Effects of an Entomopathogenic Nematode and a Nuclear Polyhedrosis Virus on Emergence of *Heliothis virescens* (Lepidoptera: Noctuidae)[1]

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ABSTRACT Cage tests were conducted during the 1993 growing season to determine the effect of incorporating an entomopathogenic nematode (*Steinernema riobravis*) in soil under cotton on subsequent emergence of tobacco budworm, *Heliothis virescens* (L.). When soil under seedling cotton was treated with 240K nematodes per m^2 on 13 May, the number of moths emerging in cages was reduced by an average of 66%, compared to the untreated control, for at least 21 days following application. When a similar rate was applied on soil under mature cotton on 12 July, the number of moths emerging in treated cages after developing as larvae on the plants was 57% less over a 39 d period compared to the untreated control. In another cage study, application of the nematodes on wild geranium, *Geranium dissectum* L., an early-season host of tobacco budworms and cotton bollworms, *Helicoverpa zea* (Boddie), reduced adult emergence by 36% compared to untreated areas, whereas a single application of baculovirus from the celery looper (600 billion polyhedra per ha) reduced the emergence by 56%. In this latter test, adult emergence was further reduced (73% less than control) when a whitening agent was added to the virus application. These studies indicate than an entomopathogenic nematode, and the use of a whitening agent with baculovirus, might be useful in tobacco budworm management programs.

KEY WORDS Pest management, tobacco budworm, baculovirus, insect, nematode

The importance of early season host plants in the delta area of Mississippi in the buildup of the F_1 generation of the cotton bollworm, *Helicoverpa zea* (Boddie), and the tobacco budworm, *Heliothis virescens* (F.) (which subsequently invades cotton) was examined by Stadelbacher (1979, 1981). These investigations showed that a species of wild geranium, *Geranium dissectum* L., was the major early-season host in the area, producing as many as 17,000 adults per hectare of host. The rationale of attacking bollworm/budworm populations during this first generation, and possibly the second, was later addressed by Stadelbacher (1982) and Knipling and Stadelbacher (1983). They theorized that a 90% reduction in the population of moths emerging from early-season hosts in the delta would result in management of these pests in cotton in that area.

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In cage studies, a single application of the bollworm nuclear polyhedrosis virus (*H*NPV) (Tradename: ELCAR®) in water to *G. dissectum* resulted in a > 90% reduction in emergence of bollworm and tobacco budworm adults (Bell 1991, Bell and Hardee 1991). These studies provided the basis for open field tests where attempts were made to treat all weeds in large areas with *H*NPV in early spring as a technique for managing these pests (Bell and Hayes 1994, Hayes and Bell 1994, Bell and Hardee 1994). Results from pheromone trap data indicated that the single application of *H*NPV reduced tobacco budworm populations emerging from early-season hosts by approximately 25-38% and bollworm populations by approximately 19-31% in a 1990 test, and tobacco budworms by approximately 42% in a 1992 test. Bioassay and spray card data indicated very poor coverage in the 1990 test, due primarily to wind drift and evaporation of the water carrier before reaching the ground. Spray coverage was improved in the 1992 test, and cage data indicated an 81% reduction in tobacco budworm moth emergence. We speculated that the pheromone trap data failed to show the true reduction in moth numbers due to immigration from the surrounding untreated areas.

Although a reduction of 81% in the first population of tobacco budworm adults that move to cotton should have a major impact on cotton insect pest management in the area, other technology to further reduce the population or improve the management strategy is needed. The studies reported here were undertaken during 1993 to examine the effectiveness of a baculovirus from celery loopers, *Anagrapha falcifera* (Kirby) (*Af*MNPV), and an entomopathogenic nematode in early season management of tobacco budworms.

Materials and Methods

The virus (Hostetter and Puttler 1991) used for this study was obtained by infecting laboratory-reared tobacco budworm larvae and collecting the polyhedra using methods described by Vail et al. (1971), except that lactose was substituted for maltose as a carrier. The viral activity was examined and standardized through bioassay methods described by Dulmage et al. (1976) as modified by Bell and Romine (1986). The entomopathogenic nematode used, *Steinernema riobravis* (Rhabditida: Steinernematidae) (Cabanillas et al. 1994), was supplied by Biosys, Inc. (Palo Alto, CA). Virus and nematode applications were made using an atomizing sprayer (Spray-Pal, Delta Industries, Philadelphia, PA). Nematode applications were made in cage tests 2 and 3 (see below) two days after rain had thoroughly wet the soil.

