas during August, and (8) SEPTFORE, collected from copperleaf in Foreman, Arkansas during September.

Topical assays were conducted with 1 ul aliquots of cypermethrin doses applied to the thoracic dorsum of early third instar larvae (~18 mg). A range of six doses and an acetone alone control were administered to three separate replicates containing 10-15 larvae each. Mortality and morbidity were measured at 24 and 48 hr posttreatment. Surviving larvae were held an additional 30 days for observations of mortality in the larval, prepupal and pupal stages.

Pupae were separated into CHECK and TREATED groups. The CHECK group included survivors from the acetone alone controls. The TREATED group included survivors from all doses of cypermethrin administered to the third instar larvae. Subsequent observations were made at emergence with adults from each replicate of each group being exposed to control (acetone alone) and diagnostic doses (5 ug/vial for *H. zea* and 10 ug/vial for H. *virescens*) in cypermethrin coated scintillation vials. Moths were placed individually into treated and control vials (in equal numbers) and observed for mortality 24 h post-exposure to the vials. Moths were considered dead if they could not right themselves and remain in an upright position. Mortality in the treated vials was corrected for mortality in the control vials.

Dose-mortality regressions were obtained by using the PROBIT procedure of SAS. Differences in survivorship of moths exposed to diagnostic doses of cypermethrin treated vials and subsequent observations of insect biology and survival were studied by AOV.

Correlation analysis was used to study preliminary results of a 2002 study comparing adult vial tests of *H. virescens* and *H. zea* across several colonies to LC50 estimates of larvae from the same colonies exposed to Cry1Ac (Ali et al. 2005). Relationships between cypermethrin LD50s at 48 h and cypermethrin LD50s at pupation, cypermethrin LD50s at 48 h and survivorship of adults in adult vial tests, and cypermethrin LD50s and Cry1Ac LC50s were studied across the range of colonies for both species by correlation analysis.

Results and Discussion

Preliminary studies of colony-to-colony variation in cypermethrin, spinosad, and Bt susceptibility showed significant correlation between % survival of *H. zea* moths exposed to spinosad (15 ug/vial) and LC50s of larvae from the same colonies exposed to Cry1Ac in diet incorporation assays (Figure 1). LC50s for Cry1Ac and Cry2Ab were correlated across the different colonies. Survival of moths exposed to cypermethrin was not correlated with survival of moths exposed to spinosad or LC50s for Cry1Ac and Cry2Ab. Similar studies with a limited number of *H. virescens* colonies (n=4) revealed no significant correlations, but interestingly, coefficients were all negative for relationships between cypermethrin and spinsosad and cypermethrin and the Bt toxins (Figure 2). Collectively these preliminary data suggested no relationship between variation in susceptibility to pyrethroids and susceptibility to Bt toxins.

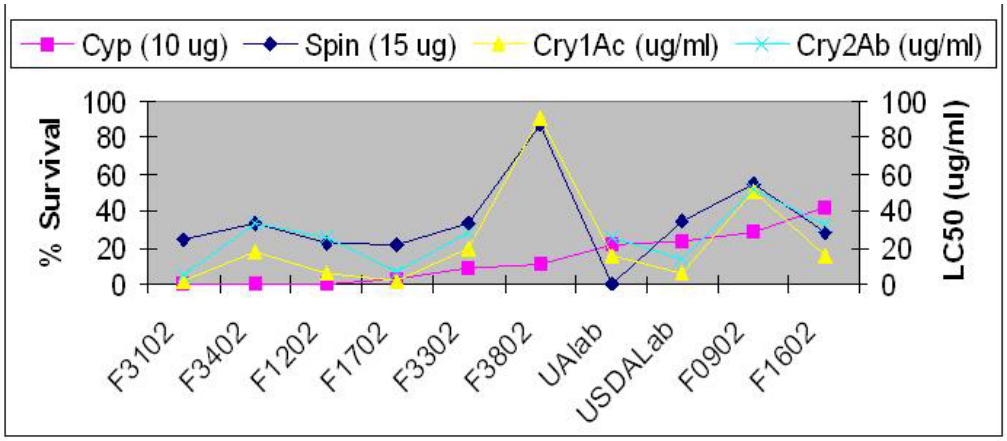

Figure 1. Relationships between mortality of *H. zea* adults in vial tests with cypermethrin

and spinosad and LC50s of larvae exposed to Cry1Ac and Cry2Ab in diet incorporation assays.

