EFFECT OF THE *HELIOTHIS* NUCLEAR POLYHEDROSIS VIRUS INFECTION ON FOOD CONSUMPTION BY *HELIOTHIS ZEA* [1,2]

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ABSTRACT

Frass production by *Heliothis zea* (Boddie) larvae infected at varying mortality levels with *Heliothis* nuclear polyhedrosis virus (NPV) was determined and used to estimate the effect of the virus on larval food consumption. Treatment in the early stages was essential to substantially reduce food consumption. Reduction in food consumption was also directly related to NPV dosage. Food consumption by larvae surviving NPV treatment was not significantly different from the untreated larvae. Field tests indicated that NPV applications must be timed against 1st- or 2nd-stage larvae to achieve an appreciable mortality level and prevent infected larvae from reaching the damaging late developmental stages. Preliminary maximum treatment levels for use of *Heliothis* NPV for suppression of *H. zea* on soybean are presented.

Key Words: *Heliothis,* soybean, nuclear polyhedrosis virus, food consumption.

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INTRODUCTION

One obstacle encountered in acceptance of the baculoviruses, which include the nuclear polyhedrosis viruses (NPV) and granulosis viruses (GV), as microbial insecticides is the crop damage that occurs as a result of feeding by the infected insect prior to cessation of feeding and death. Current treatment thresholds do not consider damage occurring during this time lag as they are based upon chemical insecticides that provide relatively rapid kill. Successful use of baculoviruses as microbial insecticides will require, among other things, adjustment of existing thresholds to compensate for the damage that occurs between infection and death of the insect host and the relative efficacy provided by the viruses on various crops.

Timing of virus applications against young lepidopterous larvae has been recognized as essential in the prevention of crop damage (Bucher 1959; Hall 1963; Smith 1967; Thompson 1963). Severe crop damage by the tobacco budworm, *Heliothis virescens* (F.), was not prevented by 100% *Heliothis* NPV-induced mortality

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because larval development was almost completed prior to death (Chamberlain and Dutky 1958). However, Ignoffo et al. (1978) reported that pod damage on caged soybean was significantly reduced when *Heliothis* NPV applications were directed against 1st- and 2nd-stage *Heliothis zea* (Boddie) larvae. Similar results have been observed on corn (Tanada and Reiner 1962) and cotton (Stacey et al. 1977).

Harper (1973) reported a decrease in consumption of artificial diet by *Trichoplusia ni* (Hubner) larvae as *T. ni* NPV dosages were increased. Tatchell (1981) obtained similar results with *Pieris rapae* (L.) larvae reared on cabbage leaves treated with the GV of *Pieris brassicae* (L.). In studies on the effect of NPV infection on molting and food consumption of *Spodoptera litura* (F.), inoculum concentration and larval age at infection affected the period of infection and, in turn, larval molting (Subrahmanyam and Ramakrishnan 1981). The occurrence of one molt before onset of symptoms was common. Both Harper (1973) and Subrahmanyam and Ramakrishnan (1981) found that when larvae were infected in the last developmental stage, larval life was prolonged by 2 - 3 days, and they consumed more food than healthy larvae.

The label for a commercial preparation of *Heliothis* NPV (Elcar®, Sandoz, Inc., San Diego, CA) was expanded to include soybean. However, treatment thresholds have not been adjusted for use of the virus on this crop. Reported herein are the results of laboratory and field studies conducted to aid in the adjustment of treatment thresholds for use with the *Heliothis* NPV on soybean.

MATERIALS AND METHODS

Heliothis zea larvae used in laboratory studies were from a stock culture maintained on a pinto bean artificial diet (Burton 1969) at the Virology-Biocontrol Laboratory, University of Arkansas, Fayetteville. Elcar (4×10^9 polyhedral inclusion bodies (PIB/g) was the source of *Heliothis* NPV in all tests.

Laboratory Study

Larval frass production was shown to be highly correlated ($r = 0.99$) with soybean foliage consumed in studies with NPV-infected soybean loopers, *Pseudoplusia includens* (Walker) (Alam 1983). Brewer (1981) presented data that showed a high correlation ($r = 0.93$) between frass produced and artificial diet consumed by *H. virescens* larvae. Thus, we choose to use frass production by larvae in the various treatments as a relative measure of food consumption.

