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BIOLOGICAL AND MICROBIAL CONTROL

Evaluation of Early-Season Baculovirus Treatment for Suppression of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) Over a Wide Area

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ABSTRACT Pheromone trap counts of F_1 male cotton bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), were used to assess the effect of areawide suppression achieved by early-season application of a *Helicoverpa/Heliothis*-specific nuclear polyhedrosis virus. Eggs (F_2) were collected from cotton and other hosts to characterize the surviving reproductive populations. Trap and egg collection sites were established at 1.6-km intervals ($n = 5$) in four cardinal directions from the center of control and treated plots (259 km^2, ≈10 by 10 mi). Traps also were placed at five additional intervals in four cardinal directions beyond the treated plot to assess the effect of dispersal. The effect of treatment was demonstrated by deviations in trap capture patterns within a year between treated and control plots and between years in the treated plot. Rates of increase between generations were calculated from the number of moths captured in one generation divided by the number from the previous generation. The rate of increase for the first field generation of *H. virescens* in the treated plot (1990) was 13% compared with 38% in the control plot (1990) and 38% in the treated plot in the year before treatment (1989). The rate of increase for the first field generation of *H. zea* was 36% in the treated plot (1990) compared with 55% in the control plot (1990) and 95% in the treated plot the year before treatment (1989). Rates of increase for both species in the subsequent generation remained low in the treated plot (1990) compared with the control plot (1990) and with the previous year (1989). Our results indicate that a single virus application can reduce the adult *H. virescens* and *H. zea* populations emerging from alternative hosts present early in the season. Other methods for improving the efficacy of treatments are discussed.

KEY WORDS *Heliothis/Helicoverpa*, baculovirus, area-wide evaluation

EVALUATION OF THE SUCCESS of suppression tactics over large areas presents serious challenges to standard small-plot or field-size experimental procedures. The task is particularly difficult in an open and heterogeneous agroecosystem, where the targets are highly polyphagous and mobile like the members of the *Heliothis/Helicoverpa*-complex. Available funding and practical considerations (operational and logistical) preclude the level of replication necessitated by the presumed plot-to-plot variability. For such large-scale entomological studies, Schneider (1989) reviewed three approaches. The optimal approach was a 2-yr study with reversal of treatment and control sites in alternate years. In this approach, both year and location served as replicates or, more accurately, standards for comparison. Other investigations, such as watershed studies, commonly use historical data and reference sites as standards for comparison.

The evaluation design for the *Heliothis* nuclear polyhedrosis virus (HNPV) pilot test discussed by Bell & Hayes (1994) followed the design described by Schneider (1989). In 1990, two 259-km^2 (16 by 16 km) plots were established in an intensive cotton production area in the delta region of Mississippi. One plot was designated the control and the other plot was designated as the treatment plot for year 1. In principle, these designations would be reversed in year 2 of the experiment. However, historical data were available for the area designated as the treatment plot from a long-term movement study conducted in approximately the same location from 1987–1989 (Hayes 1990). In the Bell & Hayes study (1994), both male adult counts from routine pheromone trap sampling and egg counts from inspections of terminals of cotton were taken. These variables were used to assess the effect of the HNPV treatment in 1990 (Hayes 1990).

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The purpose of the current study was to monitor and evaluate the tobacco budworm, *Heliothis virescens* (F.), and the cotton bollworm, *Helicoverpa zea* (Boddie), populations after treatment with an HNPV. We sought to determine if and to what extent the areawide populations of this pest complex were affected by the treatment. Here we describe results of pheromone trap counts from the parent (overwintering) generation through the second field generation F_2; we compare rates of increase of both species in the treated plot with a control and with the treated plot in the year before treatment. Results for F_2 egg collections made on cotton and other hosts are reported. We discuss ways to improve effectiveness of the treatment.

For *H. virescens* and *H. zea*, egg counts are the most valuable measure of abundance because current economic thresholds on cotton are based on egg numbers (calculated as percentage of terminals infested; e.g., Mississippi Control Guide, Mississippi Cooperative Extension Service). However, obtaining adequate infestation data is laborious and impractical on a large scale. During the early part of the growing season, data analysis is complicated by the rapid changes in host attractiveness. Field-to-field variability is high because planting times vary among growers and developmental phenology is dependent on soil and weather conditions, which fluctuate widely at this time of the year. Until cotton reaches the pinhead-square stage of development, oviposition by *H. virescens* or *H. zea* is relatively rare (Hayes et al. 1988, Hayes & Coleman 1989, Hayes 1990). Simultaneously, another solinaceous species and common weed in early-season cotton, *Abutilon theophrasti* Medikus (velvetleaf or wild cotton), is used heavily by ovipositing females. Later, as cotton becomes more attractive and cultivation of fields eliminates much of the velvetleaf, cotton is used increasingly for oviposition.