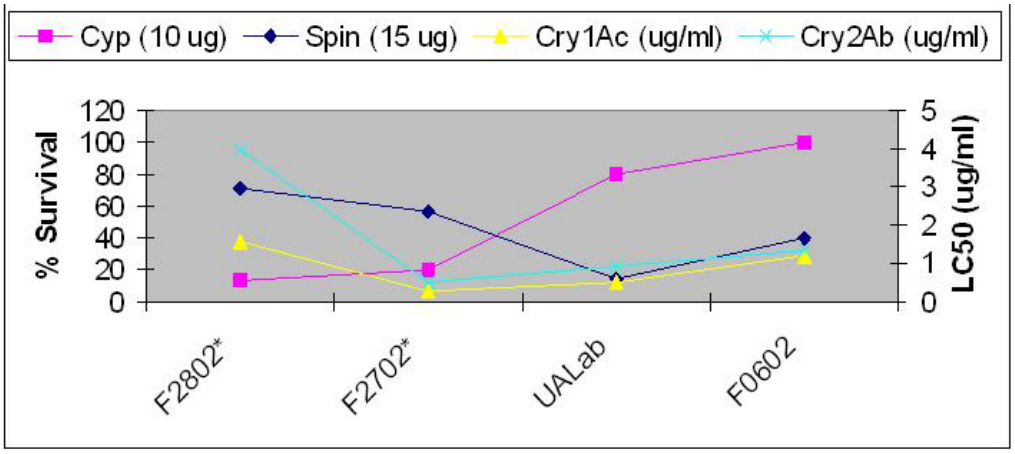

Figure 2. Relationships between mortality of *H. virescnes* adults in vial tests with cypermethrin and spinosad and LC50s of larvae exposed to Cry1Ac and Cry2Ab in diet incorporation assays.

Measurements of cypermethrin susceptibility via standard topical assays of *H. virescens* larvae revealed about a 10-fold difference in LD50s of field strains at 48 h posttreatment (Figure 3). The laboratory strain (UAVIR) was about 65-fold more susceptible than the least susceptible field strain (NC). Variations in LD50s measured at pupation showed a similar trend. LD50s of three field strains from Arkansas (F10705, F15305, and F11805) were not more susceptible than the NC strain. The LA strain was intermediate in susceptibility and the GA strain was more susceptible that the more resistant strains. The GA, LA, and NC strain had been in laboratory culture for two to three more generations than the field strains collected in Arkansas. The low susceptibility of the NC strain was not expected.