Cage Test 1. The first study was conducted in cages erected over a stand of wild geranium, primarily *G. dissectum,* using procedures previously described (Bell 1991), to examine the effect of these entomopathogens on adult emergence. The test consisted of four replicates each of four treatments, arranged in a randomized complete block design. All treatments were applied in a volume equivalent to 46.7 liters of water per ha. The treatments were: *Af*MNPV at 100 larval equivalents (100 LE) or 6×10^{11} polyhedral inclusion bodies (PIB) per ha, *Af*MNPV at 100 LE + 94 g fluorescent brightener (Blankophor BBH, Miles, Inc., Rock Hill, SC) per ha, *S. riobravis* at 240K nematodes per m^2, and an untreated control. In order to assure a test population, the areas of weeds to be caged were infested with neonate tobacco budworm larvae mixed with fine vermiculite (Bell 1991) on 3, 5,

and 7 May, applying a total of approximately 200 larvae per cage. Treatments were applied on 14 May, when the ages of released larvae ranged from 7 to 11 days. Cages (3.66 by 7.32 by 1.83 m tall) made of 20 by 20 mesh plastic insect screen were then placed over each plot on 18 and 19 May. The cages remained undisturbed until the beginning of moth emergence. Cages were then searched daily and the moths captured and removed. Numbers and species of moths were recorded. The total numbers of moths captures per cage by 2 July were analyzed by the procedures of analysis of variance (ANOVA, $P = 0.05$), and differences among means were examined using the method of least significant difference (LSD; SAS Institute 1989).

Cage Test 2. The second study was conducted to examine the effect of early-season treatment of soil with the nematode, *S. riobravis,* on emergence of tobacco budworm adults. Three random areas (2.44 by 3.35 m) in a field of seedling cotton were marked with stakes, and nematodes applied on 13 May at a rate of 200K nematodes per m^2 using 1 liter water per plot. The temperature on the day of application ranged from a low of 14°C to a high of 24°C and was 21°C at the time of application. Immediately after application, the soil was gently worked with a hoe to incorporate the nematodes into the top 5 cm of soil. Three similar areas of the same field were designated as the untreated control and were similarly tilled. After 2 h, a 0.84-m^2 plot in each of the treated and untreated areas was infested with 30 fifth-instar tobacco budworms, and the larvae were covered with loose soil. Each area was then covered with a 0.91 by 0.91 m emergence cage, consisting of a wood frame, a wire cone, and an adult capture device at the top of the wire cone. The cages were set up and left undisturbed, except for removing captured adults, until 5 d after emergence had ceased. The infesting and caging procedure was then repeated on 20 and 27 May, and 3 June (7, 14, and 21 d after nematode application) for a total of 24 cages. Emergence data were examined for significant difference using the t-test ($P = 0.05$) procedure (Steele and Torrie 1960).

Cage Test 3. The third study was conducted to examine the effect of mid- to late-season soil treatment with *S. riobravis* on tobacco budworm adult emergence. This test consisted of two nematode treatment rates and an untreated control arranged in a randomized complete block design with four replications. The two treatment rates were 200K and 1 million nematodes per m^2 of soil surface. Twelve cages (1.83 by 3.66 × 1.83 m tall) made of 20 by 20 plastic mesh screen were erected over cotton ('DES 119', planted on 6 May). The soil in each cage was tilled with a forked hoe prior to application of the nematodes on 12 July. The temperature on that day ranged from 23°C to 35°C and was 32°C when the nematodes were applied. The nematodes, suspended in 3.79 liters total volume of water per cage, were applied to the soil surface using a 5.68-liter pump sprayer. On 13 July, 30 second-instar and 30 fourth- to fifth-instar laboratory-reared tobacco budworm larvae were placed on cotton terminals and fruiting bodies, respectively, in each cage. Additional larvae (second to fourth instar) were placed on the caged plants on 26 July and 5 and 18 August for a total of 150 larvae in each cage. Cages were searched for moths daily Monday - Friday between 21 July and 8 September, and cumulative numbers of tobacco budworm moths emerging during each of the three time periods were recorded and analyzed separately. The data were analyzed by the procedures of analysis of variance (ANOVA, $P = 0.05$) and differences among means were examined using the method of least significant difference (LSD; SAS Institute 1989).

