Persistence and Efficacy of Four Nuclear Polyhedrosis Viruses for Corn Earworm (Lepidoptera: Noctuidae) on Heading Grain Sorghum[1]

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ABSTRACT The persistence and efficacy of *Helicoverpa zea* nuclear polyhedrosis virus (HzNPV) on heading grain sorghum was compared with three multiply-enveloped NPV's from other hosts to which the corn earworm, *Helicoverpa zea* (Boddie), is susceptible. Bioassay of NPV by feeding florets from sprayed heads to second instar corn earworm showed only low levels of virus remaining on heads in all of the treatments four days after application. The initial activity and persistence of a commercial preparation of *H. zea* NPV were greater than four unformulated virus preparations including unformulated HzNPV at rates of 3, 6, and 15 $\times$ 10^{11} polyhedral inclusion bodies (PIB) per ml ($P < 0.05$). Initial activity and persistence were similar for unformulated preparations of HzNPV, *Heliothis armigera* (HaNPV), and *Autographa californica* (AcNPV). Activity and persistence of *Anticarsia gemmatalis* (AgNPV) were significantly less than for the other viruses ($P < 0.05$). In a small plot test on heading grain sorghum in which viruses were applied at 1.5, 3, and 6 $\times$ 10^{11} PIB/ha, mortality of collected corn earworm larvae and larval population reduction were greater in the HzNPV treatments than in the three multiply-enveloped virus treatments. HaNPV and AcNPV preparations were more effective than AgNPV. All virus preparations required one wk or longer to significantly reduce larval populations at all rates ($P < 0.05$).

KEY WORDS Nuclear polyhedrosis virus, grain sorghum, *Helicoverpa zea, Autographa californica, Heliothis armigera, Anticarsia gemmatalis,* virus persistence, virus efficacy.

The corn earworm, *Helicoverpa zea* (Boddie), (Lepidoptera: Noctuidae) is a pest of heading grain sorghum, *Sorghum bicolor* (L.) Moench, in North America. Little is known of the effectiveness of biological insecticides for use against this pest on grain sorghum. Ignoffo et al. (1965) reported that a *H. zea* singly-enveloped nuclear polyhedrosis virus (HzNPV) of the corn earworm provided effective control of this pest on heading grain sorghum. The host range of this NPV is limited to *Heliothis* sp. and *Helicoverpa* sp. pests in the United States. The corn earworm is also susceptible to multiply-enveloped NPVs of other lepidopterous species. These NPV's have much broader host ranges and are also promising as biological insecticides. We report here the results of persistence

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and efficacy experiments on HzNPV and three multiply-embedded NPVs: *Heliothis armigera* (Hübner) (HaNPV) (Ignoffo et al. 1983), *Anticarsia gemmatalis* Hübner (AgNPV) (Carner et al. 1979), and *Autographa californica* (Speyer) (AcNPV) (Vail et al. 1978) for corn earworm on heading grain sorghum.

Materials and Methods

Test 1: Persistence of NPV's. Persistence was studied on heading grain sorghum, variety W-744-DR, planted 29 May 1989, at the Main Agricultural Experiment Station, Fayetteville, AR. Corn earworm larvae for the bioassays were obtained from a laboratory colony reared on semisynthetic diet (Burton 1969). Larvae for use in the bioassay were reared (25 per container) on diet in 270-ml wax-coated paper cups at 27 ± 1°C through the first instar.

The sources of viruses were AcNPV (P. Vail, Horticulture Crops Research Laboratory, USDA/ARS, Fresno, CA 93727), AgNPV (G. Carner, Dept. Entomology, Clemson University, Clemson, SC 29631), HaNPV (J. Hamm, IBPM Research Laboratory, USDA/ARS, Columbia, MO 65205), and a commercial formulation of HzNPV (Elcar) (Sandoz Agro Inc., Palo Alto, CA). With the exception of Elcar, the virus preparations were produced in larvae from our cultures: HzNPV and HaNPV in *Heliothis virescens* (F.), AcNPV in *Trichoplusia ni* (Hübner), and AgNPV in *A. gemmatalis*. Virus preparations other than Elcar were partially purified by homogenizing the virus-killed cadavers in deionized water and filtering through organdy. Virus in the suspension was quantified with an improved Neubauer hemacytometer. These preparations were bioassayed against first instar corn earworm using the on-diet method of Ignoffo (1965). Activity was recorded at pupation.