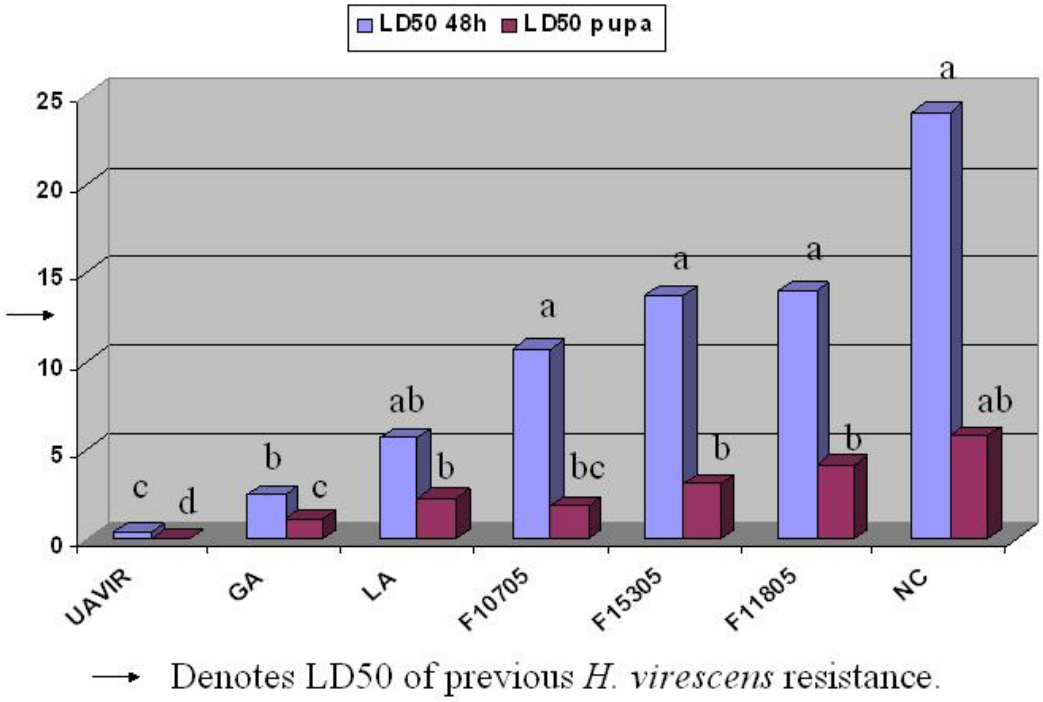

Figure 3. LD50s of *H. virescens* at 48 h posttreatment and at pupation following exposure

of early third instar larvae to cypermethrin in standard topical assays.

LD50s for *H. zea* exposed to cypermethrin in standard topical assays varied about 12-fold across the field colonies (Figure 4). The most susceptible colony was F12005 (collected from BGII cotton in Georgia during September), while the least susceptible colonies were F11005 and F10805 (collected in Arkansas during late summer). The remaining colonies, including the susceptible laboratory stain ZA and F12105 (collected on BG cotton in Georgia, were intermediate in susceptibility between F12005 and the late season Arkansas collections. F12105 was collected from a problem field in Georgia and was suspected to carry pyrethroid, but it was the fourth most susceptible colony of the eight examined in our studies.

