SIX YEARS OF AREA-WIDE MANAGEMENT OF BOLLWORM/ BUDWORM WITH PATHOGENS - WHAT DOES IT MEAN AND WHERE DO WE GO FROM HERE D.D. Hardee and M.R. Bell USDA, ARS Southern Insect Management Laboratory Stoneville, MS

#### Abstract

Research to develop improved methods of managing serious insect pests of delta crops, specifcially cotton, by use of natural insect pathogens was begun in 1987 at the USDA, ARS, Southern Insect Management Laboratory (SIML) at Stoneville, MS. Previous research had shown that non-crop hosts, particularly early-season weeds, act as hosts for the tobacco budworm, Heliothis virescens (F.), and cotton bollworm, Helicoverpa zea (Boddie), prior to the presence of crop hosts. It was theorized that tobacco budworm and cotton bollworm populations could be managed by either controlling the insects on the weeds using insecticides, or by controlling the early season hosts themselves via herbicides or mowing. Since insect pathogens (microbial insecticides) are considered to be among the safest methods of insect control, research was begun to investigate their use in a management scheme. Positive results of small field and cage tests led to large area studies, beginning with a 64,000-acre test in 1990, and culminating in 215,000-acre tests in 1994 and 1995. Results of tests to date indicate that virus application could be accomplished at a reasonable cost, and that such treatment consistently reduced the number of moths emerging from weed hosts by > 70%. Herein, we present brief results of the long-term study that led to the present question of what the future holds for this project.

#### Introduction

Stadelbacher (1979, 1981) discussed the importance of several early-season wild and domesticated host plants of the tobacco budworm [*Heliothis virescens* (F.)] and cotton bollworm [*Helicoverpa zea* (Boddie)] in the delta of Mississippi and their importance in the buildup of the first larval generation which subsequently invades cotton. The major early-season host of these insects, wild geranium (*Geranium dissectum* L.), grows primarily in disturbed areas such as ditchbanks, roadsides, and tilled but unplanted fields, and is widely distributed in the Delta. It germinates from mid-October through early spring, and flowers and sets fruit from mid-April to late May. Larval feeding is restricted to the immature fruit. In a study of larval and adult populations, Stadelbacher (1979, 1981) calculated that as many as 450,000 *Heliothis* larvae and

17,000 adults were produced per hectare (2.4 acres) of wild geranium, and theorized that wide-area control of the first larval generation could thus have a positive impact on the management of bollworm/budworm in row crops such as cotton. Additional research showed that: 1) only 3.5% of the overwintering *Heliothis/Helicoverpa* population survives to emerge as adults; 2) spring emergence of the overwintered population occurs 6 weeks before cultivated host plants are available; 3) surviving populations are restricted to and concentrated in early-season alternate host plants which occupy < 5% of the total rural area; and 4) moths emerging from these wild hosts in early to mid-June move into cotton (Knipling and Stadlebacher 1983; Snodgrass, et al. 1991). The rationale for attacking bollworm/budworm populations during the first seasonal generation, and possibly the second, was addressed by Knipling and Stadlebacher (1983). Several methods for control of this first larval generation were examined, but due to the large-area control needed, the use of safe, natural control methods such as microbial agents was deemed most appropriate for further study.

The most promising of the microbial agents available for use against first larval generation bollworms and tobacco budworms on weeds was believed to be the baculoviruses [nuclear polyhedrosis viruses (NPV) and granulosis viruses], due to their safety, relative stability, and virulence. The baculovirus from the cotton bollworm (HNPV) was the first to be registered by the Environmental Protection Agency for use on row crops, and the safety studies conducted prior to registration were considered the most indepth ever on any insect pathogen or insecticide. This virus occurs naturally in the proposed test area and is known to infect only insects in the genera Heliothis/ Helicoverpa. Although this fact has relegated it to relatively few commercial markets, this virus has a potential infectivity such that the LD<sub>50</sub> may be as low as a single polyhedron (one virus particle) per bollworm/ budworm larva (Burges 1981). The negative aspects of baculoviruses include their relatively long incubation period and problems related to ingestion of the virus by the target insect.

The following research was begun in 1987 and progressed through 1995; the various studies are briefly reported here in the step-wise manner in which they were conducted.

#### Research Studies and Results: 1987 - 1995

#### <u>1987</u>

Research by Bell (1988a) showed that a bollworm/budworm moth developed on approximately every 3 square yards of early-season wild hosts. In 1987, a dye-marking method was used to first examine the effect of NPV applications on the emergence of this generation of adults. In that study, half of field plots in an area were treated with a blue dye and  $\frac{1}{2}$  were treated with a red dye mixture. In another area,  $\frac{1}{2}$  of field plots were treated with blue dye and the

Reprinted from the Proceedings of the Beltwide Cotton Conference Volume 2:897-902 (1996) National Cotton Council, Memphis TN

other  $\frac{1}{2}$  with red dye plus *H*NPV at a rate of 2.4 x 10<sup>11</sup> polyhedral inclusion bodies (PIB)/ac. Evaluation of the effects was determined using pheromone-baited cone traps (Hartstack et al. 1979). The first marked adult was captured on 18 May and the last on 21 June. The capture of both red and blue marked tobacco budworm and bollworm adults from areas around fields treated with either blue or red dye without *H*NPV compared to only blue-dye marked moths trapped near fields treated with either the blue dye or red dye + *H*NPV indicated a significant reduction in the number of budworms emerging from the virus-treated areas (Bell 1988b).