Although we cannot ascribe an economic value to capture numbers on a day-to-day basis, pheromone traps are reliable sampling tools for monitoring *H. virescens* and *H. zea*. The primary problem with use of pheromone traps is lack of appropriate life table data to permit estimation of egg or larval densities from male capture numbers (Fitt 1989). However, extensive studies of trap performance (Hayes et al. 1988; Lopez et al. 1988; Hayes & Coleman 1989; Hayes 1990, 1991b; Witz et al. 1990, 1991) have shown that trap capture data accurately reflect the relative abundance and fluctuations in the local adult male populations. Whereas all trapping devices have inherent biases, they are far more consistent than sampling efforts by humans. Thus, we used pheromone trap counts to assess the degree of suppression achieved by early-season application of HNPV. Egg count data were used to characterize the surviving reproductive population (e.g., host, insect species composition, and distribution). The effect of treatment can be demonstrated by deviations in trap capture patterns between treatment and control plots within a year and between years in the treatment plot.

Long-range and mesoscale movement of *H. virescens* and *H. zea* are significant in areawide control (Knipling & Stadelbacher 1983, Schneider et al. 1989). For the treatment plot, we extended sampling of both adults and eggs beyond the treatment boundary to assess the effect of dispersal. Previous studies by Schneider et al. (1989) and Hayes (1991a) showed that *H. virescens* and *H. zea* can move as far as 20 km and can typically move 3-8 km per generation, depending on environmental conditions. Thus, we expected that HNPV application would be most apparent in the center of the treatment plot and would dissipate at the borders. However, spatial heterogeneity in the area is high and could obliterate the predicted effect of treatment. For both reasons, detectable suppression was likely to be *swamped out* from one generation to the next.

Materials and Methods

Preparation, application, direct evaluation of HNPV performance, a general description of the study area, and specific attributes of the treatment and control plots are described elsewhere (Bell & Hayes 1994). Only those procedures specific to the areawide field evaluation of the study are described in detail here.

Study Design. In both treatment and control plots, at least four sampling sites were established within each 1.6-km interval (radius) from the center (Fig. 1). On the basis of availability of accessible cotton fields, we spaced sampling sites per interval in different quadrants corresponding to cardinal points. In the control plot, sampling extended to 8 km (five intervals). In the treatment plot, sampling was extended to 16 km (10 intervals) to permit us to assess the effect of migration on the treated area. (Hereafter, intervals 1–5 are referred to as the treated subplot and 6–10 as the untreated subplot.) At each site, two traps (one trap per species) separated by ≈100 m were placed at the edge of a cotton field along an accessible roadway. Because fields are frequently cultivated and roadsides are often mowed or burned, traps were placed near power poles and in other protected sites. The nearby cotton field(s) was routinely searched for eggs.

Moth Sampling. Standard 75–50 hardware cloth, cone-shaped pheromone traps (Hartstack et al. 1979) were used to monitor the relative abundance and fluctuations in the adult male *H. virescens* and *H. zea* populations in the treatment and control plots. Traps were routinely monitored from 1 April to 1 August (day of year 91 to 212). This period encompassed the flights

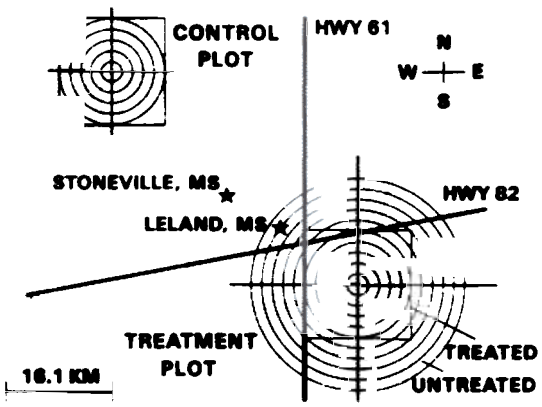

Fig. 1. Schematic diagram of 1990 HNPV early-season treatment study area near Stoneville, MS, and evaluation design; abundance of *Heliothis/Helicoverpa* adults and eggs was monitored at four locations per interval (1.6-km radius) from the center of the control and treatment (treated and untreated subplots) plots.

of the emergent overwintering or migrant parental generation (P) and first two field generations (F_1 and F_2). Using the method described by Hayes (1991a), we emptied traps three times per week (Monday, Wednesday, Friday), at which time captured moths were counted and recorded. To estimate mean per trap count per day, trap collections were divided by the number of days of operation between collections. Pheromone lures (Zealure, Hercon Environmental; Virelure, Scentry, Buckeye, AZ) were replaced biweekly. Traps were repaired as needed and the area under each trap was kept clear of vegetation.

Egg Sampling. In both control and treatment plots, one to three cotton fields near each trap location were searched routinely for F_2 eggs (and larvae, if present) during the flight of the F_1 adults. We sampled multiple sites per location to ameliorate the site-to-site variability in crop phenology. The developmental stages of cotton ranged from cotyledon to pinhead square when sampling began and from pinhead square to flowering when sampling ended. Velvetleaf was initially prevalent in many fields and was sampled along with cotton. Some sites contained corn or velvetleaf only and were sampled to assess the overall rate of oviposition and species composition in each plot more accurately.