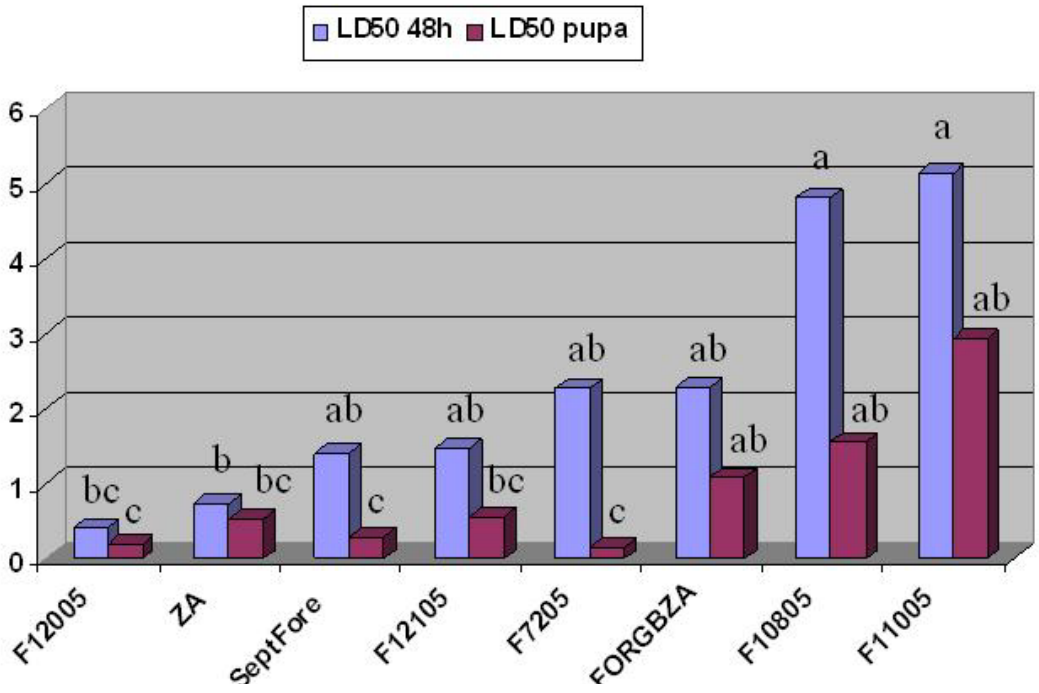

Figure 4. LD50s of *H. zea* at 48 h posttreatment and at pupation following exposure of early third instar larvae to cypermethrin in standard topical assays.

Overall, *H. virescens* was less susceptible than *H. zea*. The average LD50 for *H. virescens* at 48 h posttreatment (10.1 ug/g) was about 5-fold the average LD50 for *H. zea* (2.3 ug/g). At pupation, the LD50 for *H. virescens* (2.6 ug/g) was about that of *H. zea* at 48 h and 3-fold that of *H. zea* at pupation. Luttrell et al. (1991) reported that *H. virescens* colonies with LD50s 14-18 ug/g were resistant to pyrethroids and reduced levels of field control were associated with these levels of resistance. In our study, three of the *H. virescens* colonies had LD50s near these critical levels. None of the *H. zea* colonies had LD50s as high as those reported for *H. virescens* by Luttrell et al. (1991). Two of the *H. zea* colonies had LD50s as high as the LA colony of *H. virescens* (Figure 3).

Survival of *H. virescens* moths exposed to 10 ug/vial doses of cypermethrin (Figure 5) followed slightly different trends across the colonies than those observed with topical assays of larvae (Figure 3). Colony F10705 from Tillar, Arkansas had the greatest survival. Interestingly, the most resistant colony as measured by larval topical assays, NC, had significantly lower adult survival than the field colonies from Arkansas. The colonies from Georgia and Louisiana had statistically similar but numerically greater survival than that of the NC colony. The contrasting results of the larval and adult assays may suggest different pyrethroid resistance mechanisms working in the larval and adult stages. If this is correct, it may have implications for resistance monitoring efforts based solely on one assay method. Significant differences in survival between the check and treated groups were not observed for the individual colonies, but four of the five field strains showed numerically higher LD50s for the treated group as compared to the check group. When the data were lumped and studied across all colonies, average survival of the treated group of *H. virescens* (57.9%) was significantly greater than that of the check group (45.0%).

