IMPROVED CONTROL OF *HELIOTHIS VIRESCENS* AND *HELICOVERPA ZEA* WITH A RECOMBINANT FORM OF *AUTOGRAPHA CALIFORNICA* NUCLEAR POLYHEDROSIS VIRUS AND INTERACTION WITH BOLLGARD® COTTON

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Abstract

Development of alternative insecticides for the tobacco budworm, *Heliothis virescens* (Fabricius) (TBW) and the bollworm, *Helicoverpa zea* (Boddie), (CBW) with a recombinant form of *Autographa californica* nuclear polyhedrosis virus (AcNPV-AaIt) carrying a toxin gene from the scorpion *Androctonus australis* Hector could be useful for cotton pest management programs because the pathogen is pest specific, environmentally safe, and compatible with other insect control technologies. Greenhouse tests involving multiple sprays on squaring cotton over four weeks showed that formulations of AcNPV-AaIt effectively reduced damage by TBW and to a lesser extent, CBW. A greenhouse test with transgenic cotton (NuCotn 33) containing the insect resistant Bollgard® gene derived from *Bacillus thuringiensis* Berliner showed that AcNPV-AaIt acted additively with NuCotn 33 in reducing CBW damage; whereas, the transgenic cotton itself was sufficient to control TBW infestations. A field test showed that AcNPV-AaIt formulations effectively reduced a CBW/TBW infestation over four weeks without detrimental impact on other insects or nontarget arthropods.

Introduction

In 1995 we reported results of laboratory, greenhouse, and field tests that indicated that new genetic technologies have improved the effectiveness of the nuclear polyhedrosis virus (NPV) of the alfalfa looper, *Autographa californica*, (AcNPV) for controlling both tobacco budworm (TBW), *Heliothis virescens* (Fabricius), and the bollworm (CBW), *Helicoverpa zea* (Boddie), in cotton (Treacy and All 1996). Cotton pest managers have recognized the advantages of NPVs as insect control agents, particularly their target insect specificity and environmental safety. However, the naturally derived (feral) NPVs evaluated in cotton have had variable success. For example, the NPV of the TBW/CBW registered as Elcar® (Sandoz, Inc.) in the 1980s had erratic performance in field trials, and ultimately manufacture of the product was discontinued (Senuta 1987). Effectiveness of AcNPV-based insecticide products also have been disappointing in cotton (J. N. All unpublished data).

Poor field performance of feral NPV-based insecticides has been attributed (among other factors) to an extended incubation of 7 or more days required within an insect prior to producing death. Speed of kill by AcNPV has been decreased dramatically through genetic engineering of the baculovirus. Treacy and All (1996) reported that a recombinant form of AcNPV, (AcNPV-AaIt) carrying a toxin gene from the scorpion, *Androctonus australis* Hector, had improved control of TBW and, to an extent, CBW on cotton as compared with feral AcNPV and a *Bacillus thuringiensis* Berliner based product, Dipel® (Abbott Labs). The present study was conducted to verify 1995 greenhouse and field efficacy results with AcNPV-AaIt (Treacy and All 1996) and to evaluate the interaction of the virus product with other control technologies for TBW and CBW. A baculovirus product could be useful with transgenic cotton containing the Bollgard® gene because the toxic action of the two control methods on TBW/CBW is different.

Materials and Methods

The greenhouse experiments were conducted in the University of Georgia Pesticide Research Greenhouse in Athens using procedures described in All and Guillebeau (1991) and Treacy and All (1996). Briefly, cotton plants were grown to squaring in .25 m plastic pots and were tested over five weeks. The plants were sprayed every 5 or 7 days using a rotating boom sprayer built to simulate field application of insecticides. The machine had an adjustable height boom with three hollow cone spray nozzles. One nozzle was directed over the top of plants and the other two nozzles, mounted at 45^{o} on 10 cm drop tubes, sprayed each side of the plants. The compressed air spray system was operated at a pressure of 35 kg/cm^{2} to apply test solutions in a volume equivalent to 189 l/ha at a boom speed of 4.8 kph. Insecticide formulations were mixed in dechlorinated water and baculovirus formulations were combined with a feeding stimulant (COAX® at 3.5 l/ha). The test plants were sprayed three or four times depending on the test. The treated plants were arranged in a randomized complete block design with four or five replications in stainless steel pans flooded with 2 cm water. The test plants were separated from each other in the pans so that migrating larvae drowned.

