# DIFFERENTIAL INSECTICIDAL PROPERTIES EXHIBITED AGAINST HELIOTHINE SPECIES BY TWO VIRAL VECTORS ENCODING A SIMILAR CHIMERIC TOXIN GENE

**M. F. Treacy and P. E. Rensner**
**American Cyanamid Agricultural Research Center**
**Princeton, NJ**
**J. N. All**
**University of Georgia**
**Athens, GA**
**A. White**
**American Cyanamid**
**McCarley, MS**

## Abstract

Laboratory, greenhouse and field studies were conducted to characterize the insecticidal properties of genetically altered forms of *Autographa californica* (Speyer) nucleopolyhedrovirus (AcNPV) and *Helicoverpa zea* (Boddie) NPV (HzNPV) against selected heliothine species. The altered viruses each contained a chimeric 0.8-kb fragment encoding the insect-specific, sodium channel neurotoxin from the Algerian scorpion, *Androctonus australis* Hector (AaIT, hence recombinant viruses designated Ac-AaIT and Hz-AaIT). Based on $LD_{50}$ values, results from diet-overlay bioassays showed Ac-AaIT and Hz-AaIT to be equally virulent against larval tobacco budworm, *Heliothis virescens* (F.), but HzNPV and Hz-AaIT averaged ~ 730-fold greater bioactivity than Ac-AaIT against larval cotton bollworm, *Helicoverpa zea* (Boddie). Hz-AaIT killed larvae of both heliothine species at rates significantly faster than those imparted by HzNPV (viral $LT_{50}$ values averaged 2.4 and 4.2 d, respectively). In greenhouse studies, foliar sprays of Ac-AaIT and Hz-AaIT were equally effective in controlling *H. virescens* on cotton, however, Hz-AaIT provided control of *H. zea* on cotton at a level superior to that of Ac-AaIT. For example, following 3 weekly sessions of foliar application and *H. zea* artificial infestation, cotton treated with Ac-AaIT or Hz-AaIT at 1 x $10^{12}$ OB/ha averaged 2.5 and 16.2 non-damaged flower buds/plant, respectively. Additional greenhouse studies conducted against heliothine species on cotton showed that the quicker killing speed exhibited by Hz-AaIT led to improved plant protection versus HzNPV. Finally, results from one greenhouse and four field trials demonstrated that Hz-AaIT at 5 - 12 x $10^{11}$ OB/ha provided control of the heliothine complex in cotton at levels _ *Bacillus thuringiensis* and only slightly less than that of select macrolide, pyrethroid and carbamate insecticides. Overall, results from these studies indicate that, due to host range differences between the two wild-type viruses, HzNPV is the better vectoring agent (versus AcNPV) for designing recombinant clones as insecticides targeted at the multi-species heliothine complex. Further, these studies suggest that, if appropriately tailored for the pest complex, recombinant NPVs may be very effective, insect-specific approaches to managing pests in many cropping scenarios. Possible Hz-AaIT deployment strategies for control of heliothine species on conventional and transgenic cotton varieties are discussed.

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## Introduction

For more than 50 years, insect nucleopolyhedroviruses (NPVs) have been considered somewhat useful as microbial agents for managing certain lepidopteran pest species in forest and row-crop systems. NPVs exhibit a high degree of host specificity and can therefor provide a means of controlling pest species without causing direct adverse effects on vertebrate species or beneficial predatory and parasitic arthropods (Ignoffo 1973, Groner 1986). However, the slow speed of action exhibited by NPVs, sometimes taking a week or longer to kill infected pests, has been a deterrent to more widespread commercialization and deployment of these microbial insecticides. Two approaches to molecular design have been recently utilized to hasten the killing speed of selected NPVs, while maintaining their host specificity; (a) deletion of genes which promote viral replication and prolong host survival and (b) insertion of insect-selective toxin genes into the viral genome, thus making the virus a vectoring agent for carrying and expressing such genes into the insect body cavity.

Deletion of the NPV gene which encodes the enzyme ecdysteroid UDP-glucosyltransferase (EGT) has been shown to cause moderate improvements in insecticidal speed of certain viruses. When expressed in the viral-infected insect, EGT catalyzes conjugation of the molting hormone and its precusors (i.e., the ecdysteroids, 20-hydroxyecdysone, ecdysone and 3-dehydroecdysone) with sugar moieties from UDP-glucose or UDP-galactose (O'Reilly and Miller 1989, Evans and O'Reilly 1998). By inactivating the insect's ecdysteroids, EGT has been shown to cause prolongation or skipping of instars, increased feeding, abnormal growth and increased production of viral progeny in NPV-infected larvae (O'Reilly and Miller 1989, O'Reilly 1995, Shikata et al. 1998).

Laboratory studies have shown that EGT-deleted forms of *Autographa californica* NPV (AcNPV) and *Lymantria dispar* NPV (LdNPV) caused larvae of respective permissive lepidopteran species to consume less food and die ~ 20 - 30% sooner than those larvae infected with wild-type forms of the viruses (O'Reilly and Miller 1991, Burand et al. 1994, Riegel et al. 1994). Further, Treacy et al. (1997a) reported that, when compared to wild-type AcNPV in greenhouse and field trials, EGT-deleted AcNPV provided more consistent control of cabbage looper, *Trichoplusia ni* (Hubner), and tobacco budworm, *Heliothis virescens (F.)*, on cabbage and cotton, respectively.

A number of insect-selective toxin genes have been inserted into the genome of each of several baculoviruses, including AcNPV and *Helicoverpa zea* NPV. For example, genes encoding different invertebrate-selective, sodium channel neurotoxins have been fused into selected viral genomes; such as Lqh_IT2 from the venom of Israeli yellow scorpion, *Leiurus quinquestriatus hebraeus* Birula, AaIT from the venom of the Algerian scorpion, *Androctonus australis* Hector, µ-Aga-IV from the venom of the American funnel web spider, *Agelenopsis aperta* Gertsch, and DTX9.2 from the venom of the weaving spider, *Diguetia canities* (McCutchen et al. 1991, Zlotkin et al. 1991, Prikhod'ko et al. 1996, Hughes et al. 1997). Other neurotoxin genes, for which the pharmacodynamics of the expressed proteins have not yet been defined, and have been inserted into AcNPV, include tox34 from the straw-itch mite, *Pyemotes tritici*, and TalTX-1 from the spider, *Tegenaria agrestis* (Tomalski et al. 1989, Tomalski and Miller 1991, Hughes et al. 1997). In vitro and in vivo laboratory studies have demonstrated that the AaIT and LqhIT2 toxins are highly specific and appear to have no effect on mammals, crustaceans or arachnids (Teitelbaum et al. 1979, Zlotkin 1983, Zlotkin et al. 1991, Possee et al. 1993). Insertion of genes which encode toxins, as those described above, has been shown to cause dramatic improvements in insecticidal speed of certain viruses; causing lepidopteran larvae to die ~ 50% sooner than those larvae infected with wild-type forms of the viruses.