The pinto bean artificial diet with formalin excluded (Vail et al. 1968) was modified for use in the laboratory studies. Corn cob grits, 250g/3.78 *l* of diet, were added to facilitate separation of frass from unconsumed diet (Brewer and King 1979). Water content per batch of diet was increased from 2800 to 3600 ml to give the diet a suitable consistency.

Heliothis zea larvae (25/treatment) were exposed to *Heliothis* NPV by the diet surface treatment technique (Ignoffo 1965). Virus dosages used were determined by preliminary bioassays to result in mean mortality levels of 25, 50, 75, and 95% at pupation for neonate-, 2nd-, 3rd-, and 4th-stage larvae. Distilled water served as a 0% mortality level treatment. NPV dosages (PIB/mm^2) for neonate-, 2nd-, 3rd-, and 4th-stage larvae were 0.13, 0.48, 0.75, 4.80, respectively at the 25% mortality level; 0.35, 1.60, 2.03, 16.00, respectively at the 50% mortality level; 0.96, 4.80,

5.33, 48.00, respectively at the 75% mortality level and 3.95, 25.60, 21.33, 266.67, respectively at the 95% mortality level. Each treatment was replicated four times.

Treated larvae were held in a temperature cabinet maintained at 28°C. Larvae were observed daily and frass produced by each larva was collected after each molt until death or pupation. Larvae surviving to the 5th stage were transferred to fresh diet to insure a surplus of food at all times. Collected frass was dried by holding over anhydrous $CaSO_4$ in desiccator jars for 24 h if collected from the 1st - 4th stages or 72 h if collected from the 5th developmental stage. The dried frass was weighed on a Mettler H6T mechanical analytical balance.

Field Study

A small-plot test was conducted in southwestern Arkansas during the 1982 growing season. *Heliothis* NPV was applied at rates of 25, 50, 100 and 200 L.E./ha (L.E. = larval equivalent = 6×10^9 PIB) to "Bragg" Soybeans in R2 stage, i.e., in full bloom (Fehr et al. 1971), supporting a population of *H. zea* larvae. Plot size was approximately 0.03 ha. Each treatment, including an untreated check, was replicated four times in a randomized complete block design.

Treatments were applied with a bicycle sprayer equipped with 2 TX-3 nozzles/row. Applications were made at a spray volume of 57 l/ha and a nozzle pressure of 2.8 kg/cm^2.

At 48 h posttreatment a minimum of 100 larvae were collected per plot by shaking them onto a beat cloth on a consecutive row-m basis (Boyer and Dumas 1963). Collected larvae were placed on pinto bean artificial diet without formalin and held in a temperature cabinet at 28°C. Larvae were checked daily until pupation or death. Larval stage at collection and death and cause of death were recorded.

RESULTS AND DISCUSSION

Laboratory Study

Mean frass production by uninfected *H. zea* larvae throughout their developmental period was 882.2 mg (dry weight). Eighty-one percent of the total frass was produced in the last larval stage, followed by 14.0, 4.0, 0.9, and 0.2% by the 4th-, 3rd-, 2nd-, and 1st-stages, respectively.

Frass produced by *H. zea* larvae succumbing to NPV infection was comparable to that of uninfected larvae through the larval stages prior to the stage in which death occurred (Table 1). These data are at variance with results reported by others. Harper (1973) detected a reduction in feeding by NPV-infected *T. ni* larvae two days after infection unless the larvae were infected in the last stage. Tatchell (1981) observed a similar reduction at four days posttreatment with a GV of *P. rapae.* Ramakrishnan and Chaudhari (1974) found that food intake of NPV-infected *S. litura* began to decline four days posttreatment with a drastic reduction on the 5th day, after which the larvae began dying. In this study frass production was measured at each molt. Daily measurements of frass production may have permitted detection of a reduction in feeding just prior to death. It is also possible that the effects of baculoviruses on food consumption by their respective insect hosts may vary.

Table 1. Mean dry weight (mg) of frass produced by 1st- to 4th-stage *Heliothis zea* larvae succumbing to treatment with *Heliothis* NPV at various expected mortality levels and by uninfected 1st- to 4th-stage *Heliothis zea* larvae.