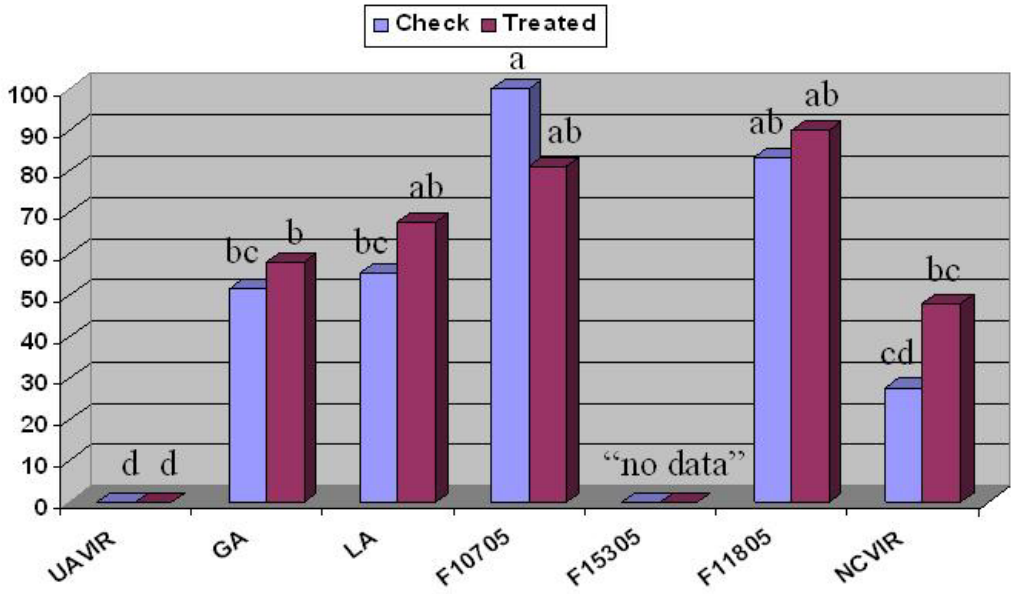

Figure 5. Corrected survival of *H. virescens* adults from check (survivors from untreated controls in larval assays) and treated (survivors of all collective doses of cypermethrin in larval assays) groups exposed to 10 ug/vial dose of cypermethrin in adult vial assays.

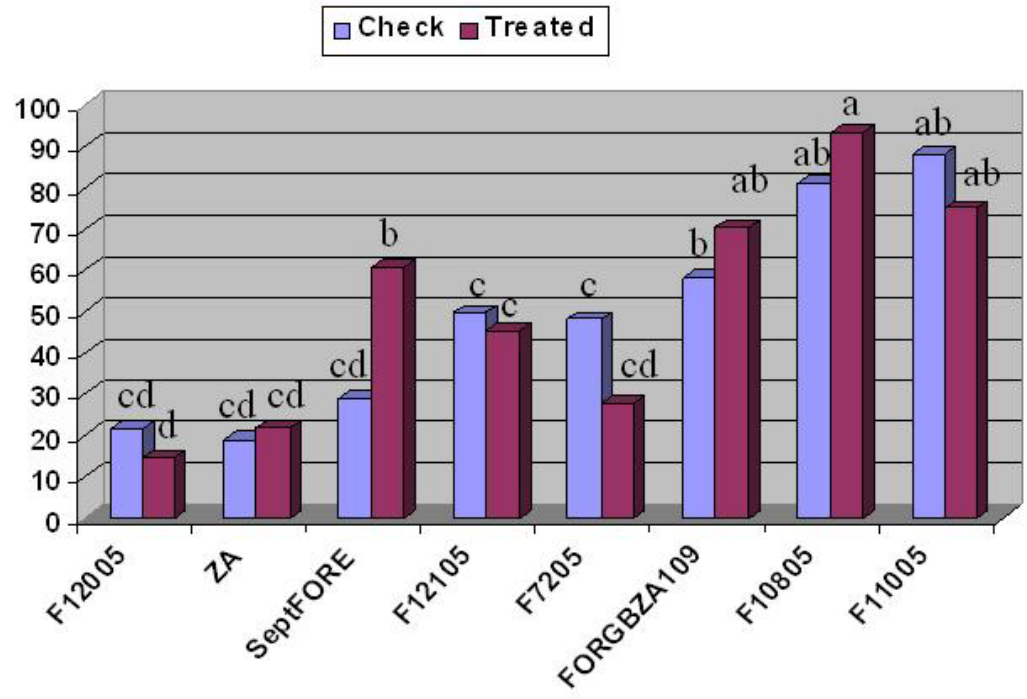

Figure 6. Corrected survival of *H. zea* adults from check (survivors from untreated controls in larval assays) and treated (survivors of all collective doses of cypermethrin in larval assays) groups exposed to 5 ug/vial dose of cypermethrin in adult vial assays.

Survival of *H. zea* moths exposed to the diagnostic dose of 5 ug/vial of cypermethrin (Figure 6) followed a similar trend across colonies as that observed with the larval assays (Figure 4). The least susceptible colonies, F10805 and F11005, were also the least susceptible colonies in the larval assays. The most susceptible colonies, F12005 from

BGII cotton and the laboratory colony ZA, were also the most susceptible in the larval assays. No consistent trend was observed between check and treated groups across the colonies with four of the eight colonies having greater survival in check groups. Average survival of the treated (57.6%) and check (58.8%) were statistical equal.