## <u>1988</u>

A cage study was conducted during April and June to more accurately measure (although under caged conditions) the effectiveness of microbials in early-season control of bollworm/ budworm (Bell 1991). This test consisted of three replicates of each of four microbial treatments and an untreated control. The four treatments examined were: NPV in water, NPV in an aqueous 10% crude cottonseed oil formulation, NPV in a COAX<sup>®</sup>-dust formulation, and an insect growth regulator (IGR) (SAN415WG354) + *Bacillus thuringiensis (Bt)* Berliner (SAN810I) formulation supplied by Sandoz Agro, Inc. (Des Plaines, IL).

Fifteen screen cages (plastic insect screen, 12 ft x 24 ft x 6 ft tall) were erected over the early-season weeds, the canopy of which was approximately 100% wild geranium, primarily G. dissectum, but also containing some G. carolinianum L. On 26, 27, and 29 April, each cage was infested with approximately 1,000 bollworm and 1,000 budworm larvae from our lab culture. Microbial treatments were applied on 2 May, when larvae were from 3-7 d old. All virus treatments were applied at a rate of 2.4 x 10<sup>11</sup> PIB/ac, and the IGR + Bt was applied at 47 gm (AI)/ac plus 1 lb/ac. The NPV-oil, NPV-water, and IGR-Bt treatments were applied in a volume equivalent to 5 gal/ac using an atomizing sprayer held approximately 20 in above the plant canopy. The dust formulation was applied at a rate of 3 lb/ac using a small garden duster. Cages remained undisturbed from 2 May until 29 May, when the first adult was observed. Cages were then searched daily and numbers, species, and sex of moths were recorded. The first adult moth was captured 1 June, and the last on 15 June.

Virus treatments resulted in overall reductions in emergence of 80.7 to 91.2% (Bell and Scott 1989), with tobacco budworm emergence reduced 78.5 to 89.2% and cotton bollworm emergence reduced 71.4 to 100%. Although the application of virus in water resulted in the greatest level of control of both budworms and bollworms (89.2 and 100%, respectively), there were no significant differences due to formulation used. Although the overall number of moths per cage in the IGR + *Bt* treatment was significantly less than the check, the level of control was significantly less than that obtained with any of the NPV treatments. These tests indicated that *HNPV* was effective in reducing bollworm/budworm emergence, and would be a good candidate for use in management systems where pests develop on early-season wild hosts.

# 1989

The success of a large-area program utilizing early-season control methods mandated that the results obtained by hand application be reproduced using aerial application. Thus, a study was undertaken to examine the effectiveness of aerially-applied virus in controlling the emergence of bollworm/ budworm adults from early-season host plants (Bell and Hardee 1991). The test was conducted in Washington County, Mississippi, with aerially-applied treatments consisting of three levels of NPV: 0, 20, and 40 larval equivalents (LE)/ac [one LE equals 6 billion polyhedral inclusion bodies (PIB)]. Treatments were applied in water on 27 April; untreated control plots were covered with plastic sheeting during application. Following application, screen cages (12 ft x 24 ft x 6 ft tall) were placed over each plot and secured. Cage searches were conducted daily after the first moth was observed, and moths were captured and removed. Numbers, species, and sex of moths were recorded.

The first adults were captured on 30 May and the last on 12 June. Budworm emergence was reduced 88% and bollworm emergence by 100% in plots treated with 40 LE/ha (Bell 1990), for a total average emergence reduction of 90%. The 20 LE rate caused reductions of 65% in budworms and 57% in bollworms, for an overall reduction of 64%. The mean total numbers of bollworm/budworm adults emerging all differed significantly from each other. The results of this test were therefore similar to those obtained when alternate host areas were treated by hand, indicating that a single aerially-applied *H*NPV treatment could result in a reduction in first-generation adult emergence of about 90%. However, a large-area test was needed to evaluate this technique as a management tool in the Mississippi delta. Such a test was planned for 1990.

# <u>1990</u>

The study consisted of aerially applying a viral insecticide (Elcar®, *HNPV*) to early-season alternate host plants of bollworm/budworm within a single area (10 x 10 mi, 64,000 acres) at a time of maximum immature seed production in a majority of the wild geranium in the test area. Emerging adult populations would be compared to those in a similar, untreated area, separated by a 10 mile buffer (Bell and Hayes 1994; Hayes and Bell 1994). The treatment area was in parts of Washington and Sunflower Counties in the Mississippi Delta. Spray coverage, infection rates of larvae on pre-season wild hosts, persistence of virus on plants, number and species of moths emerging from alternate hosts within the areas, and number and species of the first bollworm/budworm adult populations invading cotton were determined.