Larval stage at treatment	Mortality (%)		Mean dry weight (mg) frass/larva*			
	Expected	Observed	1st	2nd	3rd	4th
Neonate†	25	20	1.4	6.1	37.2	121.0
	50	42	1.4	6.3	31.0	119.3
	75	73	1.4	7.0	30.1	110.0
	95	94	1.3	7.0	40.0	150.4
	Untreated	0	1.4	7.6	33.7	127.7
Second†	25	24	–	9.5	25.6	84.6
	50	51	–	8.6	32.9	108.6
	75	79	–	9.2	41.0	137.8
	95	100	–	9.5	31.5	–
	Untreated	0	–	7.9	34.0	120.9
Third†	25	22	–	–	16.1	118.1
	50	51	–	–	17.5	123.1
	75	73	–	–	15.3	138.2
	95	98	–	–	17.4	145.7
	Untreated	0	–	–	17.7	129.1
Fourth†	25	20	–	–	–	119.7
	50	44	–	–	–	118.1
	75	71	–	–	–	112.6
	95	95	–	–	–	123.5
	Untreated	0	–	–	–	119.7

* Frass weight for the larval stage in which mortality occurred is not included.

† No significant differences in frass production within developmental stages were detected between virus mortality levels by ANOVA ($P = 0.05$).

Mean frass weights from larvae surviving treatment with the *Heliothis* NPV were not significantly different ($P = 0.05$) from the untreated check when larvae were treated in the neonate, 2nd, or 3rd stages. Frass production by 4th-stage larvae surviving treatment at the 95% mortality level was significantly higher ($P = 0.05$) than the untreated check. This difference was probably due to an inadequate sample, as only 2 larvae survived (data not shown).

Mean frass production by larvae treated at the various stages and mortality levels was estimated by combining the amount of frass produced prior to treatment with that produced after treatment (Table 2). An inverse relationship between dosages and frass production was observed regardless of larval stage at treatment, and there was significantly less ($P = 0.05$) frass production with each dosage increase. These differences were attributed to both greater and more rapid mortality at higher treatment rates (Ignoffo 1966).

Linear regression equations ($y = a + bx$) for percent reduction in frass production (y) versus NPV mortality level (x) were developed for each larval stage tested. They are as follows: neonate larvae, $y = 1.67 + 0.98$ x; 2nd stage larvae, $y = -0.48 + 0.97$ x; 3rd stage larvae, $y = 0.64 + 0.91$ x; and 4th stage larvae, $y = 2.50 + 0.79$ x. The coefficient of determination (r^2) for each equation was in

Table 2. Mean dry weight (mg) frass produced by *Heliothis zea* larvae treated at various mortality levels of *Heliothis* NPV.*†

Expected mortality	Larval stage at treatment*†			
(%)	Neonate	2nd	3rd	4th
0	882.2a	909.2a	879.5a	870.1a
25	702.6b	697.7b	705.0b	723.4b
50	508.7c	461.5c	478.7c	540.0c
75	214.5d	229.9d	314.2d	364.6d
95	71.2e	17.7e	80.9e	199.9e

* Mean frass weights for larvae treated in stages 2, 3, or 4 include mean frass weight produced by uninfected larvae prior to stage in which treatment occurred.

† Means within columns not followed by the same letter are significantly different. Duncan's [1955] Multiple-Range Test ($P = 0.05$).

excess of 0.99. The regression equations indicated that percent reduction in frass production (y) was comparable to percent mortality (x) when neonates were treated, i.e., 95% mortality resulted in approximately a 95% reduction in frass. However, at the 95% mortality level, reduction in frass production declined to 92% if treatment was applied in the 2nd stage, 87% in the 3rd stage, and 78% in the 4th stage.

Based on frass production, the laboratory study indicates that food consumption by *H. zea* larvae infected with *Heliothis* NPV was not significantly different from that of uninfected larvae through the larval stages prior to the one in which death occurred. Thus, any protection afforded by applications of the virus would be primarily due to death of the larvae. The laboratory study also indicates that food consumption by virus-treated *H. zea* larvae that died from the disease was related to both larval stage at treatment and virus dosage. These results suggest that treatment in the early larval stages is required if death is to occur prior to the late larval stages. This is essential in order to significantly reduce food consumption as most (95%) of the feeding by healthy larvae occurred in the last two larval stages.

Field Study

All *H. zea* larval stages were represented in the 48 h posttreatment collection. Second-stage larvae were the most abundant and made up 44.4% of the total collection. Third-stage larvae were the second most abundant (27.4%) followed by 1st-stage larvae (15.7%), 4th-stage larvae (9.6%) and 5th-stage larvae (2.7%). After correction for check mortality, overall larval mortality due to *Heliothis* NPV was 20.9, 27.9, 35.0 and 48.1% at the 25-, 50-, 100-, and 200-L.E./ha application rates, respectively (Table 3). Mortality from virus in the check was 4.7%. Death due to parasitism, bacteria, and fungi was low and relatively consistent across all treatments.