Results and Discussion

Laboratory bioassays and counts of the NPV used in this study showed the virus produced mortality ranges similar to a standard virus originally received from D. L. Hostetter (USDA, ARS), and indicated a polyhedral count of 24 billion PIB/g of preparation.

Cage Test 1. The first moth emerged in the caged weeds on 11 June, approximately 10 d later than the start of emergence in previous tests (Bell 1990, 1991), and emergence was complete by 30 June. The late emergence and long emergence period, compared to the previous tests, might have resulted from cooler than normal spring weather during the period. All treatments had significantly lower cumulative moth emergence than the untreated control ($F = 10.52$; df = 3, 9; $P \leq 0.05$). The dates and pattern of moth emergence in cages are illustrated in Fig. 1. Treating the wild geranium with *S. riobravis* resulted in a reduction of 36% in adult tobacco budworm emergence compared to the cumulative totals from untreated cages. Application of *Af*MNPV alone reduced the emergence by 56%, and application of the virus plus the fluorescent brightener reduced the emergence by 73% compared to the untreated cages. Increased effectiveness of *Af*MNPV due to the addition of the brightener when used for control of lepidoptera in crops was previously reported by Vail et al. (1993). Results of this test indicate that the brightener might be of value in increasing the activity of the virus when used on alternate hosts as well. *Helicoverpa zea* moth emergence in the cages was very low for unexplained reasons, and the data were not considered in this test. Although the effects of the virus treatments were somewhat less than those reported when *H*NPV was used, the decreased effect might have been due to the larvae being later instar at the time of application compared to earlier tests.

Cage Test 2. Application of nematodes to the soil in a cotton field on 13 May reduced the overall adult emergence from released tobacco budworm larvae by 66% compared to the untreated control. The effect of the nematodes persisted for the 21-d period after application (Fig. 2). Of the 270 larvae released on untreated soil on 20 and 27 May and 3 June, 46.7% emerged as adults, compared to 16.7% of those larvae placed in treated cages. No emergence was recorded from larvae placed in treated cages on 13 May; however, emergence was very low in the untreated areas (6.7%). Adult emergence was significantly less in treated cages at 7, 14, and 21 d after application based on the *t*-test procedure ($t = 4.76$, 7.09, and 3.02, respectively) ($P \leq 0.05$, df = 4). Although as great an effect would be expected at day 0, there was an apparent effect on emergence due to contamination, weather, or some factor not explained by the experiment, which obscured the treatment effect.

Cage Test 3. When tobacco budworm larvae were placed on cotton plants in cages during July and August following soil treatment with nematodes on 12 July, there was a significant reduction ($P = 0.05$, LSD) in adult emergence until 39 d after application (20 August) in all treatments compared to the untreated control (Fig. 3). The first adult tobacco budworms emerged on 23 July and the last adult was captured on 31 August. During the period of 23 July through 5 August, moth emergence in cages was reduced by 63 and 54% when the soil was treated with 1M or 200K nematodes, respectively (LSD = 7.58, df = 6). From 6 August until 20 August, adult emergence in cages treated with 1M or 200K