Since the treatment of a large, populated, non-crop area with a microbial pesticide was unprecedented, the reaction of the public to the project was of utmost concern. We believed, however, that an informed public would be an approving public. The strategy for the public information campaign was developed jointly by USDA scientists involved with the project, the USDA ARS Information Office, Delta Council, and personnel of Sandoz Crop Protection, Inc. This successful information campaign was believed to have greatly contributed to the success of the test.

Virus was applied both by aircraft and by trucks equipped with mist blowers. Both methods of application were calibrated to deliver virus at a rate of 40 LE in 2 gallons of water per acre. Since the plan was to treat only alternate host habitats, pilots attempted to treat only field edges, borders, fallow fields, treelines, etc. Water-sensitive cards were placed randomly among the weeds to determine spray coverage. Aerial application began on 24 April, a time believed to be about 7 days prior to maximum overall seed production in the G. dissectum. Aerial application was completed by 8 May, although much of the application was done during weather conditions not favorable to spotspraying sites, primarily too much wind. A series of early spring weather fronts caused many farmers to delay tilling their fields, resulting in an unusual amount of weed areas where the appropriateness of treatment was questionable. Truck-mounted mist blowers (calibrated to deliver 2 gallons per acre along the side of the truck in a 60-ft swath width at 5 MPH) were used to treat along major roadways within the test area (335 miles total) between 30 April and 4 May. Although these areas are known to be major sites for geranium (Stadelbacher 1982), they are more difficult to treat by aerial application, either because of vehicle traffic or nearby power lines.

Bioassays were made of field-collected material to estimate the efficiency of the spray application and viral persistence. To estimate the quantity of virus on the plants, *G. dissectum* terminals were cut at random from 8 plots in the treatment area as soon as possible after application. Terminals (72 terminals from each location) were fed to 6 d old tobacco budworm larvae for 48 hr, after which larvae were placed on artificial diet. Larval mortality was recorded 10 days after the beginning of the feeding period. A final bioassay was conducted on 29 May to determine the residual virus on the weeds. Random samples of remaining wild geranium were collected and fed to larvae as before, with 144 larvae fed material from treated plots and 144 fed material from untreated areas.

The methods used for the cage tests were similar to those used in the previous study. Untreated control plots were covered with plastic sheeting during application. Daily searches were conducted within the cages after the first budworm/bollworm moth was observed, and moths were captured and removed. Pheromone traps were used to

monitor the relative abundance and fluctuations in the adult male populations in the treatment and control areas. In both areas, four trap locations (1 trap/species/location) were identified near cotton fields within each 1-mi interval/radius from the center; at radii from 1-5 mi in the control plot, and from 1-10 mi in the treatment area, to assess the impact of migration on the treated area (encompassed in radii 1-5). Depending on availability of an accessible cotton field, attention was given to spacing trap locations in different quadrants. Trap locations consisted of 1 trap/species placed at the edge of a cotton field along an accessible roadway; traps were separated by about 100 yards. Because fields are frequently cultivated and roadsides are often mowed or burned, traps were placed near power poles and in other protected sites. Traps were routinely monitored from 1 April, which included the flight of the parental, F<sub>1</sub> and F<sub>2</sub> generations.

In addition to the estimation of the emerging population by adult trapping, attempts were made to estimate the number of adults by counting the egg deposition on early hosts and cotton within the test areas. This method required visually checking plants for a total of 30 min in each sample area and recording the numbers and relative populations of budworm/bollworm eggs.

When G. dissectum terminals were randomly collected from 8 different plots immediately after aerial application and fed to 6-day-old tobacco budworm larvae for 48 hours, mortalities at 10 days averaged 75.1% (67.7% to 88.7%) compared to a background mortality of 9.3% when larvae were fed terminals from untreated areas. Although the mortalities of larvae fed treated terminals were significantly greater than those fed untreated terminals, the 75.1% mortality was less than that reported in a previous study. In that study, the treated area was an open field, and the application was made in broadcast fashion, not to a strip target area. Larval mortalities in terminal bioassays averaged 95.7%. The random samples tested here were taken from along tree lines, field borders, etc., not from open fields. This lack of virus on the target hosts was also indicated by the numbers of droplets found on water-sensitive cards. A similar application rate and droplet size in the earlier test resulted in an average of 350 droplets per in<sup>2</sup>, whereas there was an average of 25 droplets per in<sup>2</sup> in the present test, indicating a 93% reduction in droplets hitting the plants. When the larvae were fed terminals collected 1, 2, 3, 5, 7, and 9 days after treatment, viral mortality averaged 77, 69, 64, 57, 41, and 38%, respectively, indicating original activity remaining (OAR, obtained by comparing mortality each day to the mortality on day 0) on those dates of 95, 85, 79, 70, 50, and 47%, respectively. Only 5% of larvae died from NPV when fed wild geranium collected from the treated area 18 days after all applications had been completed; samples from outside of the test area produced no viral mortality. Very little wild geranium remained viable by that date.