Each field was searched by one or more field crew members (who inspected terminals at random) for 30 min at intervals of 2–3 d (data were adjusted to account for number of samplers per visit and frequency of visits per site where appropriate). Crop growth stage was recorded at each visit. All eggs (or larvae) encountered were collected in 30-ml plastic cups, kept separate by location and host, and returned to the laboratory for processing. Eggs were counted and all (if <20 eggs) or a proportion (not <20% of eggs for samples >20) were placed on an artificial diet for rearing to the adult stage for species identification. If 21–100 eggs were collected, 20 eggs were placed on the diet. If 101–125 eggs were collected, 25 eggs were placed on the diet. If 126–150 were collected, 30 eggs were placed on the diet, and so on. Species composition of these subsamples were used to estimate the proportion of *H. virescens* and *H. zea* per sample.

Data Analysis. Rate of increase (r_i) between generations was calculated by taking the natural log of the total number of moths captured in a generation (n_i) and dividing the resulting number by the total number of moths captured in the previous generation ($n_{(i-1)}$). Generations (P = n_0, $F_1 = n_1$, $F_2 = n_2$) were defined with both egg collection and trap collection data, i.e., by including the duration of the oviposition period and the corresponding period of trap count increase.

Results and Discussion

Moth Sampling. Mean trap counts per day for both species over the 1990 sampling period (P, F_1, and F_2 generations) from the control and treatment (treated and untreated subplots) plots are shown in Fig. 2. Typically, we observed phenological differences between the two moth species across plots (e.g., 1989) (Fig. 3). For the F_1 or treated generation (day of year 137 to 177), a sharp increase in trap captures of *H. zea* began on DOY 152 in all plots, whereas trap captures of *H. virescens* showed a less dramatic increase 10 d later (day of year 162) (Fig. 2).

We observed differences in species composition. In the control plot, *H. zea* was the predominant species (>75% of all moths captured during the F_1 generation). In the treatment plot, the count was nearly equal (54% in treated subplot, 49% in untreated subplot). Exemplifying one of the problems with conducting controlled field trials at this scale, these differences in species composition between plots were expected given the land area involved. The control plot is more northerly and is situated in closer proximity to the Mississippi River than the treatment plot. Although the distance between the centers of the two plots was only ≈30 km, the combination of weather and soil differences may have been sufficient to account for the observed differences in both host phenologies and *H. virescens*:*H. zea* ratios.

Regardless of proportional differences in species composition, trap captures for both species increase with each successive generation until midseason under normal conditions (e.g., 1989, Fig. 3). From the parental to F_1 generations, mean captures per day may increase twofold. Visual inspection of the trap capture patterns for the sampling period revealed deviations from ex-

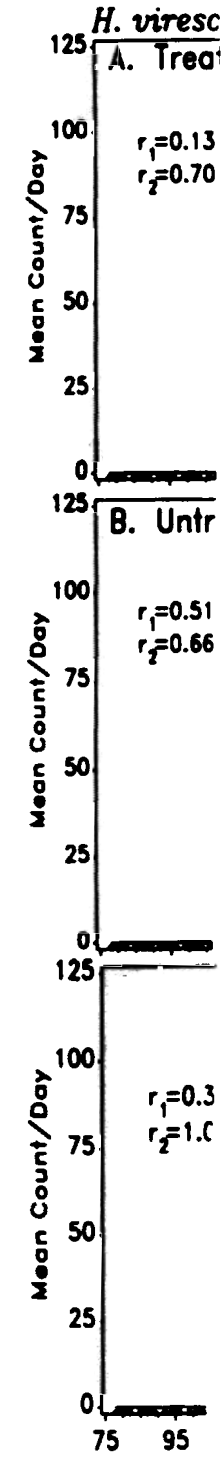

Fig. 2. Mean pheromone traps of *H. zea* males (e) untreated sub as $\ln n_i/\ln N_{i-1}$