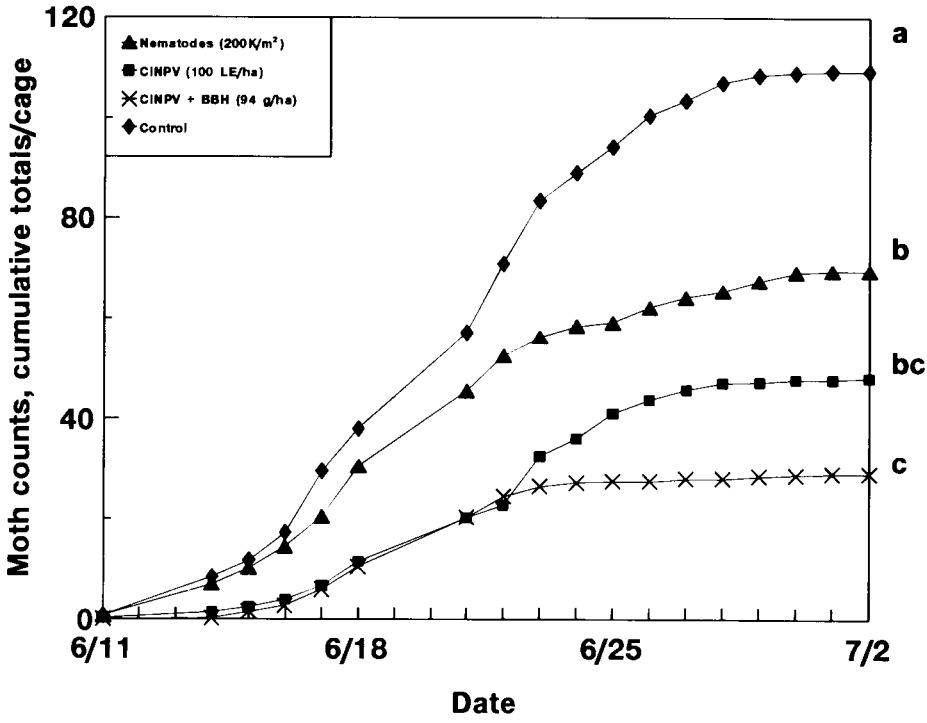

Fig. 1. Effect on emergence of *Heliothis virescens* adults from early season host plants due to a single application of various microbial treatments on 14 May (average of 4 cages per treatment)., Means shown for 2 July followed by the same letter are not significantly different (P = 0.05) (LSD, SAS 1989).

nematodes was reduced by 65 and 61%, respectively (LSD = 3.44, df = 6). Differences due to the rate of nematode application were not significant. Although the treated cages produced fewer moths, there were no significant emergence differences between treatments and the control from 40 - 50 d after application (21 - 31 August). Of the 600 larvae placed in untreated cages, 127 emerged as adults, compared to 61 of those released in cages where the soil was treated with nematodes at 200K per m^2, and 52 in cages having the 1M per m^2 treatment. The results of this test indicate a 57% reduction in adults emerging from the soil within 39 d after treatment with the 200K rate.

The results of these cage studies indicate that populations of tobacco budworms may be reduced by early-season application of the NPV from the celery looper, *Af*MNPV, on wild hosts, and further reduced by application of *S. riobravis* to the soil beneath host crops during the growing season. The celery looper NPV is known to have a broad host range compared to the virus currently under consideration for large area management of the tobacco budworm (Hostetter and Puttler 1991),

