Contamination of Arthropod Predators with *Heliothis* Nuclear Polyhedrosis Virus After Elcar™ Applications to Soybean for Control of *Heliothis* spp. (Lepidoptera: Noctuidae)[1, 2, 3]

S. Y. Young and W. C. Yearian

Department of Entomology, University of Arkansas
Fayetteville, AR 72701

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ABSTRACT Application of Elcar™ [*Heliothis* nuclear polyhedrosis virus (NPV)] to soybean at 9×10^{10} and 36×10^{10} polyhedral inclusion bodies (PIB) per ha resulted in only low levels of mortality (7% to 26.0%) from NPV in *Heliothis* spp. larvae collected up to 14 days after treatment. Bioassay of NPV in predators collected from 36×10^{10} PIB/ha-treated plots revealed that 16.9% and 14.8% were contaminated with virus 7 and 14 days, respectively, after application. The predominant predators collected were spiders (34.3%) and nabid species (29.2%) of which 11.0% and 20.2%, respectively, tested positive for NPV. Only a few predators collected from outside treated plots 14 days after treatment contained NPV. These results suggest that most predators which preyed on virus infected larvae did not move from the treated plots and did not have an important role in NPV dispersal within the treated plots.

KEY WORDS Insecta, arthropods, predators, *Heliothis* spp, soybean, baculoviruses, nuclear polyhedrosis, biological control.

Predaceous arthropods that prey on nuclear polyhedrosis virus (NPV)-infected larvae are not susceptible to their host's NPV diseases. The occluded NPV are moved along the predator's digestive tract with a meal and excreted intact (Beekman 1980). High levels of NPV activity have been found in feces of several species of arthropod predators (Franz et al. 1955, Smirnoff 1959, Vago et al. 1966, Capinera and Barbosa 1975, Beekman 1980, Cooper 1981, Abbas and Boucias 1984, Young and Hamm 1985, Young and Yearian 1987, Kring et al. 1988). Virus-contaminated feces from predators is a potential source of inoculum for secondary transmission of NPV to uninfected larval hosts. Furthermore, movement of predators fromthe epicenter of disease may also disperse NPV. Some of the scientists cited above have suggested that arthopod predators play an important role in virus dispersal and disease spread in lepidopterous larval populations. Boucias et al. (1987) found that invertebrate predators collected after application of NPV to *Anticarsia gemmatalis* Hübner populations in soybean plots contained the virus. They suggested that these predators have an important role in dissemination of the virus. Young and Yearian (unpubl. data) found that *Nabis roseipennis* Reuter that had fed on NPV infected *A. gemmatalis* larvae dispersed the virus in caged larval populations on soybean, resulting in high levels of the disease in *A.*

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[2] Published with the approval of the Director, Arkansas Agricultural Experiment Station.

[3] Use of a trade name does not imply endorsement or guarantee of the product or the exclusion of other products of similar nature.

gemmatalis larvae on the plants. Recent tests also have shown that nabids contaminated with *Heliothis* NPV transmit the virus in *Heliothis virescens F.* and *H. zea* Boddie larval populations caged on soybean (unpubl. data).

This study was designed to determine the incidence of NPV contamination in arthropod predators within and outside of soybean plots sprayed with *Heliothis* NPV for control of *Heliothis* spp.

Materials and Methods

The *Heliothis* NPV (sing nucleocapsid) preparation used was Elcar™, a commercial formulation (Sandoz, INC., San Diego, CA).

The test was conducted in a field of 'Lee' soybean in Little River County in southwestern Arkansas. The field was treated 25 Aug., 1988, when plants were predominately R_3 developmental stage (Fehr et al. 1971). The experimental design was a randomized complete block with each treatment replicated four times. Treatments included 0, 9×10^{10} and 36×10^{10} polyhedral inclusion bodies (PIB/ha) of NPV as Elcar, 93.5 liters of water per ha with a backpack sprayer equiped with a one-row boom using a single TX-10 nozzle operating at 2.1 kg/cm^2. The Elcar rates applied were selected to obtain low to moderate levels of mortality. Application rates of 9×10^{10} and 36×10^{10} PIB/ha had previously resulted in 20.9% and 35.0% larval mortality, respectively, on soybean according to Flusche et al. 1986. Each plot included 20 rows 1 m apart and 30.8 m in length. Each plot was separated by a 39 m buffer of soybean on all sides.

Heliothis spp. larvae and arthropod predators were collected within and outside of the plots using the beat cloth method (Boyer and Dumas 1963). Collections were made 4, 7 and 14 days after treatment. A total of 25 larvae and 10-25 predators were collected from each plot. When possible, collections were made from the middle two rows of plots. Additional collections, if needed, were made from adjacent rows. Collections of equal numbers outside the plots were made from rows 4-5 and 15-16 located to the east of the plots. The prevailing winds were from the southwest. *Heliothis* spp. larval instar was recorded at collection and larvae were placed on semisynthetic diet (Burton 1969) in 28-ml clear plastic cups. The larvae were held in the laboratory (27°C), and mortality from NPV and other causes was recorded at pupation. Death from NPV was determined by the cadavers appearance upon lysing (Aizawa 1963) and when symptoms were not conclusive a smear from the cadaver was examined with phase microscopy for the presence of polyhedra. Predators were placed individually in empty 28-ml clear plastic cups and frozen at -20°C until bioassays were conducted.