Treatments consisted of Elcar and four aqueous virus suspensions: HaNPV, AcNPV, AgNPV, and HzNPV, each at 3-, 6-, and 15×10^{11} polyhedral inclusion bodies per hectare (PIB/ha) and a control plot. Plots were two rows, 1.0 meter apart × 15.4 m in length. Each plot was separated by a two-row (2.0 m) buffer lengthwise and 2.9 m on each end. Heads in anthesis were tagged in each plot before application. The test was replicated four times.

Treatments were applied with a backpack sprayer equipped with a one-row boom using a single TX-10 nozzle calibrated to deliver 96 liters per hectare. A spreader-sticker, Triton CS-7 (Rohm and Haas Co. Philadelphia, PA), was added to the tank mixture at the rate of 1.0 ml per liter.

After the plots were sprayed the plants were allowed to dry, and five tagged heads were collected from each plot (Day 0). Heads were transported to a nearby laboratory for bioassay. Heads also were collected for bioassay 1, 2, 4, 7, and 14 d after treatment. Six rachis branches were clipped at random from each of the five heads per plot and placed individually in a 28-ml clear plastic cup containing a dampened filter paper disk. A single 2-day-old second instar corn earworm was placed in each cup, allowed to feed on the sorghum for 2 d, and transferred to a semisynthetic diet. Larval mortality from virus and other causes was recorded until pupation for each treatment.

Statistical analysis of the percentage of larval mortality immediately after application (day 0 bioassay mortality data) was for virus and rate and

interaction of virus × rate by analysis of variance (ANOVA, GLM procedure) with means separated by the method of protected least significant differences (LSD) using Statistical Analysis Systems Procedures (GLM, SAS Institute 1988) (Table 1). Data were corrected for untreated control mortality using Abbott's formula (Abbott 1925).

Persistence of the viruses (percentage of larval mortality in bioassays on days 1, 2, 4, 7, and 14 after application) was analyzed using the logit of mortality. Data for day 0 also were used in this analysis to account for different initial mortalities. A value of 0.01 was added to 0% mortalities and subtracted from 100% mortalities prior to performing the logit transformation. A preliminary analysis of variance was performed that included the effects of NPV, RATE, and DAY and their two- and three-factor interactions. Because we expected mortality to decrease with days and increase with rate and because our objective was to compare the persistence of five virus preparations, an analysis that accounted for these expected trends was appropriate. Therefore, the model used for each virus included a linear term for log (rate) and a linear term for log (day + 1). This reduced model accounted for most of the virus × rate and virus × day interactions. Because AgNPV was the only exception to an acceptable linear fit, an additional term was added that accounted for the difference between day 0 and the other days for this virus. This model accounted for 95% of the variation due to NPV, rate, and day in the preliminary analysis and was considered acceptable. Persistence data (% larval mortality) are presented as the estimated regressions obtained from this model (Fig. 1). The standard errors were added and subtracted on the logit scale, and the results were transformed back to original data units for purposes of presentation.

Test 2: Efficacy of NPV's. The test was conducted on Funk 1711 hybrid planted 12 May, 1989 in Lafayette Co., AR. Plots were eight rows 1.0 m apart × 30.8 m in length and separated lengthwise by a two-row buffer with a 4.6-m buffer on each end. Sprays were applied on 28 July 1989, as described in the persistence test, when panicles were predominantly in the full-bloom stage and larvae were predominantly second-instar. The 14 treatments included four aqueous suspensions of HaNPV, AcNPV, AgNPV and HzNPV each at 1.5×10^{11}, 3×10^{11}, and 6×10^{11} PIB/ha, a chemical insecticide standard (Seven XLR-Plus at 0.8 kg a.i./ha) and an untreated control.

Corn earworm larval counts were made 4, 7, and 13 d after treatment. Grain sorghum heads (20 per plot) were shaken into a 9.5-liter plastic bucket in which larvae were counted. In addition, at 4 and 7 d after treatment, 25 larvae collected from each plot were chosen at random and placed on diet in 24-ml clear plastic cups. The larvae were held in the laboratory until pupation, and the larval numbers that died of virus and other causes were recorded for each treatment replication. Because larval density in plots was below the recommended treatment threshold (6.5 larvae that are 1.28 cm or larger/m) (Johnson and Jones 1988), yield data were not taken.

Larval density within each collection date was analyzed using ANOVA and LSD procedures. In addition, larval density and the percentage of mortality for virus treatments only were analyzed for main effects (virus, rate, and day) and their interactions using the GLM Procedure and LSD (SAS Institute 1988).

Mortality data were corrected for mortality in the untreated control using Abbott's formula (Abbott 1925).