Observations of the biology and survival of check and treated groups in both species revealed significant reduction in survival of the treated groups at pupation and adult emergence, statistically similar time to adult emergence (although numerically the treated group required slightly more time), and an overall reduction in cumulative survival for the treated group that was exposed to cypermethrin as larvae and adults (Figure 7). Cumulative survival of the check and treated groups was not statistically different for *H. virescens*, but it was significantly reduced in the treated group for *H. zea*.

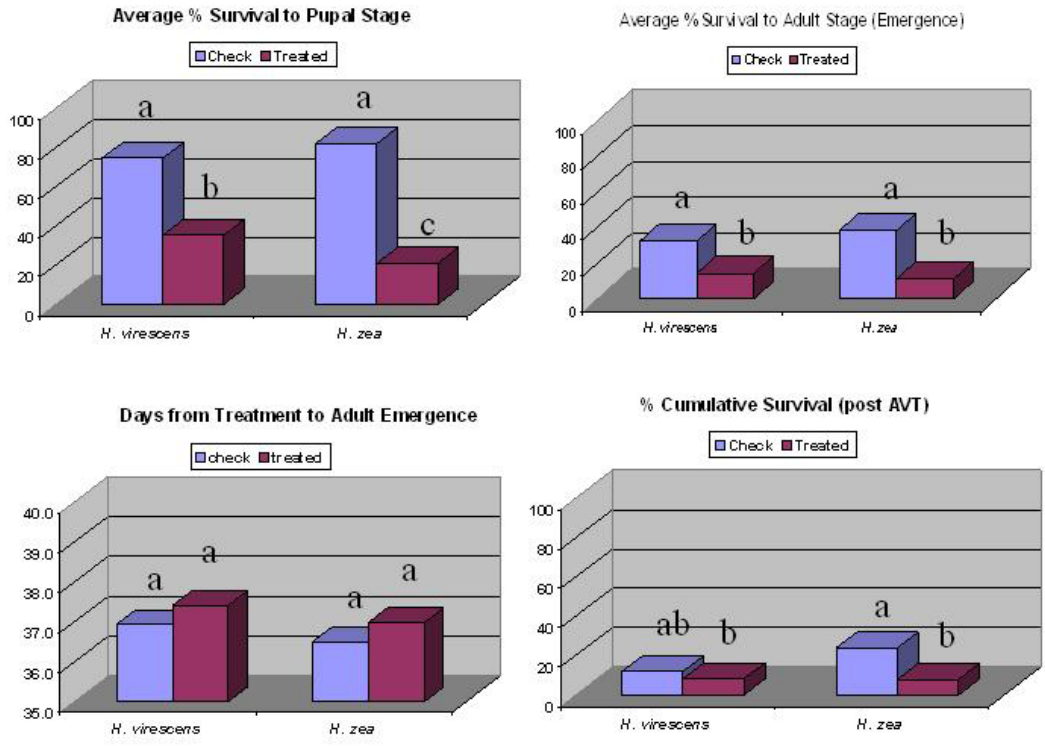

Figure 7. Survival to pupal stage, emergence of adults, days from treatment to adult emergence, and % cumulative survival for check (not exposed as larvae to cypermethrin) and treated (exposed as larvae to cypermethrin) groups of *H. virescens* and *H. zea* larvae.

LD50s for both species at 48 h and at pupation were significantly correlated (Figure 8). Survival of adults exposed to cypermethrin in vial assays was significantly correlated with LD50s for larvae exposed to cypermethrin in topical assays for *H. zea*, but not for *H. virescens* (Figure 9). Variation in the response of both species to cypermethrin was not related to variation in the response to the Bt toxin Cry1Ac.