There was a 41% total overall average reduction in emergence in the treated cages (Table 1), indicating much less effect than in the previous tests where the least overall reductions were about 88%. In the previous study, however, moth emergence was reduced only by 64% when the virus dosage was halved (20 LE/ac). The present bioassay data indicated that aerial application resulted in only about 28% as much virus on the plants as in the previous study. If these indications were real, the 41% reduction in moth emergence in this study was reasonable. These data all indicated that deposition of virus on the target area was a major problem in this test, even in the cage test areas where application was somewhat easier than in other host plant habitats.

Pheromone traps and egg counts were used to assess the degree of area-wide suppression achieved by early-season application of the *HNPV*. Eggs  $(F_2)$  were collected from cotton and other hosts to characterize the surviving reproductive populations. The effect of treatment was demonstrated by deviations in trap capture patterns within-year between treated and control plots and between years in the treated plot. Rates of increase  $(r_i)$  between generations were calculated by dividing the number of moths captured in one generation by the number from the previous generation. The results of these data indicated that the single virus application reduced the adult budworm population emerging from early season alternate hosts by 25 to 38%, and the bollworm population by 19 to 31% in the 100-mi<sup>2</sup> area. Overall budworm/ bollworm adult emergence was reduced by 41% in cage test areas. Although the treatment failed to reduce the adult population as much as expected, the results were still encouraging. This reduction in the number of moths was understandable when all factors of the study were taken into consideration, primarily the lack of spray coverage. A secondary factor was the timing of the application. Although aerial application studies had been done in which the virus was applied to alternate host areas, the conditions of this test were different in that much of the application had to be done under windy conditions. Bioassays were very informative in indicating the presence of the virus on the plants. Based on that data, the reductions in insect populations were comparable with prior tests. Although timing was less a factor than spray coverage, a 21-day application period was considered too long for most seasons.

# <u>1992</u>

A repeat of the 1990 study was planned for 1991, but the test was cancelled due to severe flooding during the spring of that year. A similar but smaller test was planned for spring of 1992 in which the goal was improved spray coverage. Preliminary tests in 1991 showed that the addition of 4% soybean oil w/emulsifier increased the amount of material reaching the surface from a spray altitude of 50 ft. These tests indicated that use of a crop oil

additive and applying the spray as a "blanket" application would increase virus deposition on the early-season hosts.

The test site was 17,591 acres in a 6- x 6-mi area used as an untreated control during 1990 and 1991 tests. Data were collected to determine spray coverage, infection rates of larvae on pre-season wild hosts, persistence of the virus on the plants, and the number and species of moths emerging from alternate hosts within the treated area and compared to the surrounding untreated area.

The virus was applied at the time of maximum immature *G. dissectum* seed production (24, 25, 27, and 28 April) using 4 fixed-wing aircraft, each calibrated to deliver virus at a rate of 40 LE in 1 gallon of water containing 4% crop oil spray additive. The lead aircraft was equipped with an electronic satellite guidance system, which recorded the aircraft's position relative to the earth's surface. The three other aircraft followed.

Bioassays were conducted on *G. dissectum* collected at various times from various locations within the treated area to estimate the efficiency of the spray application and the persistence of virus on the wild geranium. Six-day-old tobacco budworm larvae were allowed to feed on terminals for 48 h (60 larvae, 4 samples/location and time), after which they were then placed on clean artificial diet. Larval mortality due to virus was recorded 10 days after the beginning of the feeding period. The first samples were collected on 27 April and the final collected on 13 May.

Cages studies for evaluating the effectiveness of the virus application were as described previously. A total of 12 cages (6 treated and 6 untreated control) were erected in pairs. The control plots were covered with plastic sheeting during application, as in previous tests. Searches were conducted daily within the cages after the first moth was observed, and the number and species of moths captured were recorded.

Pheromone traps were used to monitor the relative abundance of the adult male bollworm/budworm populations in the treatment area (center 3-mi radius) and the untreated area (the area surrounding the treated area and 3-6 mi from center of the test area). In both areas, 26 trap locations (1 trap/ species/location) were identified near cotton fields, with 8 to 9 pairs within each 1-mi interval/radius from the center (total area encompassed in a radius of 6 mi). Traps were routinely monitored from 20 March to 31 August.

Allowing tobacco budworm larvae to feed for 48 h on *G. dissectum* terminals collected 0, 2, 3, 4, 6, 7, 8, and 11 d after treatment produced virus-induced mortalities of 83, 81, 60, 79, 75, 67, 60, and 33%, respectively. Samples collected in the treated area on May 13 (17 d after treatment) did not produce viral mortalities greater than samples from untreated areas. These results tend to verify

the improved coverage obtained in this study compared to the 1990 study, since mortalities were consistent over the areas tested. The indicated persistence of the virus also compared well with that previously reported, again indicating roughly 47% of the original activity remaining 9 days after application as reported in previous tests.

Cage data indicated that the affected generation of adults (that generation developing as larvae on the wild hosts) began emerging on 3 June (bollworm) and 12 June (budworm). A total of 36 budworms and 13 bollworms emerged in the 6 untreated areas, compared to 7 budworms and 7 bollworms in the 6 treated areas. The cage data, therefore, indicated a significant reduction in budworm emergence (80.6%) in treated areas compared to untreated hosts (Table 2). Although the bollworm emergence was 46.2% less in the treated cages, the difference was not significant.