Approximately one hour after spraying the test, plants received an equal infestation with freshly hatched TBW or CBW larvae by placing five or ten insects on the terminals and 15 or 20 at random on squares. Efficacy of treatments was determined after four or five weeks by examining terminals, squares, flowering squares, and bolls for damage and the presence of larvae.

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Three greenhouse experiments were conducted with TBW and CBW and involved the following treatments tested with NuCotn 33 and DeltaPine 90 cotton:

1. AcNPV-AaIt @ rates of 10 and 20 x 10^{11} polyhedral inclusion bodies polyhedral inclusion bodies (PIBs)/ha.
2. Esfenvalerate (Asana®, Dupont) @ 0.017, 0.025, and 0.034 kg AI/ha.

A field test was conducted at the University of Georgia Plant Sciences Farm located near Watkinsville, GA. The treatments (Table 4) were arranged in a randomized complete block design with four rows x 16.6 m long x 5 m alleys with four replications. Prior to spraying, the field was scouted once or twice each week for bollworm damage by examining 100 terminals and squares. On July 30, damage was assessed at 33% on terminals, 14% squares damaged, and three small larvae were found in 100 terminals. Spraying was applied on July 31 and continued on August 5, 9, 14, 19, and 23 using a high cycle sprayer modified with a CO_2 system for application of test insecticides. Each treatment was premixed in a separate stainless steel container and sprayed at a volume of 100 l/ha. COAX® @ 3.0% vol/vol and citric acid @ 1% wt/vol were added to all the treatments containing baculovirus. Each treatment was applied through a single spray line to a boom with three Tee-Jet® TX3 nozzles mounted so that one nozzle sprayed over the center of the row and two 0.1 m drop nozzles sprayed at 45° into the sides of a row. The cotton growth stages during the test were R6-R11 (Elsner *et al.* 1979).

Texas-style pheromone traps baited with the sex pheromone of *H. zea* and *H. virescens* were established near the test field and the number of male moths attracted to the traps was counted weekly. Efficacy ratings were made prior to each spray by examining 25 terminals and squares in the middle rows of each plot for damage and the presence of larvae. Nontarget arthropods were sampled three or four days after each spray and at 11 and 26 days after terminating spraying by conducting a timed search in the middle rows of each plot noting all insects, spiders, etc. Three entomologists searched each plot for two minutes (total of six minutes/plot) and counted all arthropods that they observed and classified them to the lowest taxonomic level possible. Any dead insects or other arthropods were collected, returned to the lab, and placed in a freezer.

Results and Discussion

In the first greenhouse experiment, both virus concentrations produced significant control of TBW infestations on cotton over the four week test period (Table 1). The higher rate of AcNpv-AaIt was not significantly better than the lower rate in the test. The two rates of esfenvalerate also produced similar control which was significantly better than the two baculovirus treatments. In the second greenhouse experiment involving both TBW and CBW, similar high amounts of damage occurred in the untreated check treatments for both insects (Table 2). AcNPV-AaIt produced significant suppression of square damage by both insects and the overall results of the test were similar to the previous experiment with TBW. The AcNPV-AaIt baculovirus is known to be less effective on CBW than TBW in laboratory assays (Treacy and All 1996) and a similar trend occurred in the greenhouse test. The percent control (1 - % damage in insecticide treatment / % damage in check treatment x 100) of TBW was 70.9% as compared with 49.5% for CBW in the test.

The third greenhouse test was designed to evaluate the interaction of the AcNPV-AaIt with transgenic cotton (NuCotn 33) containing the Bollgard® gene construct derived from *Bacillus thuringiensis* Berliner. Table 3 shows that the heavy infestation of 30 first instar larvae/week during the five weeks of testing produced heavy damage on nontransgenic (cultivar, DeltaPine 90) plants that did not receive insecticide treatment. Damage by CBW was particularly severe on nontransgenic plants, and the transgenic cotton (cultivar, NuCotn 33) also had significantly greater damage by CBW as compared with TBW. The results follow known trends for cotton cultivars containing the Bollgard® gene of having high resistance to TBW and moderate suppression of CBW (Monsanto 1995).

Four weekly AcNPV-AaIt sprays produced significant suppression of both TBW and CBW on nontransgenic, but not on the transgenic cotton (Table 3). As in the other greenhouse tests, the percent control of TBW (65.0%) was greater than for CBW (37.2%) by AcNPV-AaIt. A trend for improved suppression of CBW by AcNPV-AaIt on transgenic plants was evident (30.8% control (statistically similar to control on the nontransgenic cultivar)). Use of the genetically modified baculovirus could be a significant consideration for management of CBW on transgenic cotton since the modes of toxic action by the two agents are different and because both have minimal impact on natural enemies of CBW. Esfenvalerate at a rate of 0.025 kg AI/ha produced a high level of control of both TBW and CBW on both cotton cultivars (Table 3).