The first open field release of a baculovirus carrying a toxin gene occurred in the U.S. during 1995, and it consisted of evaluation of an AaIT-inserted form of AcNPV (Ac-AaIT) for control of *H. virescens* and the cotton bollworm, *Helicoverpa zea*, on cotton (Treacy and All 1996). In a series of subsequent field studies which were conducted on cotton, cabbage, lettuce and tobacco, it was shown that foliar sprays of Ac-AaIT at dosages ranging 0.5 - 1.2 x $10^{12}$ occlusion bodies (OBs) per hectare provided varying levels of control of *T. ni*, *H. virescens* and beet armyworm, *Spodoptera exigua* (Treacy 1997). Results from cotton trials also showed that Ac-AaIT was significantly more effective against *H. virescens* than against *H. zea* (Treacy and All 1996, All and Treacy 1997).

AcNPV is known to infect approximately 39 species of Lepidoptera (Entwistle and Evans 1985); among the most permissive to infection by this virus are *H. virescens* and *T. ni*, whereas others such as *H. zea* are only moderately sensitive to AcNPV (Vail et al. 1978, Huang et al. 1997). Conversely, HzNPV is considered to be highly virulent against *H. virescens* and *Helicoverpa* spp. (Ignoffo and Garcia 1992). Using AcNPV and HzNPV as expression vector systems for the chimeric AaIT gene, our objective was to quantify insecticidal characteristics of these recombinant clones (Ac-AaIT and Hz-AaIT) against larval *H. zea* and *H. virecsens*. Laboratory and greenhouse assays were conducted to compare Ac-AaIT and Hz-AaIT for virulence against these two heliothine species, and to assess their insecticidal attributes for protecting cotton from damage by these pests. Greenhouse and field studies were also conducted to further characterize efficacy of Hz-AaIT against heliothine species on cotton, relative to insecticidal performance of wild-type HzNPV, as well as selected commercial macrolide, pyrethroid and carbamate insecticides.

## Materials and Methods

### Nucleopolyhedroviruses

Wild-type and genetically altered nucleopolyhedroviruses evaluated in studies described herein were: (a) the V8 strain (Popham et al. 1998) of AcNPV and an isolate of HzNPV, and (b) AcNPV and HzNPV, each with approximately a 1-kb internal deletion in the ecdysteroid UDP-glucosyltransferase gene and an insertion of a 0.8-kb fragment encoding AaIT (i.e., Ac-AaIT and Hz-AaIT). Within each recombinant virus, the same promoter and signal sequence was used to control AaIT gene transcription and facilitate secretion of expressed toxin from virus-infected cells. Each of the wild-type (except Gemstar™) and recombinant viruses used in these studies were produced in vitro using insect cell lines at the Cyanamid Agricultural Research Center (CARC) in Princeton, NJ. With the exception of Gemstar™, the NPVs were formulated at CARC as wettable powders (WP) for use in greenhouse and field studies.

### Laboratory Assays

Insectary-reared *H. zea* and *H. virescens* originated from CARC. OBs of AcNPV, HzNPV, Ac-AaIT and Hz-AaIT were suspended in deionized water and 0.01% SDS. Density of OBs in each viral stock suspension was determined by using a hemacytometer (SPotlite™, model B3178-1, Baxter Healthcare Corp., McGaw Park, IL). For testing purposes, NPVs were diluted to concentrations ranging from 1 x $10^2$ - 1 x $10^8$ OB/ml.

Virulence of each NPV to each lepidopteran species was evaluated by using a diet overlay technique. Plastic bioassay trays (C-D International, Pitman, NJ) were used as test arenas. Each tray contained 32 open-faced wells. Dimensions of each well were 4.0 cm long, 4.0 cm wide, and 2.5 cm high. Five milliliters of a wheat germ-soybean flour-based artificial diet (Southland Products, Lake Village, AR) were poured into each well. After the diet hardened, 0.4 ml of an NPV suspension was pipetted onto the diet surface in each well. Virus solutions were evenly spread over surfaces of diet by picking up the tray and gently tilting it from side to side. Following application and spreading of treatments, trays were held in a vented area for ~ 2 h until water was no longer pooled on diet surfaces. A single 3-d-old *H. zea* larva or 4-d-old *H. virescens* larva was then placed on the surface of diet in each well. After larval infestation, each well was covered with an adhesive, vented, clear plastic sheet (C-D International).

All test arenas were held under constant fluorescent light and a temperature of ~ 27°C for duration of the assay. Larval mortality was rated twice per day over the first 3 - 4 d following treatment application, with a final rating taken at 10 d. At each mortality rating period, a larva was considered to be dead if it exhibited little to no movement, even after being shaken in the diet tray. Additional symptoms of larval death were liquefaction of the body, and in the case of the recombinant NPVs, contraction of the body.

Probit log-dosage and log-time regression analyses were conducted to compare biological activities of the four viruses. Data from each virus-dosage combination were pooled for statistical analyses. Regressions for dosage mortality ($LD_{50}$), as well as the relationship between time since application and mortality (i.e., predicted time for 50% mortality, $LT_{50}$), were estimated for each virus (SAS Institute 1989). Failure of 95% CLs to overlap was used as criterion for significance.

**Greenhouse Studies**

***Hz-AaIT vs. Ac-AaIT***. A study was conducted to assess insecticidal characteristics of the two recombinant baculoviruses in a test system which approximates foliar-spray and plant architecture parameters typically encountered in cotton field scenarios. The test was conducted in a greenhouse located at CARC. Wettable powder formulations of Ac-AaIT at 1 x $10^{12}$ OB/ha and Hz-AaIT at 1 x $10^{12}$ OB/ha were compared for efficacy against larvae of tobacco budworm, *H. virescens*, and cotton bollworm, *H. zea*, on cotton. To determine maximum levels of larval damage to cotton under test conditions described below, non-treated plants were also infested with either pest species. Conversely, non-treated, non-infested cotton was included in the study to establish optimal level of growth and fruit production under parameters of this study. Plants were grown from seed in plastic pots (18.5 x 17.8 cm, diameter x depth) which were filled with commercial potting soil. At the time of initial treatment application, cotton plants were just beginning to produce matchhead size flower buds (i.e., squares). Potted plants were sprayed in a laboratory-housed chamber which was equipped with an overhead, linear traveling hydraulic boom. The boom was fitted with three hollow cone nozzles (TXVS6, Spraying Systems, Wheaton, IL); one nozzle was mounted to apply material over plants and two nozzles were mounted on drop tubes angled at ~ 45° to spray each side of plants. Based on a crop row spacing of 101.6 cm, the sprayer was calibrated to deliver 192.5 liters/ha at 3.2 kg/$cm^2$ and a boom traveling speed of 3.2 km/h. Formulated materials were suspended in deionized water containing the gustatory stimulant, Coax™ (CTC Corp, Carlsbad, CA), at 3.5 liters/ha and the adjuvant, Kinetic™ (Helena Chemical, Memphis, TN), at 0.125% by volume. Plants were sprayed 3 times at 7-d intervals. Plants were arranged in a completely randomized design with 4 replications (2 plants per treatment replicate) on table-tops which were flooded with water to a depth of ~ 5 cm to prevent larval migration between plants. Each potted plant was placed on top of a 25 cm diam. plastic pedestal so that the bottom of each pot was ~ 5 cm above the waterline. Environmental parameters programmed for the greenhouse during the course of this study were an average daily low temperature of 21 - 24 °C, average daily high temperature of 28 - 32 °C, and a light:dark cycle of 14:10 h (lighting augmented with high pressure sodium lamps; SON-AGRO™ 430 watt, Phillips Lighting, Somerset, NJ).