Pheromone trap data indicated similar populations of both species within the treated and untreated areas until 3 June, after which there was an observed period of reduced response within the treated area, particularly the center 4-mi diameter. Emergence of adult budworms in the cages indicated the population emerging from 12 through 26 June most likely developed as larvae on the early season hosts. During that period, budworm traps in the 6-mi diameter treated area averaged 8.0 moths/trap/night (Table 3) compared to 11.4 moths/trap/night captured in the untreated area (3-6 mi from the center of the test), a reduction of 29.8%. However, captures averaged 6.4 moths/trap/night in the middle 4-mi diameter of the treated area, a reduction of 43.9% in that portion of the virus treated area compared to the surrounding untreated area.

The indicated reduction in budworms within the center of the test (41.9%) was an improvement over the results obtained in 1990, but the effectiveness indicated by trap data was not as good as that shown by the cage data. This difference may be explained by immigration of moths from outside the test area, reducing the indicated effectiveness of the virus application based on trap data. Red-dye-marked moths from another program were being released at least 8 mi from our test area. Trap captures in part of our test area averaged up to 33% marked moths during the release period, indicating significant movement of moths into the area. It is, therefore, reasonable to speculate that the actual reduction in budworm moth emergence from early season hosts was greater than 41% shown by the adult captures.

### <u>1994</u>

An area-wide, early-season pest management test was conducted utilizing a 314-mi<sup>2</sup> area, or twice the diameter of the 1990 test. Since no commercial virus was available, over 8 million cotton bollworm larvae were reared over a four-month period to produce enough of an EPA-labeled insect virus to treat 215,000 acres. Application of the virus to 195,000 acres was made from 28 April through 3 May

using contracted private aircraft equipped with satellite global positioning systems. Although the virus and formulating materials were tested and shown to have no effect on catfish, spray nozzles were turned off over those areas.

Evaluation of the effects of the virus application was accomplished using several methods: pheromone trapping in the treated area compared to traps in three similar untreated areas of the delta; cages placed over treated and untreated weed hosts as in previous tests; plant bioassays (feeding plant materials to 6-day-old larvae); and examining plants in selected fields within the treated area compared to the three untreated areas for differences in eggs or larvae.

Pheromone trap counts of budworm and bollworm moths in the treated area between June 10 and August 22 averaged 53% (17 to 91%) less than traps in three similar, untreated areas (Figs. 1 and 2). A total of 195 tobacco budworms emerged in 12 cages over untreated areas compared to 80 emerging in cages over treated areas, a reduction of 59%. Four bollworm moths emerged in treated areas compared to 11 in untreated areas, a reduction of 64%. Virusinduced morality of 57 larvae collected form weeds in two locations three after treatment was 85%. Bioassay data showed that 76% of larvae fed treated terminals for 48 h (average of 22 samples of weed samples collected after application had dried, 30 larvae/sample) died from virus infection. The cost of virus production and application was about \$449,162, or \$2.13/ac. Although the adult trapping counts and infection data appeared to support the significant effect of virus in reducing bollworm and budworm populations in the area, reports on cotton scouting and a possible economic impact comparing the treated area to the untreated areas did not show any positive benefit. Furthermore, counts of larvae and eggs within the treated area were equal to the untreated areas.

### 1995

As in 1994, over 8 million cotton bollworm larvae were used to produce virus to treat over 201,000 acres and evaluate the effect of such a treatment on the resulting populations of bollworms and budworms. The virus was likewise applied using contracted private aircraft with global positioning systems to treat the same area as was treated in the 1994 test. In 1995, the virus was applied from 5 May through 10 May 1995, approximately one wk later than in 1994. Evaluation methods were similar to those used in 1994, with slight modifications. First, a total of 398 tobacco budworm larvae were collected at various times from weed hosts in 21 locations. Second, pheromone traps were placed in the treatment area plus the one check area which in 1994 had captures closest to the average of the three check areas used that year. Planned cage tests were lost due to circumstances of application. An evaluation based on examinations of cotton fields for presence of eggs and larvae was conducted.

The mortality of the collected larvae due to virus infection by date of collection is shown in Table 4. It was impressive that the larvae collected on 11 and 12 May were all infected (100% mortality due to virus). By the next week, the percentage of infected larvae decreased; but by that time, many of the infected larvae would have been dead and therefore would not have been collected. The persistence of virus over the following 10 days could indicate disease transmission from diseased and dying larvae to previouslyuninfected larvae. In 1994, overall counts of tobacco budworm and bollworm moths in the treated area between 10 June and 22 August averaged 53% less than in traps in the untreated areas. During the same period in 1995, the overall counts averaged 66% less in the treated area (Table 5). Trap captures of tobacco budworms and bollworms are illustrated in Figures 3 and 4. We expected the effects of the early virus treatment to be expressed during the emergence of moths in the June generation. That is what was indicated by the 1994 H. virescens trap captures (7 June through 8 July). The 1995 trap data not only indicated the reduction in June, but may have also indicated continued reductions through the July population. Although we felt that the timing of our application was too late to cause reductions of the first population of H. zea, trap capture data suggests this was not the case. We found larvae easily during the period of 11 May - 19 May, and the amount of virus on the plants from dying budworm larvae might have affected the bollworms if they were in a second generation at that time.