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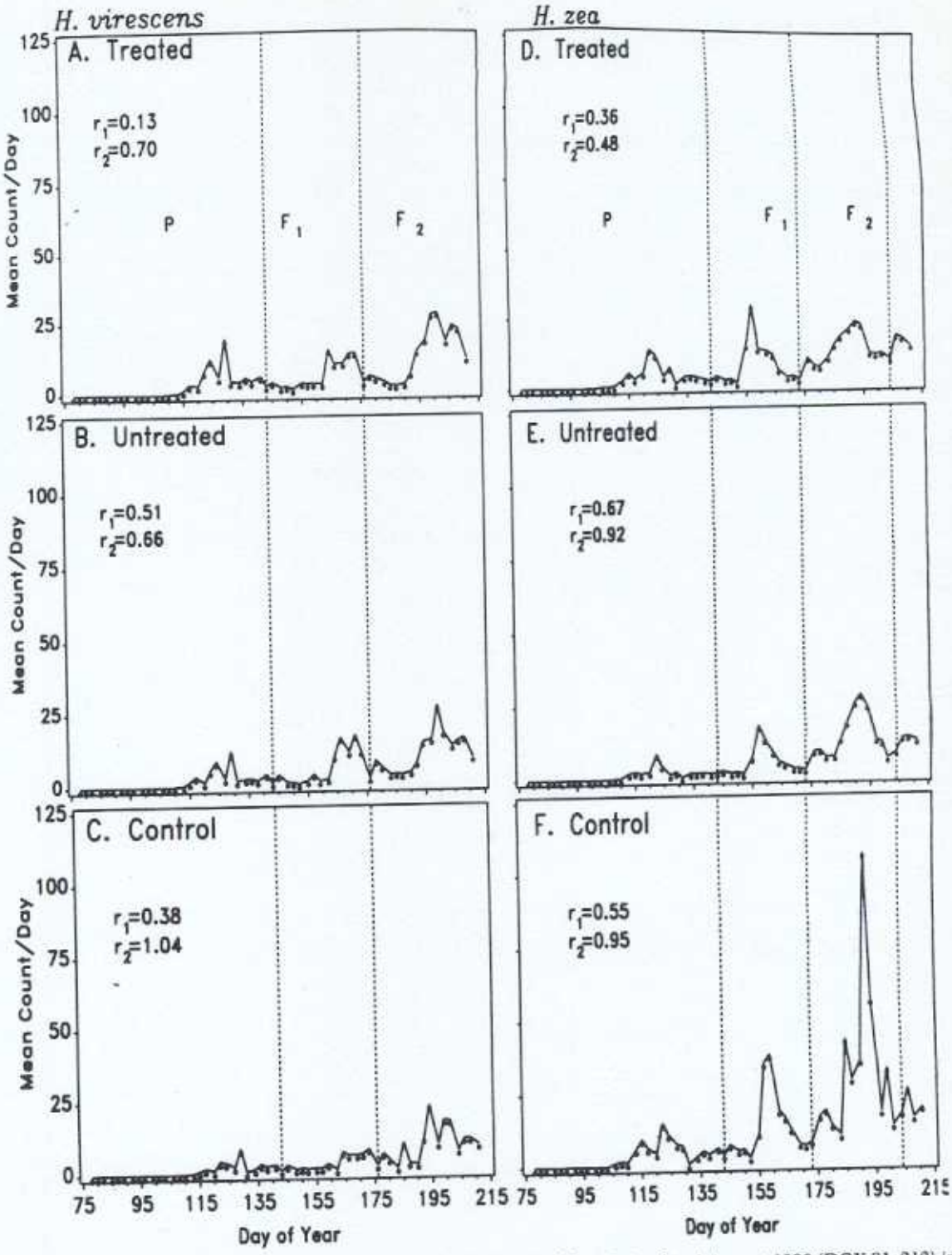

Fig. 2. Mean number per day of *H. virescens* males captured from 1 April to 1 August 1990 (DOY 91–212) in pheromone traps in the (a) control plot, (b) untreated subplot, and (c) treated subplot; and mean number per day of *H. zea* males captured from 1 April to 1 August (DOY 91–212) in pheromone traps in the (d) control plot, (e) untreated subplot, and (f) treated subplot. Rates of increase (r_i) for the first and second generations (calculated as $\ln n_i/\ln N_{i-1}$ where n_i is number of moths per generation, P, F_1, and F_2) are given.

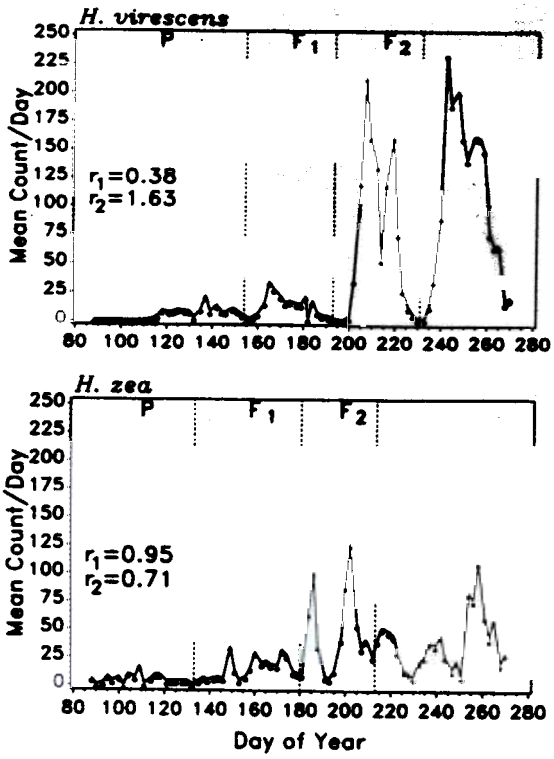

Fig. 3. Mean number per day of (a) *H. virescens* and (b) *H. zea* males captured in 1989 (DOY 91–212) in pheromone traps in the area treated by HNPV in 1990. Rate of increase (r_i) for the first and second generations (calculated as $\ln n_i/\ln n_{i-1}$ where n_i is number of moths per generation, P, F_1, and F_2) are given.