Cotton was infested with laboratory-reared, 1-day-old *H. virescens* or *H. zea* larvae at ~ 2 h after each spray session (i.e., individual plants devoted to either *H. virescens* or *H. zea* infestations for duration of the study). Upon hatching, neonates were held on cotton leaves in plastic petri dishes for one day prior to release on plants in the greenhouse (i.e., this process conditioned larvae for feeding and surviving on cotton plants). With the use of a small paint brush, larvae were placed on or near the terminal of each cotton plant. A total of 2 - 3 larvae were placed on each plant following each of the three spray sessions. Artificial placement of larvae on plants was designed to approximate natural distribution of eggs and small larvae of these pest species on cotton (Farrar and Bradley 1985). Efficacy of treatments applied to cotton was determined 7 (replicates 1 and 2) or 8 days (replicates 3 and 4) after the 3rd application/infestation session (i.e., 20 - 21 days after initiating the study) by recording number of non-damaged squares (_ 1/3-grown) on each plant and measuring height of each plant (i.e, length of mainstem; indicative of degree of larval feeding on meristematic plant tissue).

Significant differences among treatments in injury to cotton by *H. virescens* and *H. zea* were determined by a general linear model procedure (PROC GLM; SAS Institue 1989). Treatment means were separated by Duncan's multiple range test (DMRT; SAS Institute 1989).

***Hz-AaIT vs. wild-type HzNPV.*** Using similar methodology and the same equipment as described above, a second greenhouse assay was conducted at CARC to compare Hz-AaIT WP and a commercial, liquid-concentrate formulation of wild-type HzNPV (Gemstar™ LC, Crop Genetics International, Columbia, MD) for efficacy against the same two heliothine species on cotton. Treatment application and pest infestation sessions were initiated later in the crop's phenology in this study vs. the previous one, i.e., at the 1/3-grown square stage of development.

***Hz-AaIT vs Non-Viral Insecticides***. The test was conducted in the Entomology Pesticide Research Greenhouse at the University of Georgia in Athens, GA. A wettable powder formulation of Hz-AaIT at 5 x $10^{11}$ and 1.2 x $10^{12}$ OB/ha, a commercial formulation of *B. thuringiensis* subsp. *kurstaki* (Dipel™ 2X; CryIA(b) endotoxin, Abbott Laboratories, Chicago, IL) at 1121 g (WP)/ha, spinosad (Tracer™, Dow Agrosciences, Indianapolis, IN) at 76 g (AI)/ha and lambda-cyhalothrin (Karate™, Zeneca Ag Products, Wilmington,

DE) at 34 g (AI)/ha were compared for efficacy against larvae of tobacco budworm and cotton bollworm on cotton (*var*. Delta and Pineland 90). Plants were grown from seed in 3.8-liter plastic pots which were filled with commercial potting soil. Treatment applications were initiated in this study about 5 weeks after planting of cottonseed. Potted plants were sprayed in a laboratory-housed chamber which was equipped with an overhead, rotary hydraulic boom. The boom was fitted with 3 hollow cone nozzles (TX3, Spraying Systems, Wheaton, IL); one nozzle was mounted to apply material over plants and two nozzles were mounted on drop tubes angled at ~ 45° to spray each side of plants. The sprayer was calibrated to deliver 189 liters/ha at 3.5 kg/cm$^2$; compressed air was used as the spray propellant. Formulated materials were suspended in dechlorinated water containing the gustatory stimulant, Coax™ at 3.5 liters/ha and the adjuvant, Kinetic™ at 0.125% by volume. Plants were sprayed 3 times at 7-d intervals. Plants were arranged in a completely randomized design with 5 replications (1 plant per treatment replicate) on table-tops which were flooded with water to a depth of ~ 2 cm to prevent larval migration between plants. Environmental parameters programmed for the greenhouse during the course of this study were an average daily low temperature of 27 °C, and an average daily high temperature of 35 °C.

Cotton was infested with laboratory-reared, neonate *H. virescens* or *H. zea* at ~ 1 h after each spray session (i.e., individual plants devoted to either *H. virescens* or *H. zea* infestations for duration of the study). With the use of a small paint brush, larvae were placed on leaves throughout the upper portion of each cotton plant. A total of 30 freshly hatched larvae were placed on each plant following each of the 3 spray sessions. Efficacy of treatments applied to cotton was determined 9 days after the 3rd application/infestation session (i.e., 23 days after initiating the study) by inspecting aborted and affixed squares of each plant for larval feeding damage. Additionally, degree of larval feeding damage to shoot terminals of each plant was assessed. Significant differences among treatments in injury to cotton by *H. virescens* and *H. zea* were determined by a analysis of variance (ANOVA; SAS Institute 1989). Treatment means were separated by Tukey's studentized range test (SAS Institute 1989).

**Field Trials**

***Mississippi Test Site, 1998.*** A small-plot field experiment was conducted on "Stoneville 474" cotton grown near Itta Bena, MS. Hz-AaIT WP at 5 and 12 x $10^{11}$ OB/ha, *B. thuringiensis* subsp. *kurstaki* (Condor™, Ecogen Inc., Langhorne, PA) at 2.4 formulated liters/ha, spinosad at 76 g (AI)/ha and cyfluthrin (Baythroid™, Bayer Corp., Kansas City, MO) at 37 g (AI)/ha were compared for efficacy against *H. zea*. Formulated materials were suspended in tap water; Coax™ was added to the NPV and Condor™ treatments at 3.5 liters/ha. Treatments were applied to plots (2-row by 12 m) of cotton, which was planted 7 May on 101.6-cm row spacing. Treatments and non-treated control were replicated 4 times in a randomized complete block design. Applications were made on 10, 16 and 20 July with a hand-held $CO_2$-powered spray wand calibrated to deliver 85 liters/ha through 2 hollow-cone nozzles (TX10, Spraying Systems) per row at 3.8 kg/cm$^2$.