Data on efficacy of the *Heliothis* NPV on soybean are limited, and results have been quite variable. Luttrell et al. (1982b) obtained mortality levels of 66.4 and 88.3% in two field tests at an application rate of 100 L.E./ha. Only 35.0% larval mortality was obtained at the same application rate in this study. Size composition of the larval populations could account for part of the difference. However, the highest mortality level we obtained at this dosage was 42.5% mortality for larvae that were in the third stage at treatment (Table 3).

Table 3. Corrected percent mortality from virus infection of field-collected *Heliothis zea* larvae following treatment with *Heliothis* NPV on soybean.

Larval stage at collection*	Estimated larval stage at treatment	Heliothis NPV Dosage (L.E./ha)†‡ 25	50	100	200	Mean¶
1st	egg-neonate	17.3a	23.6ab	23.3ab	18.6ab	20.8A
2nd	1st	21.8ab	23.7ab	36.3bc	50.4c	32.5B
3rd	late 1st - 2nd	22.3ab	36.5bc	42.5c	62.3c	41.2C
4th	3rd - early 4th	18.0a	33.0abc	31.6abc	56.5c	35.2BC
Total¶§		20.9A	27.9AB	35.0B	48.1c	

* Larvae collected 48 h posttreatment.

† Check mortality corrected for by Abbot's (1925) formula.

‡ Individual larval stage: *Heliothis* NPV dosage means not followed by the same lower case letter are significantly different. Duncan's [1955] Multiple Range Test ($P = 0.05$).

¶ Includes 5th-stage larvae, none of which succumbed to the virus.

§ Column or row means not followed by the same upper case letter are significantly different. Duncan's [1955] Multiple Range Test ($P = 0.05$).

Heliothis NPV mortality of field-collected larvae by larval stage is given in Table 3. Due to the 48-h lag period between treatment and collection, both larval stage at collection and estimated stage at treatment are given. Larvae estimated to be in the late 1st to 2nd stages at treatment exhibited the highest level of mortality from virus. Mortality of this group of larvae average 41.2% and was significantly ($P = 0.05$) higher than that for larvae estimated to be in the 1st-stage at treatment. Mortality of larvae estimated to be in the 3rd to early 4th stages at treatment was intermediate between the 1st-stage and late 1st- to 2nd-stage groups. Mortality averaged 20.8% for those larvae estimated to be in the egg-neonate stage at treatment and was significantly ($P = 0.05$) less than that of the other larval groups in which mortality from virus occurred. None of the larvae estimated to be in the 4th or 5th stages at treatment succumbed to virus infection. Luttrell et al. (1982a) found that mortality from NPV treatments on cotton decreased as larval age or stage increased. Larvae estimated to be in the egg-neonate stages at treatment were most susceptible. This was not the case in this study, as larvae estimated to be late 1st - 2nd instars at treatment were most susceptible. The lack of apparent susceptibility of egg-neonate stages may have been partially due to the small larvae feeding in the unexpanded soybean terminals and thus avoiding ingestion of the virus.

The mortality data for field collected larvae show a weak, but significant overall dosage response (Table 3). Similar responses were evident for larvae estimated to be in the 1st, late 1st to 2nd, or 3rd to early 4th stages at treatment but not for those estimated to be in the egg-neonate stage. Absence of a dosage response when larvae were in the egg-neonate stages may have been due to inactivation of the virus before hatching as well as the small larvae feeding in the terminals. Approximately half of *Heliothis* NPV activity on soybean may be lost within 24 h and two-thirds after 48 h (Young and Yearian 1974).

Of the larvae estiamted to be in the 1st and late 1st - 2nd stages at application of *Heliothis* NPV, 91.4 and 70.6%, respectively, of those that died from virus did so by the 3rd stage. These larvae were thus prevented from reaching the highly damaging 4th and 5th stages. Treatment of the 3rd - early 4th stages resulted in

74.5% of the larvae that died reaching the 5th stage before death (data not shown).