In the field test infestations by TBW/CEW were low until late July and increased greatly during August, averaging 24% infested squares in nontreated cotton during the period that the baculovirus and cypermethrin were applied in the test. The numbers of male moths captured in the pheromone traps increased during the period. Higher numbers of TBW than CBW (102 and 261, respectively) were counted during the first week (7/29) of spraying, but the proportion changed dramatically in favor of CBW during the week of (8/5) (162 TBW and 553 CBW/trap, respectively), and the ratio of the two species remained higher in CBW than TBW throughout the remainder of the season. Leonard et al. (1989) reported that adult moth data collected from pheromone traps correlates with CBW/TBW

proportion infesting nearby cotton, and thus it was assumed that a higher percentage CBW to TBW larval population infested the cotton during field test with the baculovirus treatments.

Overall the data demonstrated that AcNPV-AaIt produced significant control of the CBW/TBW infestation (Table 4) during the 24 day spray period (Table 4). All three rates of the AcNPV-AaIt formulation significantly reduced the infestation and, although the two higher rates were numerically superior to the lowest rate, the difference was not statistically significant. The three baculovirus treatments had statistically similar efficacy as compared with two cypermethrin rates of 0.045 and 0.09 kg AI/ha. There was no significant difference in yield among the various treatments in the test, but a trend for greater yield was apparent for AcNPV-AaIt and the high rate of cypermethrin as compared with the nontreated check.

A total of 37 nontarget arthropod groups were quantified during the test period, and the data in Table 4 reflects average numbers observed during six minutes in each plot every four to five days during the spray period. Table 4 does not include data taken at 11 and 26 days after sprays were terminated. Additionally, aphid counts were not included in the data since the high numbers counted on certain plants skewed the overall totals in various plots that had aphid populations. Overall, the presence of cypermethrin in a treatment tended to reduce nontarget arthropod numbers, and treatments that had only baculovirus sprays did not affect any nontarget insects, nor spiders, mites etc. that were observed during the test. The samples taken 11 and 26 days after spraying had no significant difference among treatments, indicating that recolonization by nontarget arthropods in cypermethrin treatments had occurred when residues of the pyrethroid diminished. The results are similar to a cotton field test conducted in 1995 in Georgia (Treacy and All 1996) and confirmed laboratory findings that genetically modified baculoviruses have low impact on nontarget arthropods.

In conclusion, the greenhouse and field research had similar results using AcNPV-AaIt formulations and verified that the genetically modified baculovirus has potential for utilization in management of TBW/CBW on cotton. The data indicated that the AcNPV-AaIt products were more effective on controlling TBW, but had moderate effectiveness on CBW. AcNPV-AaIt had a positive interaction with cultivar NuCotn 33 containing the Bollgard® gene and significantly reduced CBW infestations on the transgenic cotton. In the field test, care was taken to note any possible detrimental effects of AcNPV-AaIt on natural enemies of TBW/CBW and on other nontarget arthropods, because the mode of action is different from feral baculovirus. No detrimental effect of AcNPV-AaIt on nontarget arthropods was observed, the diversity and numbers of arthropods in plots sprayed with three rates of AcNPV-AaIt were similar to nonsprayed plots.

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References

All, J. N. and L. P. Guillebeau. 1991. New ideas for screening insecticides on squaring cotton in the greenhouse and in field trials. Proc. Beltwide Cotton Prod. and Research Conf. National Cotton Council (Memphis, TN). pp. 756-757.

Elsner, J. E., C. W. Smith, and D. F. Owen. 1979. Uniform stage descriptions in upland cotton. Crop Sci. 19: 361-363.

Leonard, B. R., J. B. Graves, E. Burris, A. M. Pavloff, and G. Church. 1989. Heliothis spp. (Lepidoptera: Noctuidae) captures in pheromone traps: species composition and relationship to oviposition in cotton. J. Econ. Entomol. 82: 574-579.

Monsanto Corp. 1995. Bollgard: cotton's self defense system against worms. Monsanto Corp. St. Louis, MO. Sales brochure 004-9520A. 16pp.

Senuta, A. 1987. Are microbials viable? Agrichem. Age. 31: 21-24.