At 3 - 5 d intervals beginning on 20 July, shoot terminals and squares were inspected on randomly selected plants in each plot for presence of damage due to feeding by larvae. Significant differences among treatments in percentage of squares and terminals damaged by *H. zea* were determined by GLM. Treatment means were separated by Duncan's multiple range test (SAS Institute, 1989). Percentile data were arcsine-transformed for statistical analysis.

***North Carolina Test Site, 1998.*** A small-plot field experiment was conducted on "Paymaster 1220RR" cotton grown near Jamesville, NC. Hz-AaIT WP at 5 and 12 x $10^{11}$ OB/ha, Dipel™ 2X at 1121 g (WP)/ha, thiodicarb (Larvin™, Rhone-Poulenc Ag Co., Research Triangle Park, NC) at 560 g (AI)/ha and lambda-cyhalothrin at 28 g (AI)/ha were compared for efficacy against *H. zea*. Formulated materials were suspended in tap water; Coax™ was added to the NPV and Dipel™ treatments at 2.4 liters/ha. Treatments were applied to plots (4-row by 7.5 m) of cotton, which was planted 21 May on 90-cm row spacing. Treatments and non-treated control were replicated 4 times in a randomized complete block design. Applications were made on 27, 30 July, 4 and 8 August, 1998, with a hand-held spray wand calibrated to deliver 129.6 liters/ha through 2 hollow-cone nozzles (TX12, Spraying Systems) per row at 4.2 kg/cm$^2$; $CO_2$ was used as the spray propellant.

At 3 - 6 d intervals beginning on 4 August, squares and bolls were inspected on randomly selected plants in each plot for presence of damage due to feeding by larvae. Significant differences among treatments in percentage of fruit forms damaged by *H. zea* were determined by ANOVA. Treatment means were separated by Duncan's multiple range test (SAS Institute, 1989). Percentile data were arcsine-transformed for statistical analysis.

***Georgia Test Site No. 1, 1998.*** A small-plot field experiment was conducted on "Delta Pine 90" cotton grown near Watkinsville, GA. Hz-AaIT WP at 10 x $10^{11}$ OB/ha, *B. thuringiensis* subsp. *kurstaki* (Dipel™2X) at 1121 g (WP)/ha, spinosad at 76 g (AI)/ha and lambda-cyhalothrin at 34 g (AI)/ha were compared for efficacy against a mixed infestation of *H. zea* and *H. virescens*. Formulated materials were suspended in tap water; Coax™ was added to the NPV and Dipel™ treatments at 2.4 liters/ha. Treatments were applied to plots (6-row by 7.5 m) of cotton, which was planted 20 May on 96.5-cm row spacing. Treatments and non-treated control were replicated 4 times in a randomized complete block design. Applications were made on 6 and 12 August with a hand-held $CO_2$-powered spray boom calibrated to deliver 94.6 liters/ha through 2

hollow-cone nozzles (TX3, Spraying Systems) per row at 2.1 kg/cm$^2$.

At 5 - 7 d intervals beginning on 12 August, shoot terminals and squares were inspected on 10 randomly selected plants in each plot for presence of damage due to feeding by larvae. Significant differences among treatments in percentage of squares and terminals damaged by heliothine larvae were determined by ANOVA. Treatment means were separated by Duncan's multiple range test (SAS Institute, 1989). Percentile data were arcsine-transformed for statistical analysis.

***Georgia Test Site No. 2, 1998.*** A small-plot field experiment was conducted on "Delta Pine 90" cotton grown near Midville, GA. Hz-AaIT WP at 5 and 12 x $10^{11}$ OB/ha, *Bacillus thuringiensis* subsp. *kurstaki* (Dipel$^{TM}$2X) at 1121 g (WP)/ha, spinosad at 76 g (AI)/ha and lambda-cyhalothrin at 34 g (AI)/ha were compared for efficacy against *H. zea*. Formulated materials were suspended in tap water; Coax$^{TM}$ was added to the NPV and Dipel$^{TM}$ treatments at 2.4 liters/ha. Treatments were applied to plots (6-row by 7.5 m) of cotton, which was planted 12 May on 96.5-cm row spacing. Treatments and non-treated control were replicated 4 times in a randomized complete block design. Applications were made on 23 and 30 July with a hand-held $CO_2$-powered spray boom calibrated to deliver 94.6 liters/ha through 2 hollow-cone nozzles (TX3, Spraying Systems) per row at 2.1 kg/cm$^2$.

At 7 - 8 d intervals beginning on 27 July, shoot terminals and squares were inspected on 10 randomly selected plants in each plot for presence of damage due to feeding by larvae. Significant differences among treatments in percentage of squares and terminals damaged by bollworm larvae were determined by ANOVA. Treatment means were separated by Tukey's studentized range test (SAS Institute, 1989).

## Results and Discussion

### Laboratory Assays

Based on final larval mortality ratings taken 10 d after treatment application in an artificial diet assay, and resultant $LD_{50}$ values, HzNPV was ~ 8000-fold more virulent against larval *H. zea* than AcNPV (Table 1). Magnitude of difference in potency between the two toxin-expressing recombinants was similar to that found for the respective wild-type viruses. $LD_{50}$ values for Hz-AaIT and Ac-AaIT against *H. zea* were ~ 60 and 70,000 OB/16cm$^2$, respectively. Bollworm infected with Hz-AaIT died sooner than did larvae infected with HzNPV. When 3-d-old *H. zea* were exposed to artificial diet which was surface-contaminated with either virus at a rate of 4000 OB/16cm$^2$ (i.e., dosage required to obtain ~ 90% population mortality at 10 d), LT50 values for HzNPV and Hz-AaIT were 3.8 and 2.0 days, respectively (non-overlapping confidence limits).

The wild-type and recombinat NPVs tested herein were equal in potency against larval *H. virescens*, as $LD_{50}$ values ranged ~ 60 - 570 OB/16cm$^2$ with overlapping confidence intervals, against this pest species (Table 2). Both recombinants killed budworm larvae at a rates significantly faster than the respective wild-type NPVs. Against *H. virescens* larvae, $LT_{50}$ values were 4.7, 5.1, 2.7 and 2.9 d for HzNPV, AcNPV, Hz-AaIT and Ac-AaIT, respectively.