pected catch in the treated subplot (treated in 1990) compared with the previous year (1989) and with the control plot and untreated subplot for 1990 (Figs. 2 and 3). In the treated subplot, *H. zea* initially increased during the F_1 generation; however, F_1 *H. virescens* failed to reach a mean capture rate per day that exceeded the parental generation peak. At the same time, in the adjacent untreated subplot, both *H. virescens* and *H. zea* patterns appeared normal (i.e., peak mean captures per day during the F_1 generation exceeded the parental generation peaks). In the control plot, the mean captures per day peak exceeded the parental generation peak and continued to climb after the treated and untreated subplot values showed a decline.

Calculations of rate of increase (r_i) confirmed our visual assessments of trap capture data (Figs. 2 and 3). In general, the rate of increase for both species in all plots in 1990 was lower than those measured for 1989; the rate of increase for *H. zea* in both years was higher than that for *H. virescens*. Both species had lower rates of increase in the treated subplot compared with the untreated subplot and control plot. The rate of increase for *H. virescens* in the treated subplot was 13% compared with 38% for the control plot and 51% for the untreated subplot. The rate of increase for *H. zea* in the treated subplot was 36% compared with 55% for the control plot and 67% for the untreated subplot.

For both species in 1989 (Fig. 3), the rate of increase between the second and third generations (r_2) remained high; *H. virescens* showed a substantial increase which coincided with the typically observed high field infestation around 4 July. The 1990 results show comparably high r_2 values for both species in the control plot and in the untreated subplot for *H. zea* (Fig. 2). In the treated subplot area, the change in r values (r_1 versus r_2) is comparable (i.e., ≈60% for *H. virescens* and 15–20% in *H. zea*); however, neither species appeared to recover completely from the loss during the next generation (i.e., *H. virescens* in the control plot showed a 104% rate of increase versus 70% in the treated subplot; *H. zea* in the control plot had a 95% increase versus 48% in the treated subplot). Relatively low values in the untreated subplot (especially for *H. virescens*) may reflect the influence of the neighboring treated subpl into the untreated su

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Egg Sampling. H tectable level of sup HNPV treatment, we (F_2 generation) to pr about the reproduc (treated) generation and abundance of o tion for the control a parent in the compa ple site over the sar were detected first earlier in the treat than in the control pl in local planting co

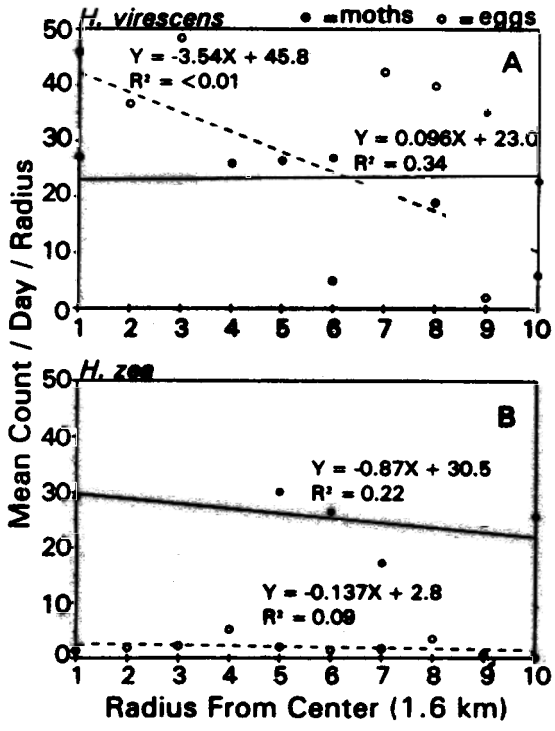


Fig. 4. Frequency of moths (mean number trapped per day; solid circles) and eggs (mean number per site per day; open circles) for: (A) *H. virescens* and (b) *H. zea* collected at intervals (1.6-km radius) from center of treatment plot. Line fitted by regression (dashed-line, moths; solid line, eggs): for *H. virescens* moths, r^2 = 0.04209 and eggs, r^2 = 0.5852, and for *H. zea* moths, r^2 = 0.4684 and eggs, r^2 = 0.3000.