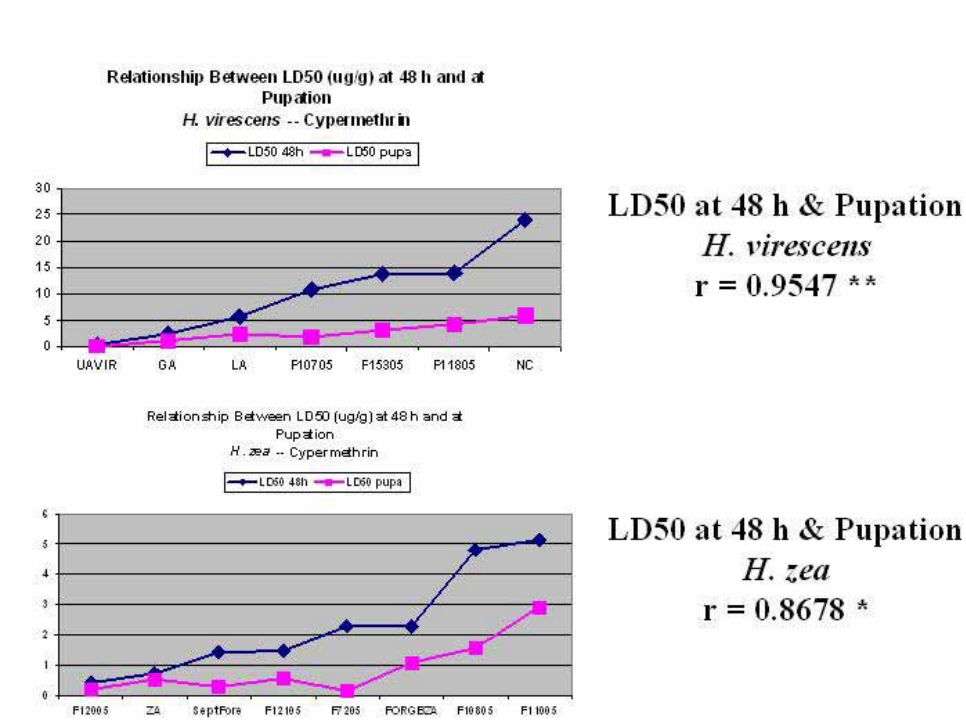

Figure 8. Relationships between LD50s at 48 h and at pupation for different colonies of *H. virescens* and *H. zea* exposed to cypermethrin in standard topical assays.