### Greenhouse Studies

***Hz-AaIT vs. Ac-AaIT***. Artificial infestations of *H. virescens* on non-treated cotton in the greenhouse produced extreme damage to plants. The number of non-damaged squares on non-treated cotton by the end of the 3-wk study averaged only 0.5 per plant, and height of these non-treated plants averaged 55.8 cm (Table 3). Conversely, cotton treated with WP formulations of Ac-AaIT and Hz-AaIT, each at 1 x $10^{12}$ OB/ha, averaged 19.6 and 19.9 squares per plant, respectively, at the end of the study. Plants treated with these two recombinant viruses were also significantly taller than untreated plants at the end of the study, with mainstem lengths of 76.9 and 76.8 cm for Ac-AaIT and Hz-AaIT treated cotton, respectively. Cotton infested with *H. virescens* and treated with either rNPV also exhibited plant heights and square production at levels statistically equal to cotton plants which were never infested with larvae during the study, i.e., non-treated, non-infested cotton averaged 22.8 squares per plant and a mainstem height of 81.2 cm.

Compared to control of *H. virescens* described above, foliar sprays of AcNPV-AaIT at 1 x $10^{12}$ OB/ha provided significantly less control of *H. zea* on cotton. This finding was not surprising, since the previously described laboratory assays showed that, based on $LD_{50}$ values, larval *H. virescens* were > 300 times more susceptible to Ac-AaIT than *H. zea*. Cotton infested with *H. zea* and sprayed with Ac-AaIT averaged 2.5 non-damaged squares per plant and a mainstem height of 58.6 cm at the end of the 3-week study. Conversely, Hz-AaIT was as efficacious against *H. zea* as it was against *H. virescens* in this study. As noted in the laboratory assays reported herein, *H. zea* and *H. virescens* were equally permissive to Hz-AaIT. In the greenhouse, cotton which was infested with *H. zea* and sprayed with Hz-AaIT at weekly intervals averaged 16.2 non-damaged squares per plant and a mainstem height of 77.4 cm at the end of the study. Non-treated cotton which was infested on a weekly basis with *H. zea* larvae averaged 1.0 square per plant and a mainstem height of 55.6 cm.

***Hz-AaIT vs. wild-type HzNPV.*** Foliar sprays of Hz-AaIT at 1 x $10^{12}$ OB/ha provided significantly better control of *H. zea* on cotton than HzNPV (Gemstar$^{TM}$LC) (Table 4). Cotton infested with *H. zea* and sprayed with HzNPV-AaIT averaged 16.7 non-damaged squares and bolls per plant and a mainstem height of 79.3 cm at the end of the 3-week study. Conversely, cotton infested with *H. zea* and sprayed with 1 x $10^{12}$ OB/ha of HzNPV at weekly intervals averaged 7.0 non-damaged squares and bolls per plant and

a mainstem height of 67.0 cm at the end of the study. Non-treated cotton which was infested on a weekly basis with *H. zea* larvae yielded an average of only 1.0 square per plant.

Artificial infestations of *H. virescens* also caused severe damage to non-treated cotton plants. The number of non-damaged squares on non-treated cotton by the end of the 3-wk study averaged only 0.8 per plant (Table 4). Conversely, cotton treated with a WP formulation of Hz-AaIT at 1 x $10^{12}$ OB/ha averaged 14.0 squares and bolls per plant. Although not significantly different, there was a numerical trend which indicated that Hz-AaIT exhibited slightly better activity against *H. virescens* than HzNPV, as cotton treated with the recombinant virus yielded approximately two more squares and bolls per plant, and had ~ 2 cm more in mainstem growth, than cotton treated with HzNPV.

In the series of diet-overlay assays described above, it was demonstrated that, at the similar ~ $LD_{90}$ dosage of 4000 OB/16 $cm^2$, the recombinant form of HzNPV killed larvae of both heliothine species at a rate ~ 2 days faster than that imparted by wild-type counterpart. Therefor, it is postulated that, when compared to HzNPV-treated cotton in this greenhouse study, the slightly increased fruit-load (~ 12%) on cotton treated with Hz-AaIT and infested with *H. virescens*, and the significantly increased fruit-load (2.4-fold) on cotton treated with Hz-AaIT and infested with *H. zea*, was likely due to the faster killing speed exhibited against these pests by the recombinant NPV.

***Hz-AaIT vs Non-Viral Insecticides***. Although not significantly different in this greenhouse study, Hz-AaIT WP at 5 x $10^{11}$ OB/ha provided control of artificial *H. zea* infestations on cotton at a level which was generally better than that imparted by Dipel™ 2X at 1121 g WP/ha (Table 5). For example, at the end of the 23-d long, three-application (7-day intervals) study, cotton treated with Hz-AaIT and Dipel™ averaged 0 and 60% feeding damage to shoot terminals, respectively. Further, HzAaIT- and Dipel™-treated cotton averaged 16 and 26% square-damage, respectively. Performance of Hz-AaIT against *H. zea* in this study was also similar to that of spinosad at 76 g ai/ha; the later treatment exhibiting 20 and 14% damage to shoot terminals and squares, respectively. Bollworm-infested cotton which was treated with cyhalothrin at 34 g ai/ha had 20% shoot-damage and 5% square-damage at the end of the study. Non-treated cotton was severely damaged by bollworm in this study, as it sustained 100% feeding damage to shoot-terminals and 45% square-damage. The 1.2 x $10^{12}$ OB/ha dosage of HzAaIT performed against *H zea* at a level similar to that of the lower dosage of this recombinant virus.

HzAaIT and Dipel™ showed similar levels of efficacy against *H. virescens* on cotton in this greenhouse trial. For example, cotton treated with HzAaIT at 5 x $10^{11}$ OB/ha, 1.2 x $10^{12}$ OB/ha and Dipel™ 2X at 1121 g WP/ha averaged 22, 17 and 17% damaged squares, respectively (Table 6). Untreated cotton had 36% of the squares damaged by budworm in this study. Only cotton which was treated with spinosad and cyhalothrin had significantly fewer squares damaged by budworm than non-treated cotton. Spinosad- and cyhalothrin-treated cotton averaged 11 and 2% budworm-damaged squares, respectively.

**Field Trials**

***Mississippi Test Site, 1998.*** Throughout the course of this three-application (4- to 6-d intervals), 17-d long trial, Hz-AaIT at 5 x $10^{11}$ OB/ha provided control of light-to-moderate infestation of *H. zea* at a level equal to that of the commercial *B. thuringiensis* product, Condor™ at 2.4 liters/ha. When plant-terminal damage was averaged over two post-application sample dates, cotton treated with Hz-AaIT at 5 x $10^{11}$ OB/ha and Condor™ had 14 and 16% injury levels, respectively (Table 7). Non-treated cotton averaged 29% damaged terminals, which was significantly greater than the two aforementioned treatments. When damage to squares was averaged across three post-application sample dates, cotton treated with Hz-AaIT at 5 x $10^{11}$ OB/ha and Condor™ had similar means of 4% damaged buds (Table 8). With a three-date mean of 8% damaged squares, non-treated cotton had a significantly greater level of damaged squares than cotton treated with either biological insecticide. Although not significantly different from Hz-AaIT at 5 x $10^{11}$ OB/ha, spinosad and cyfluthrin both had lower seasonal mean levels of damaged squares and plant terminals (~ 2 and 7% for the two parameters, respectively). The higher dosage of Hz-AaIT evaluated in this study (12 x $10^{11}$ OB/ha) showed no performance advantage over that of the 5 x $10^{11}$ OB/ha rate of this recombinant NPV.