### **Summary of Research Findings**

All studies have shown that application of baculovirus (NPV) to early-season hosts of the tobacco budworm and cotton bollworm resulted in reduction in the numbers of adults that emerge from treated areas compared to adjacent untreated areas. This fact is demonstrated both by the significant reduction in the numbers of moths in cages placed over treated areas (compared to untreated areas), and by the infection rates of larvae collected from treated early-season weeds. Based on those results, we estimate that a properly timed NPV treatment with good coverage consistently resulted in viral-induced mortality of least 70%, and possibly as high as 100%.

In the largest tests (1994 and 1995), the rates of infection of larvae on the hosts as well as the reductions shown in moth emergence did not translate to a significant difference in the numbers of eggs/larvae on the cotton in the treated area compared to the surrounding untreated areas. During both years, numbers of adults during the crop season in the entire area of the study was relatively low. Much of the theory regarding the use of this pest management system is related to the distance and timing of moth flights. The high percentage of marked moths released at least 8 mi from trap sites in 1992 indicated significant moth movement. Mobility of the target pest makes evaluation of the success of suppression tactics difficult over large areas of the agroecosystem, and it will definitely play a part in the use of this method as a management tool. In addition, because the area under study had a history of the highest numbers of these insects in the Delta, movement from the treated area to untreated areas may have been reduced.

## **Considerations for Future Programs**

It is important to remember that the methods for mass production of the baculovirus and for accurately applying the virus to large areas are already in place. What is not known at this time is the cost:benefit ratio of such a program.

### **Estimated Cost and Availability of Virus**

Until recently, *HNPV* was registered only by Sandoz Agro, Inc. (Elcar®), and they had not produced it as a commercial product for a number of years. The virus used in the 1994 and 1995 tests was produced at this laboratory in cooperation with the USDA Gast Rearing Laboratory at Mississippi State University under an agreement with Sandoz. Since Elcar® is presently registered by Sandoz, they are interested in resuming production of that product, if needed. However, due to the current methods of virus production, any producer of NPV will need considerable lead time between the order for large amounts of the virus and the expected delivery date.

Another product (GEMSTAR®, also the baculovirus from the bollworm) was recently registered by biosys, Inc. biosys is currently producing limited quantities of this virus but could increase production given enough advance notice of need; biosys had stated they could supply enough virus to treat 500,000 acres for delivery in mid-April of 1996, if an order had been placed by mid-November of 1995. A following order for 1 to 3 million acre-treatments could be prepared for delivery in 1997, if ordered by August 1, 1996. The estimated cost of the biosys virus for the 1996 season is \$2.50/ac, and the estimated cost of an order for 1997, if 3 million or more acre-treatments were ordered, is \$2.00/ac.

The estimated grower-cost of a pilot program would be based on the number of cotton acres within the program area. For example, it was estimated that about 50% of the 201,000 acres in the 1994 and 1995 tests was in cotton production. The cost of virus to treat that area, based on the biosys estimates, would be \$502,500 (201,000 acres @ \$2.50). Therefore, the cost of virus per acre of cotton in the program would be \$5.00, or  $401,500 \div 100,500$  acres. It has been estimated that at least 1/3 of the better cottongrowing area of the delta is planted in cotton. It is known that about 1/4 of the 4 million acres of the Delta of Mississippi is in cotton production. Therefore, if a program were developed which included the entire Delta of Mississippi, virus costs (based on present estimates) would be \$8 million (4 million acres @ \$2 ), and the cost to cotton growers would be \$8 per acre of cotton. The cost of

application within the program area, divided by the number of cotton acres within the area, would determine the growers' final program costs.

## **Estimated Cost of Aerial Application**

The cost of aerial application and the crop oil additive used to increase coverage during the 1994-95 tests was ca. \$1.00/ac (\$0.60 for application, \$0.40 for 1 pint oil adjuvant). Application costs could possibly be reduced if large areas are treated; however the cost of the oil would be based on current soybean and cottonseed oil prices. For example, the estimated cost of application using large aircraft to deliver the same volume used in the 1995-95 tests was \$0.25/acre (\$0.65/ acre, including oil). Based on these figures, the estimated cost of virus and application for 4 million acres would be \$10.6 million (\$8 million plus \$4.6 million), or \$10.60/ac of cotton.

# **Supplementary Cultural Practices**

In the Mississippi River Delta, noncropped areas consist mostly of disturbed margins of cultivated fields or the shoulders of roads. Wild geranium is very abundant in this relatively small noncropped area, and here it serves as an excellent host plant for the tarnished plant bug, and as the main host for the first generations of the bollworm and tobacco budworm. Small plot research has shown that bollworm and tobacco budworm populations can be reduced by as much as 97% with one mowing, and by 99% with one herbicide application. Mowing can reduce plant bug adult levels by 40% and nymphs by 79%, while a herbicide treatment can cause reductions of 65% for adults and 73% for nymphs. Both control methods have been tested only in small plots, and the effects of the treatments on pest and beneficial arthropods found in the noncropped areas has not been evaluated on large areas. Any supplemental mowing or herbicide treatments by growers would be an added benefit.