The bioassay procedures were as follows. The predators were individually homogenized in 0.1 ml of 2.0% KCL solution in a 12 ml conical centrifuge tube using a 0.5 cm dia. glass rod. The homogenate was diluted to 1.0 ml with additional 2.0% KCL. Viral activity of the homogenate was determined using the diet surface-treatment technique (Ignoffo 1966) with individual neonate *H. virescens* larvae. A total of 10 larvae were used per homogenate. The bioassay larvae were obtained from a culture maintained in the laboratory on semisynthetic diet. Mortality from NPV and from other causes were recorded. A predator was considered positive for NPV contamination when two or more assay larvae died of the virus. A comparable group of 10 control larvae was reared on diet.

Data were analyzed by analysis of variance (ANOVA), and means were separated by Duncan's (1955) multiple range test.

Results and Discussion

Heliothis spp. larval density 4 days after treatment was low, did not vary significantly between control-and virus-treated plots (Table 1) and averaged 1.8 larvae per meter of row across all treatments. Seven days after treatment mean larval density was significantly ($P < 0.05$) lower in the 9×10^{10} PIB/ha (0.9 larvae/m) and 36×10^{10} PIB/ha (0.8 larvae/ha) treated plots than in the control plots (1.8 larvae/m). Mean larval densities from rows 4-5 and 15-16 outside the plots did not differ significantly between treatments. At 14 days after treatment the mean larval density did not differ between treatments ($P < 0.05$) (Table 1) or at locations outside the plots.

Table 1. Mean density (larvae/1.5 row meter) of *Heliothis* spp. larvae on soybean within and outside plots treated with Elcar (nuclear polyhedrosis virus).

Elcar rates	Days after treatment†		
PIB/ha*	4	7	14
		Within plot	
0	2.1 a	1.8 ab	0.5 d
9×10^{10}	1.8 ab	0.9 cd	0.3 d
36×30^{10}	1.5 abc	0.8 cd	0.3 d
		Rows 4-5 east of plot	
0	3.1 ab	1.8 ab	0.4 d
9×10^{10}	1.6 ab	1.4 bc	0.5 d
36×30^{10}	1.4 bc	1.3 bc	0.3 d
		Rows 15-16 east of plot	
0	1.7 ab	1.4 abc	0.4 d
9×10^{10}	2.0 ab	1.4 abc	0.5 d
36×30^{10}	1.4 bc	0.9 cd	0.4 d

* PIB = polyhedral inclusion bodies.

† Means not followed by a common letter(s) are significantly different ($P \leq 0.05$; Duncan's [1955] multiple range test.

Mortality from NPV occurred in larvae collected in virus-treated plots on all collection days with up to 12.9% (day 14) and 26.0% (day 7) mortality in 9×10^{10} PIB/ha and 36×10^{10} PIB/ha treatments, respectively (Table 2). Larval mortality did not differ significantly with collection date or treatment rate. Although mortality levels were low, there was a significant dosage response (Table 2). Mortality from NPV in larvae collected outside the plots was very low and was significantly higher compared with control plots only when larvae were collected on day 14 from plots treated with 36×10^{10} PIB/ha ($P < 0.05$) (Table 2).

Table 2. Mean mortality (%) from NPV of *Heliothis* spp. larvae collected from soybean within and outside of plots treated with Elcar (nuclear polyhedrosis virus).

Elcar rates	Days after treatment†		
PIB/ha*	4	7	14
		Within plot	
0	0.0 e	0.0 e	1.0 e
9×10^{10}	8.0 cde	7.0 cde	12.9 bc
36×30^{10}	24.0 a	26.0 a	19.4 ab
		Rows 4-5 east of plot	
0	0.0 e	0.0 e	0.0 e
9×10^{10}	0.0 e	1.1 e	1.1 e
36×30^{10}	0.0 e	0.0 e	11.4 bcd
		Rows 15-16 east of plot	
0	0.0 e	0.0 e	0.0 e
9×10^{10}	0.0 e	7.5 cde	0.0 e
36×30^{10}	0.0 e	1.0 e	2.5 de

* PIB = polyhedral inclusion bodies.

† Means not followed by a common letter(s) are significantly different ($P \leq 0.05$; Duncan's [1955] multiple range test.

Larval mortality in the virus treatments was comparable to that reported by Flusche et al. (1986) at similar dosage rates. It was anticipated that these low mortality levels would allow an assessment of predator contamination as a factor in epizootic development. However, viral epizootics did not develop. This may be influenced by larval density. Ali et al. (1987) reported rapid epizootics in *H. zea* caged on soybean, but the population levels in their test were higher than those in the field test.