***North Carolina Test Site, 1998.*** Based on levels of square and boll protection, Hz-AaIT WP at 5 and 12 x $10^{11}$ OB/ha provided relatively good control of a severe infestation of *H. zea* in this cotton field trial. Cotton sprayed with the two dosages of the recombinant NPV did not significantly differ in percent damaged squares over the course of this study (Table 9). The level of insecticidal efficacy demonstrated by the aforementioned viral treatments was equal to that exhibited by Dipel™ 2X at 1121 g (WP)/ha, and only slightly less than that of thiodicarb at 560 g ai/ha and lambda-cyhalothrin at 28 g ai/ha. For example, when damaged squares and bolls were averaged among the four sample dates (i.e., 3 - 9 days after each of the four spray sessions), cotton treated with Hz-AaIT (combined dosages) and Dipel™ had means of ~ 9 and 8% damaged fruit, respectively. Non-treated cotton averaged ~ 22% damaged squares and bolls over the four sample dates. Further, cotton treated with Hz-AaIT at 12 x $10^{11}$ OB/ha and Dipel™ had significantly less square damage than non-treated cotton on three of the four dates (4, 11 and 17 Aug). Cotton treated with thiodicarb or lambda-cyhalothrin averaged 2% damaged squares over the four post-application sample dates. However, cotton treated with thiodicarb did not significantly differ from cotton treated with Hz-AaIT at 12

x $10^{11}$ OB/ha in levels of damaged squares on any of the post-application assessment dates. Cyhalothrin-treated plants had significantly fewer damaged squares than cotton treated with Hz-AaIT at 12 x $10^{11}$ OB/ha on only one of the four dates (11 Aug).

***Georgia Test Site No. 1, 1998.*** HzAaIT at 10 x $10^{11}$ OB/ha provided relatively good control of a light-to-moderate infestation of *H. zea* and *H. virescens*. For example, when data were averaged across the three post-application sample dates, cotton treated with HzAaIT, DipelJ 2X, spinosad, and cyhalothrin averaged 3, 7, 3, and 6% damaged squares, respectively, vs. 9% damaged squares in non-treated cotton. On one of the sample dates (12 August), HzAaIT-, DipelJ 2X- and spinosad-treated cotton had significantly fewer damaged squares than non-treated cotton (2, 4, 3, and 13% damaged squares, respectively) (Table 10). Treated cotton also incurred less damage to shoot-terminals than non-treated cotton. On 12 August, HzAaIT, DipelJ 2X, spinosad, and cyhalothrin each had significantly fewer damaged terminals than the non-treated control.

***Georgia Test Site No. 2, 1998.*** HzAaIT at 5 x $10^{11}$ OB/ha provided good control of a severe infestation of cotton bollworm in this cotton field trial. For example, when data were averaged for the last two post-application sample dates (3 and 11August), cotton treated with Hz-AaIT at 5 x $10^{11}$ OB/ha, DipelJ 2X, spinosad, and cyhalothrin had 15, 16, 13, and 12% damaged squares, respectively, vs. a two-date mean of 36% damaged squares in non-treated cotton (Table 11). On one of the sample dates (3 August), HzAaIT at 5 x $10^{11}$ OB/ha, spinosad, and cyhalothrin had significantly lower levels of square-damage than non-treated cotton (12, 10, 9, and 36% damaged squares, respectively). Treated cotton also incurred significantly less damage to shoot-terminals than non-treated cotton on 3 and 11 August. When averaged across all three post-application sample dates, cotton treated with HzAaIT at 5 x $10^{11}$ OB/ha, DipelJ 2X, spinosad, and cyhalothrin exhibited 17, 17, 15, and 20% damaged terminals, respectively, vs. a three-date mean of 70% damage in non-treated cotton. The higher dosage of HzAaIT evaluated in this study (12 x $10^{11}$ OB/ha) showed no performance advantage over that of the 5 x $10^{11}$ OB/ha rate of this recombinant NPV.

In conclusion, results from laboratory, greenhouse and field studies described herein suggest that use of nucleopolyhedroviruses which have been engineered to express chimeric toxin genes may be a promising, insect-specific approach to pest management in certain crops. Two characteristics of gene-inserted NPVs which impact their usefulness as an effective insecticide are (a) the host range of the vector [since host range is determined by viral gene products and not those of the introduced gene] and (b) the speed with which they exert pesticidal or pestistatic effects on the infected host. One way to design a recombinant baculovirus with a desirable target profile is to insert the insecticide-expressing gene into a wild-type virus which naturally has high levels of virulence against the pest complex which needs to be controlled. Since, as demonstrated herein, HzNPV is equally virulent against *H. virescens* and *H. zea*, the recombinant Hz-AaIT has potential to be a more effective bioinsecticide than Ac-AaIT for control of the heliothine complex in cotton (i.e., as a vectoring agent, AcNPV is weakly pathogenic to *H. zea* relative to *H. virescens*). Further, when compared to Gemstar™ in a greenhouse study on cotton, it was shown that the quicker heliothine killing speed inherent to Hz-AaIT led to improved plant protection versus that provided by the wild-type counterpart. Finally, results from one greenhouse and four field trials demonstrated that Hz-AaIT at 5 - 12 x $10^{11}$ OB/ha provided control of the heliothine complex in cotton at levels _ *B. thuringiensis* and only slightly less than that of select macrolide, pyrethroid and carbamate insecticides.

Select recombinant NPVs could become excellent candidates for use in IPM programs because they exhibit desired levels of pesticidal activity and, like their wild-type counterparts, are non-pathogenic to beneficial insects and other non-target organisms (Bishop et al. 1995, Heinz et al. 1995, McCutchen et al. 1996, Huang, et al. 1997, Treacy et al. 1997b, M.F.T., non-published data). For example, since Hz-AaIT was demonstrated in tests described herein to be as effective as foliar-applied *B. thuringiensis* in controlling heliothine species, this recombinant NPV could serve as a bioinsecticide alternative to the aforementioned commercial bacterium products. Additionally, since pathology of the viral vector and pharmacology of the expressed toxin are different from that of *B. thuringiensis*, Hz-AaIT could also be utilized in *B. thuringiensis* resistance management programs for both conventional and transgenic cotton production systems. A binary, transgenic host-plant resistance/biocontrol system such as Bollgard™-transformed cotton and foliar deployment of Hz-AaIT could be a highly effective and pest-specific method for control of severe outbreaks of *H. zea*. Also, spray mixtures of toxin-gene expressing NPVs with synthetic insecticides may provide additional means for enhanced control of a broad range of lepidopteran pest species, as Treacy (1997) reported that binary mixtures of cypermethrin or chlorfenapyr with Ac-AaIT exhibited potentiating effects against larval *H. virescens*.