Maximum Treatment Level Calculations

Maximum Treatment Levels (MTL) for use of *Heliothis* NPV on soybean were developed using data from this study. The methods used were a modification of a hypothetical model developed by Luttrell and Yearian (1981) for cotton. One feeding unit (F.U.) was assigned for each mg of frass produced as determined by the laboratory study. Uninfected larvae produced an average of 882.2 mg of frass before pupation and were assigned a value of 882.2 F.U. Based upon a threshold of 13 medium-sized *H. zea* larvae/row-m (Nester 1983), a total of 11,469 F.U. could be tolerated. Feeding units for larvae treated with *Heliothis* NPV were calculated from the percent reduction in feeding observed in the laboratory by inserting field mortality levels into the regression equations given earlier. Thus, the 50.4% field mortality of 1st-stage larvae, at an application rate of 200 L.E./ha, resulted in a 51.1% reduction in feeding and an expected average of 431 F.U./larvae. MTL calculation for 1st-stage larvae would be as follows: 431 x = 11,469, giving x = 27 1st-stage larvae. Therefore, populations of 1st-stage larvae above 27/row-m could not be adequately suppressed by an application of *Heliothis* NPV at 200 L.E./ha. The *Heliothis* NPV MTL for the first three larval stages at application rates of 100 and 200 L.E./ha are listed in Table 4. A decrease in application rates resulted in lower MTL's due to a decrease in larval mortality.

Table 4. Estimated Maximum Treatment Levels for use of *Heliothis* NPV at 100 or 200 L.E./ha for control of the first 3 larval stages of *Heliothis zea* on soybean.

Larval stage	% Expected* mortality	% Reduction† in feeding	Mean expected‡¶ feeding units/larvae	Maximum§ treatment level (larvae/row-m)
		100 L.E./ha		
1st	36.3	37.2	54	21
2nd	42.5	40.7	21	22
3rd	31.6	29.4	623	18
		200 L.E./ha		
1st	50.4	51.4	431	27
2nd	62.3	59.9	354	32
3rd	56.5	52.1	423	27

* From Table 3.

† Reduction (%) in feeding (y) determined as follows: First-stage larvae, y = 1.67 + 0.98 x; second-stage larvae, y = −0.48 + 0.97 x; third-stage larvae, y = 0.64 + 0.91 x; x @ % expected mortality.

‡ The feeding unit (F.U.) = 1 mg, frass (dry weight) produced. Uninfected larvae produced a mean of 882.2 F.U. during the larval development period.

¶ Mean expected feeding units/larvae (Z) determined as follows: Z = y (882.2) ÷ 100; y = % reduction feeding.

§ Maximum treatment level (MTL) determined as follows: MTL = maximum feeding units that can be tolerated (M) ÷ mean expected feeding units (Z). M = accepted chemical treatment threshold, in this case 13 larvae/row meter times 882.2.

Heliothis larval populations in soybean are not generally of uniform stage. The feasibility of using *Heliothis* NPV to suppress a larval population with mixed sizes can be determined by calculating the number of feeding units expected to occur following a *Heliothis* NPV application, taking into account all larval stages present, their respective densities, and expected mortality levels. For example, a larval population of 30/row-meter made up of 8, 4, and 18 1st-, 2nd-, and 3rd-stage larvae, respectively, would result in a total of 12,478 F.U. if treated with *Heliothis* NPV at 200 L.E./ha. This population would be above the MTL and could not be adequately suppressed with the virus. A similar population consisting of 4, 20, and 6 1st-, 2nd-, and 3rd-stage larvae, respectively, treated at the same virus rate would result in 11,342 F.U. and would be adequately suppressed. Treatment of both populations at 100 L.E./ha would result in 17,730 and 16,374 F.U., respectively, and neither population would be adequately suppressed by the virus.

The above examples deal only with the activity of the *Heliothis* NPV. Natural mortality factors are not taken into consideration. Inclusion of these should result in somewhat higher MTL's than those for *Heliothis* NPV alone. The treatment levels presented are preliminary and based upon very limited data. We assumed that food consumption by *H. zea* larvae was proportional to frass production and that plant tissue consumption is strongly correlated with artificial diet consumption. Both of these assumptions may be subject to error. Development of accurate, workable MTL's will require considerably more research to address these assumptions and to more accurately correlate field application rates with larval mortality.

A better understanding of the natural biological control agents and other mortality factors affecting *H. zea* populations in soybean is critical. Methods of predicting the subsequent impact of *H. zea* populations in the early developmental stages are also essential. Results obtained from this study are a preliminary step in obtaining the information needed.

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