### **Transgenic Cottons**

With the anticipated planting of large acreages of transgenic cotton in 1996, use of a large-scale program of this type may be a valuable tool in managing resistance development in Bt cottons.

### **Future Developments**

A series of options has been presented to the Delta Council and area producers for a decision on expansion, alteration, adding new research concepts, etc. A decision will be reached by mid-February as to the future of this program.

## References

Bell, M.R. 1988a. *Heliothis virescens* and *H. Zea*: Feasibility of using oil-soluble dye to mark populations developing on early-season host plants. J. Entomol. Sci. 23:223-228.

Bell, M.R. 1988b. Oil-soluble dyes for marking and estimating the effectiveness of microbial control of *Heliothis* spp. developing on early season wild geraniums. Proc. Beltwide Cotton Prod. Conf., pp. 254-257.

Bell, M.R. 1990. Use of baculovirus for the control of *Heliothis* spp. in area-wide management programs. Proc. 5th Inter. Coll. for Inv. Pathol.

Bell, M.R. 1991. Effectiveness of microbial control of *Heliothis* spp. developing on early season wild geraniums: Field and field cage tests. J. Econ. Entomol. 84:851-854.

Bell, M.R. and W.P. Scott. 1989. Microbial control of *Heliothis* spp. developing on early-season hosts: a cage study. Proc. Beltwide Cotton Prod. Conf., pp. 366-368.

Bell, M.R. and D.D. Hardee. 1991. Spray efficiency of aerial application of a nuclear polyhedrosis virus in areawide treatment of early season hosts of bollworms/budworms. Proc. Beltwide Cotton Prod. Conf. pp. 624-627.

Bell, M.R. and J.L. Hayes. 1994. Area-wide management of cotton bollworm/tobacco budworm through application of a nuclear polyhedrosis virus on early-season alternate hosts. J. Econ. Entomol. 87:53-57.

Burges, H.D. 1981. Strategy for the microbial control of pests in 1980 and beyond. *In* Microbial Control of Pests and Plant Diseases, 1970-1980. H. Burges (ed.), Academic Press, NY.

Hartstack, A.W., J.A. Witz, and D.R. Buck. 1979. Moth traps for the tobacco budworm. J. Econ. Entomol. 72:519-522.

Hayes, J.L. and M.R. Bell. 1994. Evaluating early-season baculovirus treatment for wide-area suppression of *Heliothis/Helicoverpa*. J. Econ. Entomol. 87:58-66.

Knipling, E.F., and E.A. Stadelbacher. 1983. The rationale for area wide management of *Heliothis* (Lepidoptera; Noctuidae) populations. Bull. Entomol. Soc. Am. 29:29-37.

Snodgrass, G.L., E.A. Stadelbacher, and J.W. Smith. 1991. Distribution and abundance of early-season wild host plants and bollworm and tobacco budworm populations (Lepidoptera: Noctuidae) in an intensively cropped area of the mid-delta of Mississippi. J. Entomol. Sci. 26:9-16.

Stadelbacher, E.A. 1979. *Geranium dissectum*: an unreported host of the tobacco budworm and bollworm and its role in their seasonal and long-term population dynamics in the delta of Mississippi. Environ. Entomol. 8:1153-1156.

Stadelbacher, E.A. 1981. Role of early-season wild and naturalized host plants in the buildup of the F<sub>1</sub> generation of Heliothis zea and H. virescens in the delta of Mississippi. Environ. Entomol. 10:766-770.

Stadelbacher, E.A. 1982. An overview and simulation of tactics for management of Heliothis spp. on early-season wild host plants. Proc. Beltwide Cotton Prod. Conf., pp. 209-121.

Table 1. Number of moths emerging from G. dissectum after treatment with NPV

| Treatment  | No. moths per cage, mean $\pm$ SEM1 |                   |                 |  |
|--|-------------------------------------|-------------------|-----------------|--|
|  | H. virescens                        | H. zea            | Totals          |  |
| Untreated  | 8.11 ± 1.31 a                       | $1.44 \pm 0.56$ a | $9.56 \pm 1.23$ |  |
| a  |                                     |                   |                 |  |
| Aerial application   | $5.00\pm1.76~b$                     | $0.67\pm0.37\ ab$ | $5.67 \pm 1.75$ |  |
| b  |                                     |                   |                 |  |
| LSD  | 147                                 | 1.08              | 332             |  |
| $^{1}$ n = 9 Cages/treatment. Values within columns followed by the same |                                     |                   |                 |  |

letter are not significantly different (P > 0.05, Fisher's LSD).

Table 2. Bollworm and budworm emergence from G. dissectum aerially treated with NPV1

| $1.17 \pm 0.7$     |
|--------------------|
| $2.21 \pm 0.9^{1}$ |
|                    |

Values represent numbers of moths emerging per cage (6 cages/treatment). Means denoted by \* are significantly different from the control (P = 0.05).