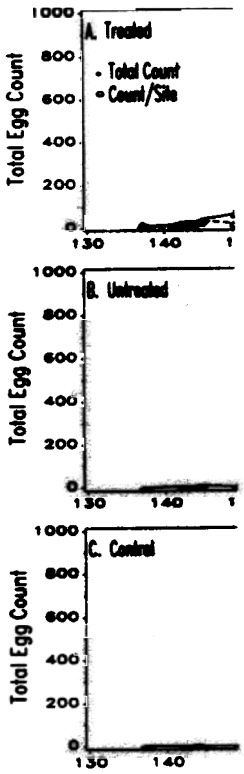


Fig. 5. Total numb site) of eggs (F_2) colle the flight period of F_1 (B) untreated subplot,

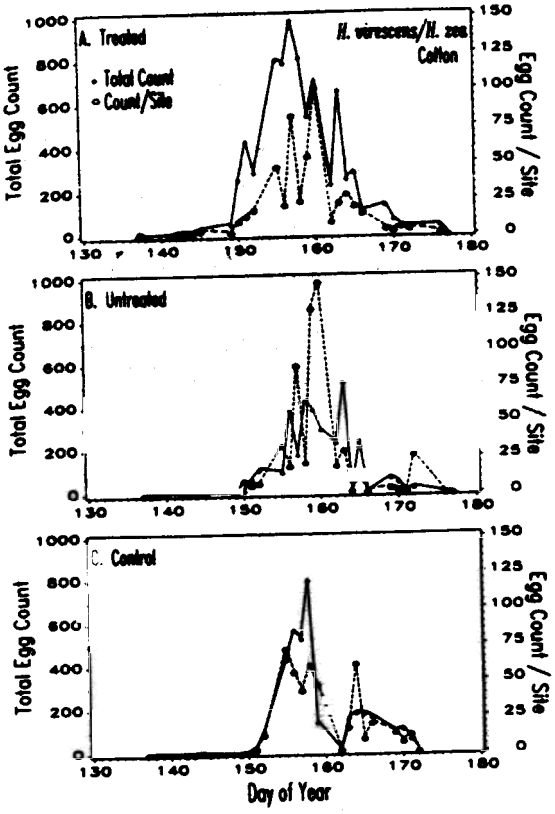


Fig. 5. Total number and frequency (mean count/site) of eggs (F_2) collected from cotton fields during the flight period of F_1 adults in the (A) control plot, (B) untreated subplot, and (C) treated subplot.

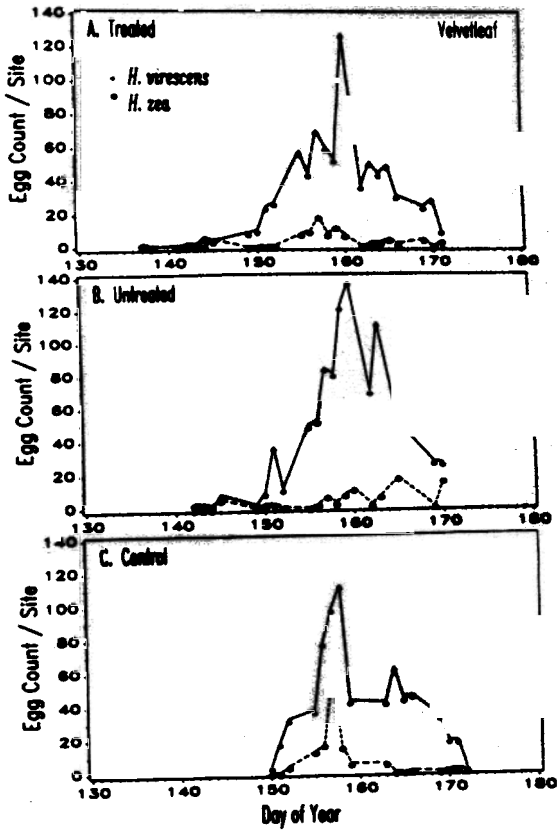


Fig. 6. Frequency of oviposition by *H. virescens* and *H. zea* on velvetleaf in: (A) control plot, (B) untreated subplot, and (C) treated subplot.

boring treated subplot (i.e., fewer insects moved into the untreated subplot).

The possible effect of movement was not apparent in the pheromone trap collection data (Fig. 4). Neither linear nor nonlinear regression analysis indicated significant relationships between mean captures of either species and distance from the center of the treatment plot. This result is not surprising given the high degree of spatial heterogeneity on the area and the high degree of temporal heterogeneity imposed by the elongated applications period (21 d).

Egg Sampling. Having determined that a detectable level of suppression was achieved with HNPV treatment, we analyzed egg sampling data (F_2 generation) to provide additional information about the reproductive effort of the affected (treated) generation (F_1). Differences in timing and abundance of oviposition by the F_1 generation for the control and treatment plots were apparent in the comparison of mean eggs per sample site over the sampling period (Fig. 5). Eggs were detected first at low levels nearly 2 wk earlier in the treatment plot (treated subplot) than in the control plot. In addition to differences in local planting conditions, a major source of difference may have been the availability of early-season hosts. Overall, nearly twice as many eggs were recovered from the treatment plot compared with the control plot (57% of all eggs were recovered from the treated subplot versus 22% from the untreated subplot and 21% from the control plot). In any year, however, local differences in abundance during early generations may disappear during the season as population levels build in subsequent generations, particularly as host crops become more prevalent.