Treacy, M. F. and J. N. All. 1996. Impact of insect-specific AaHIT gene insertion on inherent bioactivity of baculovirus against tobacco budworm, *Heliothis virescens*, and cabbage looper, *Trichoplusia ni.* Proc. Beltwide Cotton Prod. and Research Conf. National Cotton Council (Nashville, TN).pp. 787-793.

Table 1. Greenhouse evaluation of AcNPV-AaIt for control of *H. virescens* on DeltaPine 90[a].

Insecticide	Rate[b] kg AI/Ha or PIBs/Ha	% damaged squares[c]
Check		73.5 a
AcNPV-AaIt	10×10^{11}	17.6 b
AcNPV-AaIt	20×10^{11}	16.4 bc
Esfenvalerate	0.017	1.6 d
Esfenvalerate	0.034	1.1 d

a. Plants were sprayed three times on February 16, 26, and March 4, followed one hour later with infestation of 30 (February 16) or 20 (February 26 and March 4) freshly hatched larvae/plant.

b. Rates are expressed as kg AI esfenvalerate/ha and number polyhedral inclusion bodies (PIBs)/ha.

c. Means followed by the same letter are not significantly different in Duncan's new multiple range analysis ($P < 0.05$).

Table 2. Greenhouse evaluation of AcNPV-AaIt for control of *H. virescens* and *H. zea* on DeltaPine 90 cotton. [a]

Insecticide	Rate [b] kg AI/Ha or PIBs/Ha	% damaged squares [c] *H. virescens*	*H. zea*
Check		79.4 a	76.0 a
AcNPV-AaIt	10 x 10^{11}	23.1 b	38.4 b
Esfenvalerate	0.025	0.0 c	7.6 c

a. Plants were sprayed three times on May 16, 23, and 30, followed one hour later with infestation of 20 freshly hatched larvae/plant.

b. Rates are expressed as kg AI esfenvalerate/ha and number polyhedral inclusion bodies (PIBs)/ha.

c. Means followed by the same letter are not significantly different in Duncan's new multiple range analysis ($P < 0.05$).

Table 3. Greenhouse evaluation of AcNPV-AaIt for control of *H. virescens* and *H. zea* on NuCotn 33 and DeltaPine 90 cotton. [a]

Insecticide	Rate [b] kg AI/ha or PIBs/ha	% damaged squares [c] *H. virescens* NuCotn 33	*H. virescens* DP 90	*H. zea* NuCotn 33	*H. zea* DP 90
Check		1.7 de	64.2 b	35.1 c	92.0 a
ACNPV-AaIt	10 x 10^{11}	3.6 de	22.5 cde	24.3 cde	57.8 b
Esfenvalerate	0.017	0.0 e	0.0 e	0.9 e	2.9 de

a. Plants were sprayed three times on April 5, 12, and 19, followed one hour later with infestation of 20 freshly hatched larvae/plant.

b. Rates are expressed as kg AI esfenvalerate/ha and number polyhedral inclusion bodies (PIBs)/ha.

c. Means followed by the same letter are not significantly different in Duncan's new multiple range analysis ($P < 0.05$).

Table 4. Field test results expressed as a seasonal average of bollworm control and impact on nontarget arthropods of formulated AcNPV-AaIt at different rates on DeltaPine 90 cotton near Athens, GA. [a]

Insecticide	Rate [b] kg AI/Ha or PIBs/ Ha	% damaged squares [c]	Mean no. nontarget arthropods /six minute search [c]	Yield kg/ha x 10^3
Check		24.0 a	30.0 a	13.4 a
AcNPV-AaIt	5 x 10^{11}	17.0 b	30.0 a	14.1 a
AcNPV-AaIt	12.5 x 10^{11}	13.0 bc	32.0 a	14.1 a
AcNPV-AaIt	20 x 10^{11}	13.0 bc	35.0 a	13.0 a
Cypermethrin	0.045	9.0 cd	29.0 ab	13.9 a
Cypermethrin	0.09	10.0 bcd	20.0 b	12.0 a

a. Cotton was sprayed on 7/31, 8/5, 8/9, 8/14, 8/19, and 8/23 and efficacy data was taken as number infested square/25 squares examined in the two middle rows of each plot. Nontarget arthropod estimates were made on 8/2, 8/7, 8/13, 8/16, and 8/21 by doing a timed search of six minutes in the middle rows of each plot. The above data does not include aphid counts.

b. Rates are expressed as kg AI esfenvalerate/ha and number polyhedral inclusion bodies (PIBs)/ha.

c. Means followed by the same letter are not significantly different in Duncan's new multiple range analysis ($P < 0.05$).