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Table 1. Mortality of 3-d-old larval *H. zea* after exposure to wild-type and recombinant NPVs in laboratory bioassays.

| Treatment | *n* | $LD_{50}$ (95% CL) | $LT_{50}$ in days (95% CL) |
|---|---|---|---|
| HzNPV | 144 | 2.4 | 3.8 |
| | | (0.8-4.8) | (3.1 - 4.7) |
| AcNPV | 509 | 19600.0 | NA |
| | | (11200.0-35200.0) | |
| Hz-AaIT | 144 | 0.6 | 2.0 |
| | | (0.3-1.0) | (1.8-2.5) |
| Ac-AaIT | 511 | 700.0 | NA |
| | | (400.0-1100.0) | |

Three replicates per three concentrations of Hz-based viruses (16 insects per treatment replicate).
Four replicates per four concentrations of Ac-based viruses (30-32 insects per treatment replicate).
$LD_{50}$ = (OB per 16 $cm^2$ diet surface area) x 100.
$LT_{50}$ values estimated at dosage of 4000 OB/16 $cm^2$ (i.e., ~ $LD_{90}$).
NA=not applicable, <50% larval mortality at 4000 OB/16 $cm^2$.

Table 2. Mortality of 4-d-old larval *H. virescens* after exposure to wild-type and recombinant NPVs in laboratory bioassays.

| Treatment | *n* | $LD_{50}$ (95% CL) | $LT_{50}$ in days (95% CL) |
|---|---|---|---|
| HzNPV | 288 | 5.7 | 4.7 |
| | | (4.4-7.3) | (4.6-5.8) |
| AcNPV | 512 | 0.6 | 5.1 |
| | | (0.1-5.1) | (4.7-5.4) |
| Hz-AaIT | 144 | 2.2 | 2.7 |
| | | (0.8-6.5) | (2.4-2.9) |
| Ac-AaIT | 144 | 1.8 | 2.9 |
| | | (0.5-5.1) | (2.2-3.2) |

Three to four replicates per three concentrations of NPVs (16-32 insects per treatment replicate).
$LD_{50}$ = (OB per 16 $cm^2$ diet surface area) x 100.
$LT_{50}$ values estimated at dosage of 4000 OB/16 $cm^2$ (i.e., ~ $LD_{90}$).

Table 3. Greenhouse evaluation of recombinant NPVs for control of *H. zea* and *H. virescens* on cotton, Princeton, NJ, 1998.

| Pest species & treatment | Mean no. non-damaged squares per plant | Mean plant height in cm |
|---|---|---|
| *AH. virescens@* | | |
| Hz-AaIT WP @ 1 x $10^{12}$ OB/ha | 19.9 ab | 76.8 a |
| Ac-AaIT WP @ 1 x $10^{12}$ OB/ha | 19.6 ab | 76.9 a |
| Non-treated/infested | 0.5 c | 55.8 b |
| *AH. zea@* | | |
| Hz-AaIT WP @ 1 x $10^{12}$ OB/ha | 16.2 b | 77.4 a |
| Ac-AaIT WP @ 1 x $10^{12}$ OB/ha | 2.5 c | 58.6 b |
| Non-treated/infested | 1.0 c | 55.6 b |
| Non-treated/non-infested | 22.8 a | 81.2 a |

Within a column, means followed by a common letter are not significantly different as determined by Duncan's multiple range test (P≤0.05).
Treatments were sprayed onto cotton 3 times at 7-d intervals, plants were artificially infested with either *H. virescens* or *H. zea* (1-d-old larvae) approx. 2 h after each treatment application session.
Cotton plant height and numbers of non-damaged squares affixed to each plant were assessed 7-8 d after the final application/infestation session.

Table 4. Greenhouse evaluation of recombinant and wild-type forms of HzNPV for control of *H. zea* and *H. virescens* on cotton, Princeton, NJ, 1998.

| Pest species & treatment | Mean no. non-damaged squares per plant | Mean plant height in cm |
|---|---|---|
| *AH. virescens@* | | |
| Hz-AaIT WP @ 1 x $10^{12}$ OB/ha | 14.0 bc | 76.7 ab |
| HzNPV LC @ 1 x $10^{12}$ OB/ha | 12.3 c | 74.2 abc |
| Non-treated/infested | 0.8 e | 72.2 bcd |
| *AH. zea@* | | |
| Hz-AaIT WP @ 1 x $10^{12}$ OB/ha | 16.7 ab | 79.3 a |
| HzNPV LC @ 1 x $10^{12}$ OB/ha | 7.0 d | 67.0 d |
| Non-treated/infested | 1.0 e | 69.7 cd |
| Non-treated/non-infested | 17.8 a | 71.5 bcd |

Within a column, means followed by a common letter are not significantly different as determined by Duncan's multiple range test (P≤0.05).
Treatments were sprayed onto cotton 3 times at 7-d intervals, plants were artificially infested with either *H. virescens* or *H. zea* (1-d-old larvae) approx. 2 h after each treatment application session.
Cotton plant height and numbers of non-damaged squares affixed to each plant were quantified 8 d after the final application/infestation session.

Table 5. Greenhouse evaluation of Hz-AaIT and selected commercial insecticides for control of *H. zea* on cotton, Athens, GA, 1998.

| Treatment | Mean % larval feeding damage to terminals | Mean % cumulative square damage |
|---|---|---|
| Hz-AaIT WP @ 5 x $10^{11}$ OB/ha | 0 b | 16 bc |
| Hz-AaIT WP @ 1.2 x $10^{12}$ OB/ha | 0 b | 18 bc |
| Dipel™ 2X @ 1121 g (WP)/ha | 60 ab | 26 b |
| Spinosad @ 76 g AI/ha | 20 b | 14 bc |
| Cyhalothrin @ 34 g AI/ha | 20 b | 5 c |
| Non-treated | 100 a | 45 a |

Within a column, means followed by a common letter are not significantly different as determined by Tukey's studentized range test (P≤0.05).
Treatments were sprayed onto cotton 3 times at 7-d intervals, plants were artificially infested with *H. zea* neonates approx. 1 h after each treatment application session.
Cumulative square damage and plant terminal damage were quantified 9 d after the final application/infestation session.