Velvetleaf is heavily used as a host and appeared to be more prevalent in the treatment plot. Using the species proportions generated from subsamples reared for identification, we determined the distribution of eggs by species per host for each plot (Figs. 6 and 7). In addition to cotton fields, where both cotton and velvetleaf were sampled, a few small patches of corn were searched for eggs. Corn is a preferred host of *H. zea*, and nearly all (99%) of the eggs recovered were *H. zea*. However, given the relatively small area devoted to corn production, this alternative host is not likely to be sufficient to account for the large number of adult *H. zea* moths captured in traps (compared with the few eggs recovered).

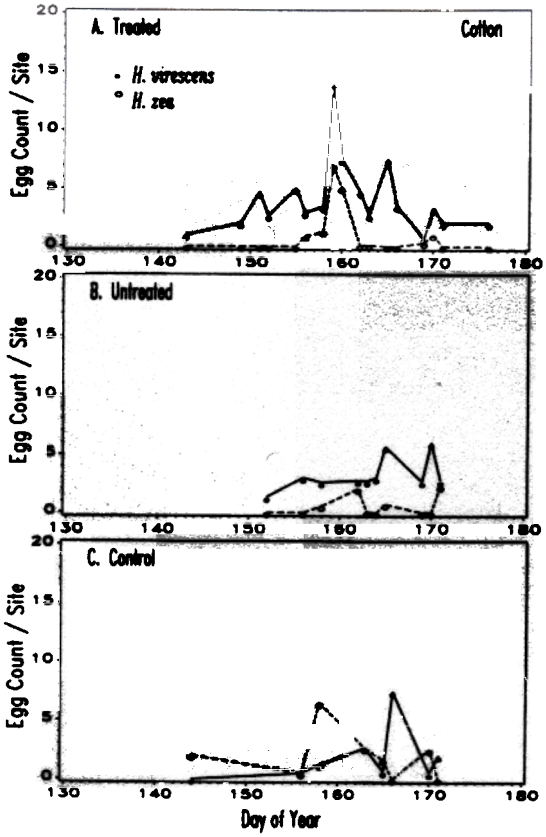


Fig. 7. Frequency of oviposition by *H. virescens* and *H. zea* on cultivated cotton in: (A) control plot, (B) untreated subplot, and (C) treated subplot.

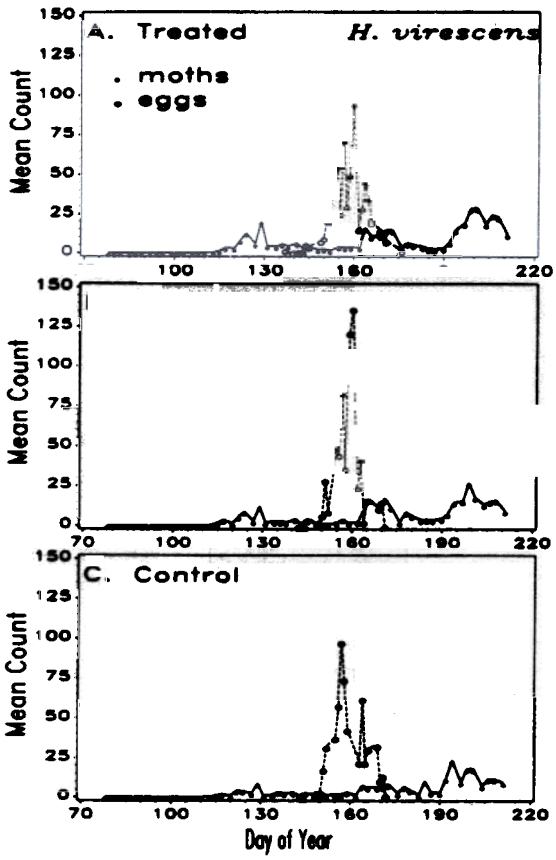
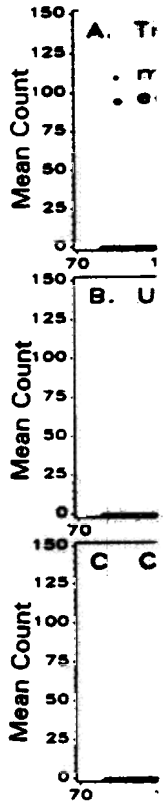


Fig. 8. Mean number per day of *H. virescens* males captured and eggs collected during the sampling period in the: (A) control plot, (B) untreated subplot, and (C) treated subplot.

The obvious predominance of *H. virescens* revealed by rearing is consistent with results from previous studies in this area (e.g., Hayes 1991b). Despite the capture of relatively high numbers of *H. zea* in pheromone traps (i.e., often equal to or higher than *H. virescens*), the incidence of oviposition by *H. zea* remained significantly lower than *H. virescens* in cotton fields throughout the growing season. Previous studies show that this difference in oviposition persists in subsequent generations and throughout the growing season (Hayes 1990, 1991b). Differences in composition of adult (male) between plots was not as apparent in species composition of eggs collected from nearby fields (14% versus 10% *H. zea* in control versus treatment plots).