Results and Discussion

Virus Persistence Test. Mortality from virus bioassayed immediately after application (day 0) varied widely between treatments and at 3×10^{11} PIB/ha ranged from 16.1 to 74.0% for AgNPV and Elcar treatments, respectively (Table 1). Mortality was significantly affected by virus ($F = 38.28$; df = 4, 59; $P = 0.0001$) and rate ($F = 21.55$; df = 2, 59; $P = 0.0001$), but there was not a significant virus × rate interaction ($F = 0.86$; df = 8, 59; $P = 0.5548$) (Table 1). Across all rates, Elcar resulted in significantly higher and AgNPV significantly lower mortality than other viruses. HzNPV, HaNPV and AcNPV activity did not differ significantly among viruses on day 0 after treatment. Across all viruses, there was a significant increase in mortality with each increase in rate. The greater activity shown by HzNPV, as compared to HaNPV and AcNPV on semisynthetic diet in our laboratory (Table 2) and in previous reports (Ignoffo et al. 1983, Carner et al. 1979, Vail et al. 1978), was not evident. The commercial product Elcar resulted in higher mortality against corn earworm on grain sorghum than did the aqueous preparations of HzNPV, although bioassays on diet showed that viral activity was similar (Table 2).

Table 1. Percentage of corrected mortality ± SEM from NPV in second instar corn earworm bioassayed on heading grain sorghum immediately (Day 0) after application in the field.

Treatment Rate/ha ($\times 10^{11}$ PIB)	Virus preparation*					
	Elcar	HzNPV	AgNPV	AcNPV	HaNPV	Mean
3	74.0 ± 5.0	48.7 ± 5.0	16.1 ± 4.8	54.0 ± 6.7	53.0 ± 9.2	49.2 C
6	92.1 ± 1.8	60.1 ± 4.9	21.0 ± 5.0	60.1 ± 2.3	67.1 ± 10.5	60.1 B
15	93.2 ± 2.3	77.7 ± 6.7	48.5 ± 1.7	72.5 ± 5.2	74.2 ± 6.6	73.2 A
Mean	86.4 a	62.2 b	28.5 c	62.2b	64.8b	

* Means in a row (lowercase) or column (uppercase) not followed by the same letter are significantly different (PROC GLM, 5% level; LSD [SAS Institute 1988]). Ac, *Au californica,* Ag, *An gemmatalis;* Ha, *H. armigera*; Hz, *H. zea* and Elcar (a commercial preparation of HzNPV).

Table 2. Dose mortality response (polyhedral inclusion bodies per mm² of diet surface) for the first instar corn earworm to five nuclear polyhedrosis virus (NPV) preparations on semi-synthetic diet.

Nuclear polyhedrosis virus*	LC_{50}	Fiducial limits	Slope
Elcar	0.046	0.031 - 0.065	1.311
HzNPV	0.049	0.035 - 0.065	1.258
HaNPV	0.106	0.087 - 0.129	1.726
AcNPV	0.290	0.228 - 0.387	1.391
AgNPV	12.734	10.407 - 15.662	1.406

* Ac, *Au. Californica;* Ag, *An. gemmatalis;* Ha, *H. armigera;* Hz, *H. zea.*

Larval mortality from virus infection decreased rapidly in all treatments after application (Fig. 1). In AgNPV treatments larval mortality from NPV was negligible by day 2. With the other virus preparations, larval mortality 2 d after application was reduced to less than 20% and 40% at the 3.0 and 6.0 $\times$ 10^{11} PIB/ml rates, respectively. After four days, larval mortality remained above 30% only at the 15.0 $\times$ 10^{11} PIB/ml rate of Elcar. Little virus activity was detected in any of the treatments at 7 or 14 d after application.

The three-factor interaction (virus, rate, time) was not significant; the two-factor interactions were significant, but the dominant sources of variation, i. e. sums of squares, were the main effects. Significant main effects were observed for NPV, rate and time (Table 3). Across all rates and days, Elcar was more persistent and AgNPV less persistent than the other virus preparations. Persistence did not differ significantly among HzNPV, HaNPV, and AcNPV treatments. Larval mortality at 3 $\times$ 10^{11} PIB/ml was significantly less than that at the higher concentrations; these resulted in similar mortalities. Larval mortality decreased significantly with time, except between days 4 and 7.