Table 6. Greenhouse evaluation of Hz-AaIT and selected commercial insecticides for control of *H. virescens* on cotton, Athens, GA, 1998.

| Treatment | Mean % larval feeding damage to terminals | Mean % cumulative square damage |
|---|---|---|
| Hz-AaIT WP @ 5 x $10^{11}$ OB/ha | 100 a | 22 ab |
| Hz-AaIT WP @ 1.2 x $10^{12}$ OB/ha | 60 ab | 17 ab |
| Dipel™ 2X @ 1121 g (WP)/ha | 60 ab | 17 ab |
| Spinosad @ 76 g (AI)/ha | 20 b | 11b |
| Cyhalothrin @ 34 g (AI)/ha | 0 b | 2 b |
| Non-treated | 100 a | 36 a |

Within a column, means followed by a common letter are not significantly different as determined by Tukey's studentized range test (P≤0.05).
Treatments were sprayed onto cotton 3 times at 7-d intervals, plants were artificially infested with *H. virescens* neonates approx. 1 h after each treatment application session.
Cumulative square damage and plant terminal damage were quantified 9 d after the final application/infestation session.

Table 7. Field evaluations of Hz-AaIT for control of *H. zea* on cotton near Itta Bena, MS, 1998.

| Treatment | Rate per ha | Mean % terminals damaged | | |
|---|---|---|---|---|
| | | 20 July | 24 July | 2-date |
| Hz-AaIT WP | 5 x $10^{11}$ OB | 16 bc | 12 bc | 14 bc |
| Hz-AaIT WP | 12 x $10^{11}$ OB | 21 ab | 21 ab | 21 ab |
| Condor™ | 2.4 L | 14 bc | 19 ab | 16 b |
| Spinosad | 76 g (AI) | 6 c | 6 c | 6 c |
| Cyfluthrin | 37 g (AI) | 12 bc | 4 c | 8 c |
| Non-treated | --- | 33 a | 25 a | 29 a |

Within a column, means followed by a common letter are not significantly different as determined by Duncan's multiple range test (P≤0.05).
Treatments applied to cotton on 10, 16 and 20 July, 1998.

Table 8. Field evaluations of Hz-AaIT for control of *H. zea* on cotton near Itta Bena, MS, 1998.

| Treatment | Rate per ha | Mean % terminals damaged | | | |
|---|---|---|---|---|---|
| | | 20 July | 24 July | 27 July | 3-date |
| Hz-AaIT WP | 5 x $10^{11}$ OB | 2 a | 2 a | 8 abc | 4 b |
| Hz-AaIT WP | 12 x $10^{11}$ OB | 2 a | 3 a | 11 ab | 5 ab |
| Condor™ | 2.4 L | 2 a | 5 a | 5 bcd | 4 b |
| Spinosad | 76 g (AI) | 1 a | 1 a | 1 d | 1 c |
| Cyfluthrin | 37 g (AI) | 3 a | 2 a | 2 cd | 2 bc |
| Non-treated | --- | 3 a | 5 a | 16 a | 8 a |

Within a column, means followed by a common letter are not significantly different as determined by Duncan's multiple range test (P≤0.05).
Treatments applied to cotton on 10, 16 and 20 July, 1998.

Table 9. Field evaluations of Hz-AaIT for control of *H. zea* on cotton near Jamesville, NC, 1998.

| Treatment | Rate per ha | Mean % squares & bolls damaged | | | |
|---|---|---|---|---|---|
| | | 4 Aug | 7 Aug | 11 Aug | 17 Aug |
| Hz-AaIT WP | 5 x $10^{11}$ OB | 9 ab | 9 ab | 8 bc | 10 b |
| Hz-AaIT WP | 12 x $10^{11}$ OB | 6 bc | 10 ab | 12 b | 8 b |
| Dipel™ 2X | 1121 g (WP) | 6 bc | 8 ab | 11 b | 6 b |
| Thiodicarb | 560 g (AI) | 1 c | 2 b | 2 bc | 2 b |
| Cyhalothrin | 28 g (AI) | 2 b | 2 b | 1 c | 1 b |
| Non-treated | --- | 18 a | 21 a | 22 a | 26 a |

Within a column, means followed by a common letter are not significantly different as determined by Duncan's multiple range test (P≤0.05).
Treatments applied to cotton on 27, 30 July, 4 and 8 August, 1998.

Table 10. Field evaluations of Hz-AaIT for control of *H. zea* and *H. virescens* on cotton near Watkinsville, GA, 1998.

| | % terminals damaged | | | % squares damaged | | |
|---|---|---|---|---|---|---|
| **Treatment & dosage/ha** | **12 Aug** | **19 Aug** | **24 Aug** | **12 Aug** | **19 Aug** | **24 Aug** |
| Hz-AaIT WP @ 10 x $10^{11}$ OB | 5 b | 0 a | 15 a | 2 b | 1 a | 7 ab |
| Dipel™ @ 2X @ 1121 g (WP) | 5 b | 0 a | 25 a | 4 b | 4 a | 14 a |
| Spinosad @ 76 g (AI) | 0 b | 0 a | 15 a | 3 b | 0 a | 5 ab |
| Cyhalothrin @ 34 g (AI) | 0 b | 5 a | 5 a | 12 ab | 3 a | 3 b |
| Non-treated | 25 a | 10 a | 15 a | 13 a | 2 a | 12 ab |

Within a column, means followed by a common letter are not significantly different as determined by Duncan's multiple range test ($P \leq 0.05$).
Treatments applied to cotton on 6 and 12 August, 1998.

Table 11. Field evaluations of Hz-AaIT for control of *H. zea* on cotton near Midville, GA, 1998.

| | % terminals damaged | | | % squares damaged | | |
|---|---|---|---|---|---|---|
| **Treatment & dosage/ha** | **27 July** | **3 Aug** | **11 Aug** | **27 July** | **3 Aug** | **11 Aug** |
| Hz-AaIT WP @ 5 x $10^{11}$ OB | 25 a | 5 b | 20 b | 30 a | 12 b | 19 a |
| Hz-AaIT WP @ 12 x $10^{11}$ OB | 20 a | 15 b | 25 b | 30 a | 17 ab | 14 a |
| Dipel™ @ 2X @ 1121 g (WP) | 20 a | 10 b | 20 b | 33 a | 16 ab | 16 a |
| Spinosad @ 76 g (AI) | 15 a | 0 b | 30 b | 30 a | 10 b | 17 a |
| Cyhalothrin @ 34 g (AI) | 35 a | 5 b | 20 b | 17 a | 9 b | 15 a |
| Non-treated | 55 a | 75 a | 80 a | 43 a | 36 a | 36 a |

Within a column, means followed by a common letter are not significantly different as determined by Tukey's studentized range test ($P \leq 0.05$).
Treatments applied to cotton on 23 and 30 July, 1998.