The high incidence of oviposition on velvetleaf by *H. virescens* (and to a lesser extent *H. zea* [Fig. 6]) suggests that velvetleaf has high potential for population management. Apparently, well-timed cultivation would contribute substantially to suppression of moth numbers in subsequent generations. More sophisticated trap crop systems may be designed to take advantage of this attractive host (at least during this time frame) and to draw substantial oviposition away from cultivated cotton and into a relatively easily managed situation (e.g., with limited pesticide or herbicide treatment and mowing).

Comparison of egg and trap count data (Figs. 8 and 9) confirmed previously observed temporal relationships (Hayes et al. 1988, Hayes & Coleman 1989, Hayes 1990, Witz et al. 1990). The significant correlation coefficients for *H. zea* egg and trap counts over time were obtained by comparison of same day (egg DOY = trap DOY) or 1-d lag (egg DOY = trap DOY 1), suggesting that pheromone traps might be useful devices to indicate an increase in number of damaging immatures of this species within a field. However, significant correlations for *H. virescens* were obtained with a delay of 7 and 8 d (egg DOY = trap DOY 7 or 8); the highest coefficient was obtained at a delay of 8 d in the treated subplot (r = 0.8235; n = 9; $P < 0.0064$). Thus, trap collections showed a corresponding increase in moth numbers a full week or more after egg counts began to increase; therefore, traps do not provide timely information for prediction of infestations of *H. virescens*.



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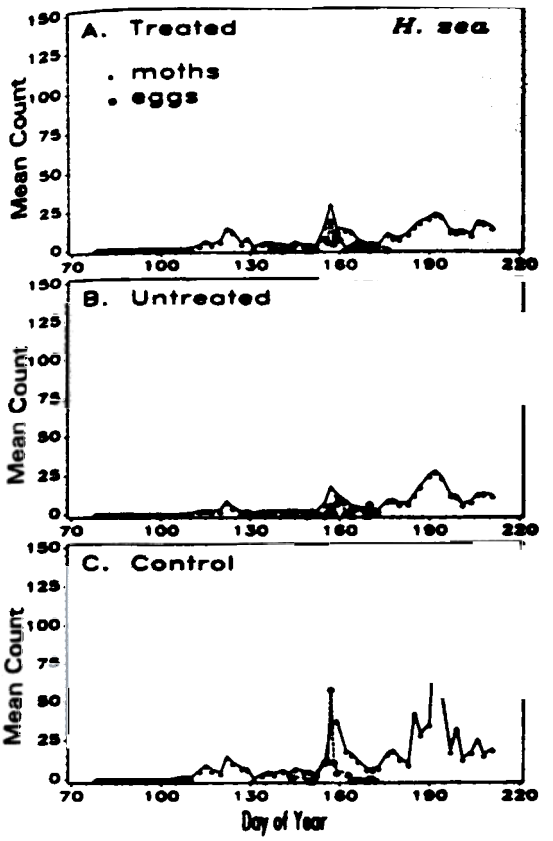

Fig. 9. Mean number per day of *H. zea* males captured and eggs collected during the sampling period in the: (A) control plot, (B) untreated subplot, and (C) treated subplot.

In summary, our results indicated that the single virus application reduced the adult *H. virescens* and *H. zea* populations emerging from alternative hosts present early in the season. Although the treatment failed to reduce the adult population as much as was expected on the basis of preliminary small-scale studies, these results are encouraging. The primary factors affecting the overall success of the treatment were lack of spray coverage and timing of application. Spray coverage was adversely affected by windy conditions during the application period. Because of high winds, appropriate aerial application conditions were also limited to narrow time periods, with the result that the application period extended for 21 d. During this time, eggs laid earliest in the season would reach pupal stage before treatment reached all areas of plot and would thereby escape. This pattern may explain why the *H. zea* population in the treated subplot appeared to experience a sudden drop in the rate of increase because this species was the first to emerge in the plot. Ideally, the application of baculovirus would be made when the last eggs laid are hatching and the larvae of the first eggs laid are in the last instar. Use of additional aircraft may dramatically decrease the application time. Unfortunately, poor application conditions cannot be overcome as simply, but could be offset by multiple rather than single application; doubling the application rate would ensure improvement in coverage.

These results also have important implications for evaluation technology, particularly the value of pheromone traps for this purpose. Trap calibration still has serious problems, but trap capture data appear to reflect the fluctuations in the population abundance necessary to monitor response to treatments. Additional studies of adult demography (dispersal and survivorship) are needed to unravel the relationship between oviposition and male flight. Finally, the high incidence of oviposition on velvetleaf versus cotton suggests a second and environmentally sound suppression tactic to compliment the use of HNPV.

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