Because of rapid inactivation of the viruses on the sorghum head, little control could be expected beyond two days after treatment. Thereafter, larval control provided by these viruses would be due primarily to virus inoculum released from virus-killed cadavers of larvae infected during the first two days. Roome and Daoust (1976) reported better persistence of HaNPV on grain sorghum heads than obtained in our investigation. In their study, *H. armigera* larval mortality on grain sorghum heads fell to approximately 30% after two days at a rate of 3.3 $\times$ 10^{11} PIB/ha but was approximately 75% at 1.2 $\times$ 10^{12} PIB/ha. The improvement in HzNPV activity in Elcar suggests that formulation of the multiply-embedded NPV (HaNPV, AcNPV, and AgNPV) may improve their effectiveness. The spray-dry technique used in the Elcar formulation has

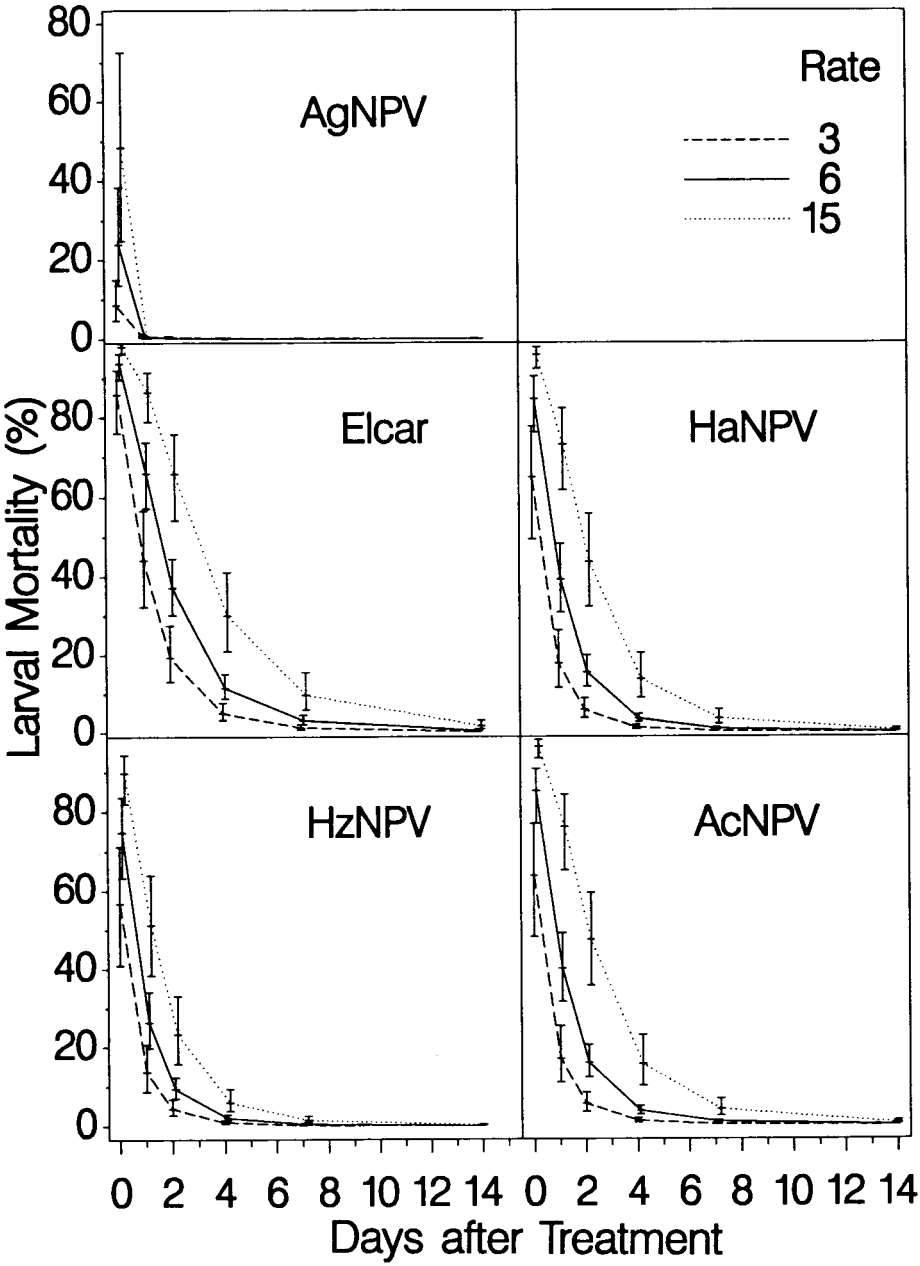

Fig. 1. Percentage of mortality (± SEM) from NPV in second instar corn earworm larvae placed on heading grain sorghum collected after virus application in the field. Data are back transformed from regression lines ploted from the log of the rates and log of day + 1. The virus preparations (NPV) are Ac, *Au. californica;* Ag, *An. gemmatalis*; Ha, *H. armigera;* Hz, *H. zea* and Elcar (a commercial preparation of HzNPV).