Larval mortality from causes other than NPV ranged from 12.0% to 29.9% with a mean of 19.2%. This mortality did not differ significantly between treatments within a collection date, but mean mortality of all treatments on day 4 (16.3%) was significantly lower than that on days 7 (21.2%) and 14 (20.3%) (data not shown). It was observed that mortality was primarly due to parasitism, particularly from tachinid flies. The mean larval instar collected from within treated plots did not differ between rates on any collection date, indicating that distribution of larval instars within the population was not influenced by NPV treatment (data not shown).

Bioassay of homogenates of predators collected from plots indicated that few predators collected on day 4 after treatment were contaminated with virus (Table 3). Failure to detect NPV on day 4 in predators collected from plots that were sprayed with the highest rate of NPV shows that predators collected in this test were not contaminated with the NPV that had been sprayed on plants. In collections made 7 and 14 days after treatment some predators from the treated plots were contaminated with NPV. Contamination of predators from the 36×10^{10} PIB/ha plots was significantly ($P < 0.05$) higher than in the control plots on day 7

Table 3. Percentage of NPV-contaminated predators (all species combined) collected from soybean fields after treatment with Elcar (nuclear polyhedrosis virsus) sprays against *Heliothis* spp.

Elcar rates	Days after treatment†		
PIB/ha*	4	7	14
		Within plot	
0	0.0 b	0.0 b	0.0 b
9×10^{10}	3.0 b	5.2 b	2.2 b
36×30^{10}	0.0 b	16.9 a	14.8 a
		Rows 4-5 east of plot	
0	0.0 b	0.0 b	2.1 b
9×10^{10}	0.0 b	0.0 b	4.2 b
36×30^{10}	0.0 b	0.0 b	1.8 b
		Rows 15-16 east of plot	
0	0.0 b	0.0 b	0.0 b
9×10^{10}	0.0 b	0.0 b	0.0 b
36×30^{10}	0.0 b	0.0 b	0.b b

* PIB = polyhedral inclusion bodies.

† Means not followed by a common letter(s) are significantly different ($P \leq 0.05$; Duncan's [1955] multiple range test.

and 14 post treatment, with 16.9% and 14.8% contamination, respectively (Table 3). NPV-contaminated predators were detected outside the treatment plots only on day 14 in rows 4-5. The source of the virus in predators near control plots does not appear to be the control plots because no larval mortality from NPV or contaminated predators were detected within these plots. The virus contaminated predators near these control plots may have dispersed from treated plots.

A total of 1997 predators were collected: spiders (34.3%), *Geocoris* spp. (9.4%), *Orius* spp. (9.7%), coccinellid larvae (12.5%), *Nabis* spp. (29.2%), and *Reduvid* spp. (4.8%). One or more individuals from each predator group bioassayed positive for NPV. However, the predators most often testing positive for NPV were spiders and *Nabis* spp. In day 7 and day 14 collections from the 36×10^{10} PIB/ha plot, 11.0% of the spiders and 20.2% of the *Nabis* spp. tested positive for NPV. This information supports previous research that showed these groups to be important predators of lepidopterous larvae in soybean (Turnipseed and Kogan 1976, Harper et al. 1983).

The results indicated that at least some of the predators that preyed on virus infected larvae in the treated plots became contaminated. However, the contaminated predators were not important in virus dissemination within the plots. Because < 4.2% of predators from outside the treated plots were contaminated with virus, most predators apparently emigrated little from the treated plots. Detectable levels of virus have been shown to remain in contaminated predators for several days after they have attacked a virus-infected larva (Young and Yearian 1987, Kring et al. 1988). Previous studies on caged soybean using higher *H. zea* densities showed tht NPV contaminated nabids effectively dispersed virus to

healthy *H. virescens* larvae (unpubl. data). Up to 60% larval mortality from NPV occurred in larval collections from these plants. The low levels of dispersal of the viral inoculum by predators in this test could be related to density of predators and prey. The density of predators may not have been sufficient to cause dispersal that would be necessary to adequately disseminate the NPV. Also, with the low *Heliothis* spp. larval densities, there may not have been sufficient primary infected larvae to contaminate large numbers of predators. There were other prey in the field, e.g., other species of lepidopterous larvae. Predation on alternative prey would have reduced attacks on *Heliothis* spp. and, therefore, contamination by *Heliothis* NPV.

In summary, low doses of Elcar resulted in mortality from virus in *Heliothis* spp. populations on soybean. Predator groups collected from Elcar-treated plots, but rarely from adjacent areas of the fields, contained virus-contaminated individuals. However, predators at these low pest-population densities did not appear to be important in disease spread within the treated plots or in the dispersal of the virus from treated plots, at least not in the specific situation represented in this test.

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