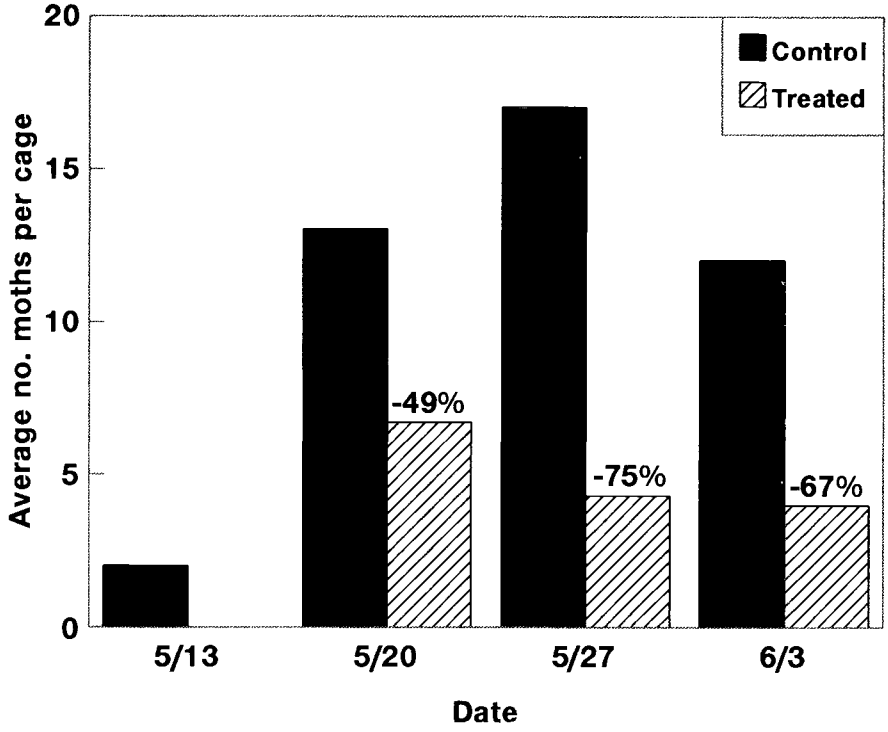

Fig. 2. Effect of treatment with *Steinernema riobravis* (200 K nematodes per m^2) on emergence of *Heliothis virescens* adults from larvae placed on soil at various dates after treatment on 13 May (average of 3 cages per treatment).

and, therefore, its use in large area management programs might produce benefits in managing other lepidopterous insect pests. Although the present cost of producing the nematodes is considered prohibitive to its utilization in large area programs, results of these tests indicate that it could be used to further reduce populations of tobacco budworms during the growing season. A combination of treating early-season hosts with virus, followed by application of nematodes in host crop areas, should produce additive effects toward seasonal management of this pest. For instance, if a wide area application of virus on early season hosts that reduced the first seasonal generation by 70% was followed by an application of nematodes in all crop areas, thus reducing the first generation developing on crops by 60%, the combined effect on the second generation in crops should approach an 88% reduction (70% plus 60% of the remaining 30%) compared to the expected population.

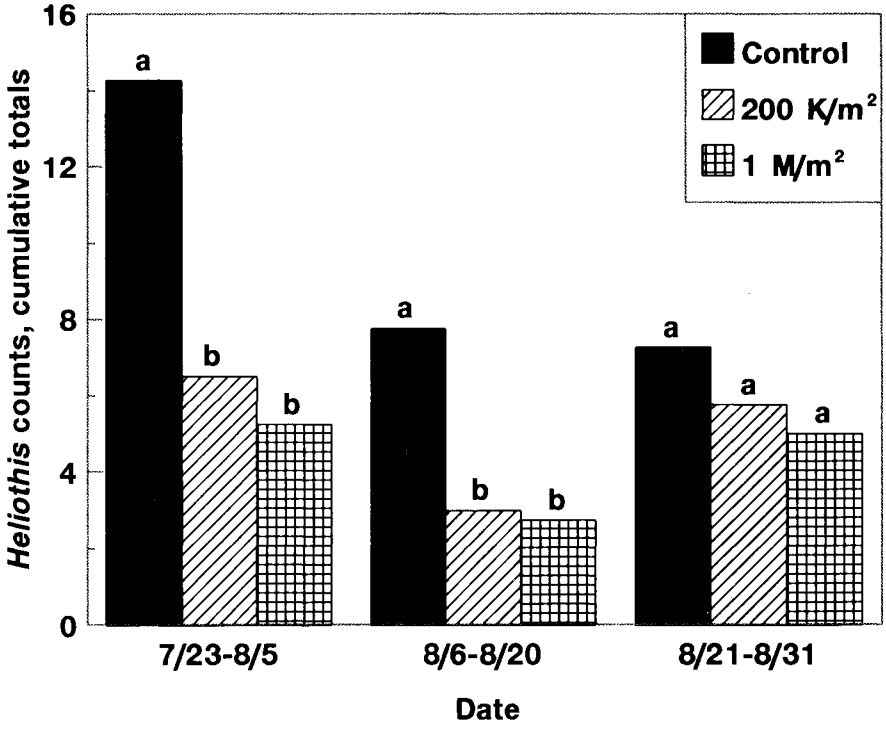

Fig. 3. Effect of soil treatment with *Steinernema riobravis* on emergence of *Heliothis virescens* adults from larvae placed on caged cotton plants at various dates after treatment with nematodes on 12 July (average of 4 cages per treatment). Means followed by the same letter within each time period are not significantly different ($P = 0.05$) (LSD, SAS 1989).

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