Table 3. Mean number of budworm captures by pheromone traps per night within treated and untreated areas of the Mississipi Delta in 1992

|                             | 6/12 | 6/15   | 6/17/ | 6/19  | 6/22   | 6/24 | 6/26 | X     |
|-----------------------------|------|--------|-------|-------|--------|------|------|-------|
| Untreated Area*             | 6.58 | 14.79  | 14.83 | 15.96 | 9.22   | 9.92 | 8.23 | 11.36 |
| Treated Area A*. **         | 4.83 | 11.36  | 10.65 | 11.83 | 3 5.92 | 6.12 | 5.31 | 8.00  |
| Treated Area B <sup>†</sup> | 3.3  | 35 10. | 00 7. | .92 1 | 1.35 4 | 4.49 | 4.42 | 3.26  |
| 6.40                        |      |        |       |       |        |      |      |       |
| Prob. of sig. diff.         |      |        |       |       |        |      |      |       |
| between Treated A           |      |        |       |       |        |      |      |       |

and untreated (X<sup>2</sup>)<sup>+</sup> 0.72 0.47 0.98 0.22 0.76 0.88 0.76 \* The treated area (center 3-mi radius) received an aerial application of bollworm NPV on 24-28 April at a rate of  $\approx 40$  LE/ac.

\*\* Data based on 26 traps in each area. Treated Area A = 3-mi radius area. Untreated area = 3 to 6 mi radius area surrounding treated area.

† Data based on 13 traps located in center 2 mi radius of treated area (Treated Area B).

‡ Probabilities of significant differences based on frequency distributuions (Chi-square test).

Table 4. Mortality due to NPV in budworm populations collected from treatment area. Virus treatment applied 5-10 May 19951

|                 | # Larvae  | # Larvae killed | %NPV      |
|-----------------|-----------|-----------------|-----------|
| Collection date | collected | by NPV          | mortality |
| 11 May          | 25        | 25              | 100       |
| 12 May          | 20        | 20              | 100       |
| 15 May          | 86        | 40              | 47        |
| 16 May          | 122       | 59              | 48        |
| 17 May          | 14        | 54              | 45        |
| 19 May          | 18        | 8               | 44        |
| 22 May          | 5         | 4               | 80        |
| 23 May          | 8         | 5               | 63        |
| Total/overall   | 398       | 215             | 54        |

<sup>1</sup> Samples represent larvae collected from 21 of the 25 treated sectors.

Table 5. Pheromone trap counts, 20 traps/species/treatment

| Treated       |                      |                   |                         |                       | Unt                     | Untreated                          |       |
|---------------|----------------------|-------------------|-------------------------|-----------------------|-------------------------|------------------------------------|-------|
| -             | #                    |                   |                         |                       |                         |                                    |       |
| Dates         | Trap<br><u>nites</u> | Species           | Total<br><u># moths</u> | × Moths/<br>trap/nite | Total<br><u># moths</u> | $\bar{\times}$ Moths/<br>trap/nite | %     |
| 6/7-<br>7-8   | 35                   | H. vire-<br>scens | 6084                    | 8.69                  | 15205                   | 21.72                              | 59.99 |
|               |                      | H. zea            | 8538                    | 12.2                  | 20566                   | 29.38                              | 58.48 |
| 7/12-<br>8/16 | 39                   | H. vire-<br>scens | 15589                   | 19.99                 | 21744                   | 27.88                              | 28.31 |
|               |                      | H. zea            | 12547                   | 16.09                 | 34181                   | 43.82                              | 63.29 |
| 6/7-<br>8/16  | 74                   | H. vire-<br>scens | 21673                   | 14.64                 | 36949                   | 24.97                              | 41.34 |
|               |                      | H. zea            | 21085                   | 14.25                 | 54747                   | 36.99                              | 61.49 |
| 6/7-8/        | 16 Tot               | al moths          | 42758                   |                       | 91696                   |                                    | 53.37 |
| 1995          |                      |                   |                         |                       |                         |                                    |       |
| 6/7-<br>7/12  | 38                   | H. vire-<br>scens | 2725                    | 3.59                  | 8245                    | 10.85                              | 66.95 |
|               |                      | H. zea            | 1867                    | 2.46                  | 5696                    | 7.49                               | 67.22 |
| 7/14-<br>8/16 | 35                   | H. vire-<br>scens | 6559                    | 9.37                  | 16275                   | 23.25                              | 59.70 |
|               |                      | H. zea            | 11375                   | 16.25                 | 35622                   | 50.89                              | 68.07 |
| 6/7-<br>8/16  | 73                   | H. vire-<br>scens | 9284                    | 6.36                  | 24520                   | 16.79                              | 62.14 |
|               |                      | H. zea            | 13242                   | 9.07                  | 41318                   | 28.30                              | 67.95 |
| 6/7-8/        | 16 To                | otal moths 2      | 22526                   |                       | 65838                   |                                    | 65.79 |