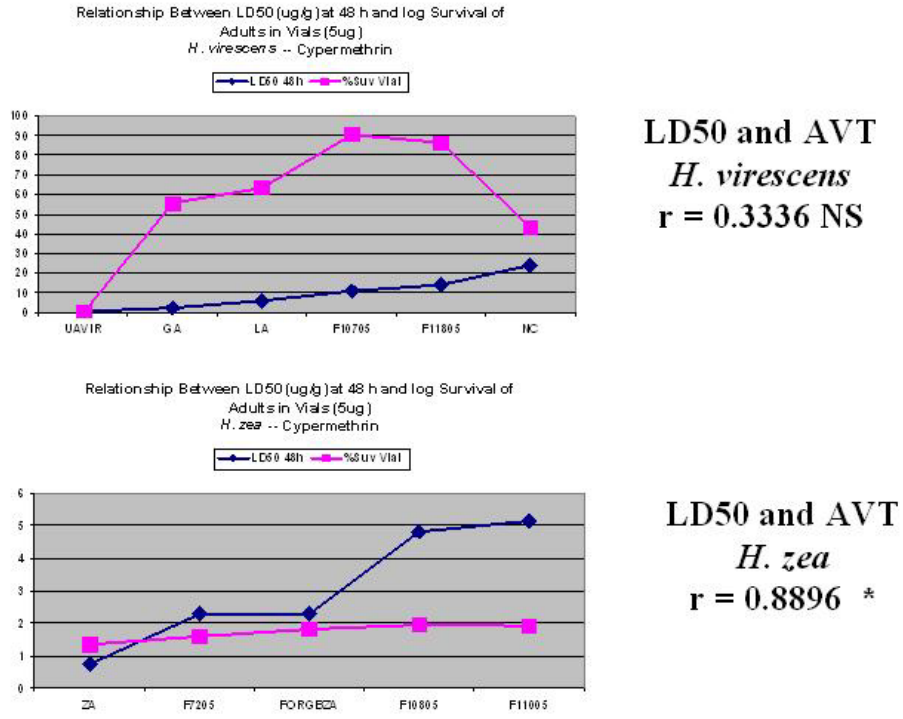

Figure 9. Relationships between LD50s for topical assays of larvae at 48 h and mortality of adults in vial tests with appropriate diagnostic doses for different colonies of *H. virescens* and *H. zea* exposed to cypermethrin.

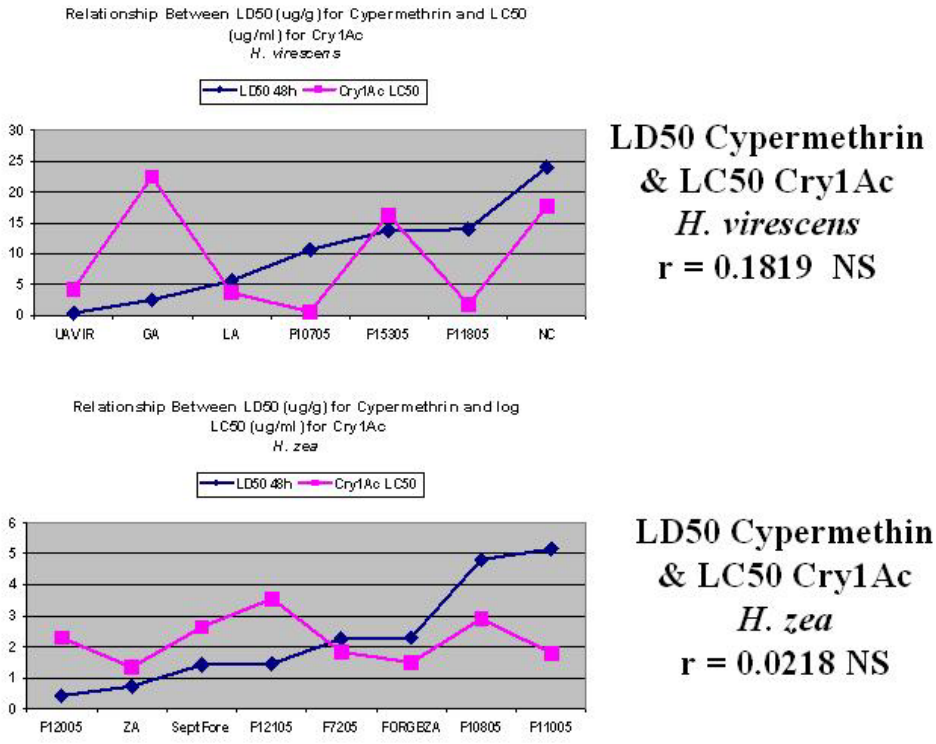

Figure 10. Relationships between LD50s for topical assays of larvae at 48 h of exposure to cypermethrin and LC50s for diet incorporation assays of larvae at 7 d of exposure to Cry1Ac for different colonies of *H. virescens* and *H. zea*.

Collectively these studies illustrate variability in the response of *H. virescens* and *H. zea* to cypermethrin. *H. virescens* is generally less susceptible to cypermethrin than *H. zea*. Susceptibility of larvae appears to be related to susceptibility of adults with *H. zea*, but not with *H. virescens*. This may indicate a number of different resistance mechanisms involved in *H. virescens* resistance to cypermethrin. Vial assays of *H. zea* adults appear to be good indicators of larval susceptibility to cypermethrin in the colonies studied, but they do not appear to be reliable indicators of larval susceptibility with *H. virescens*. Susceptibility to cypermethrin was not related to susceptibility to Bt, but interestingly the *H. zea* colony collected on BGII cotton (F12005) was extremely susceptible to cypermethrin.

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