Table 3. Results of Analysis of Variance main effects and interactions for Figure 1.

Source	f ratio	df*	p**
Virus (V)	33.35	4,359	0.0001[b]
Rate (R)	17.55	2,359	0.0001[b]
Day (D)	85.49	5,359	0.0001[b]
VxR	1.98	8,359	0.0487[a]
VxD	1.91	20,359	0.0117[a]
RxD	2.05	10,359	0.0285[a]
VxRxD	0.92	40,359	0.6042

* Source and corrected total degrees of freedom, respectively.
** Significantly different at the 0.05 ([a]) and the 0.01 ([b]) level using ANOVA (PROC GLM, SAS Institute 1988) and LSD).

been shown to have several desirable characteristics including increased coverage and persistence due to the presence of sunlight screens (Ignoffo et al. 1976).

Efficacy of NPV's. Larval densities in the control plots decreased during the study from 5.46 per meter of row four days after treatment to 1.46 per meter 13 days after treatment (Table 4). This decrease in larval density in the control plots appeared to be due to both natural mortality factors and pupation. Larval density in the Sevin XLR-plus treatment was significantly reduced to 0.16 and 0.49 per meter on days 4 and 7, respectively ($P < 0.05$), but increased to 1.33 per meter on day 13 and did not differ significantly from control on that date. Densities of larvae were not reduced significantly below that in the control in any virus treatment on day 4 and only at the highest rate of HzNPV and HaNPV on day 7. By day 13, larval density was significantly reduced ($P < 0.05$) at all rates of HzNPV and in some HaNPV and AcNPV treatments but not in any of the AgNPV-treated plots.

Examination of virus treatments only for group effects (virus, rate, and date) and their interactions on density revealed significant effects for virus and time and a significant interaction only between virus and rate (Table 5). Examination of the significant interactions revealed that across all collection dates the viruses did not differ significantly among themselves in mean larval density at 1.5×10^{11} PIB/ha. The only significant differences were that density of AgNPV was higher than HzNPV and AcNPV at 3×10^{11} PIB/ha and HzNPV and HaNPV at 6×10^{11} PIB/ha. In addition, mean larval densities for all virus treatments decreased significantly between collection dates with day 13 < day 7 < day 4.

Table 4. Density of corn earworm larvae collected from heading grain sorghum after application of nuclear polyhedrosis viruses (NPV).

Treatment**	Treatment rate/ha ($\times 10^{11}$ PIB)†	Larvae/row-meter*		
		4 days	7 days	13 days
AgNPV	1.5	5.56 bcd	3.97 ab	2.05 a
	3.0	8.06 a	5.30 a	1.76 ab
	6.0	6.83 abc	4.26 ab	1.95 a
HaNPV	1.5	5.23 cd	3.64 abc	0.85 d-g
	3.0	5.20 cd	3.48 bcd	0.91 cf
	6.0	4.32 d	1.82 de	0.52 f-h
AcNPV	1.5	7.83 ab	3.74 abc	0.68 e-h
	3.0	4.45 cd	2.70 bcd	1.08 c-f
	6.0	5.30 cd	3.09 bcd	1.24 b-e
HzNPV	1.5	6.31 a-d	3.54 a-d	0.26 gh
	3.0	4.65 cd	2.96 bcd	0.26 gh
	6.0	4.97 cd	2.15 de	0.23 h
Seven XLR-Plus	4.0 Pts.	0.16 e	0.49 e	1.33 bcd
Control		5.46 bcd	3.71 abc	1.46 abc

* Means within a column not followed by the same letter(s) are significantly different (5% level; by LSD).
** Ac, *Au. californica; Ag, An. gemmatalis*; Ha, *H. armigera;* Hz, *H. zea.*
† NPV rates only are $\times 10^{11}$ PIB/ha.

Table 5. Results of Analysis of Variance main effects and interactions for Table 3.

Source	f ratio	df*	p**
Virus (V)	14.43	3,143	0.0001[b]
Rate (R)	2.99	2,143	0.0545
Time (D)	216.40	2,143	0.0001[b]
VxR	2.35	6,143	0.0360[a]
VxD	0.56	6,143	0.7575
RxD	1.19	4,143	0.3190
VxRxD	1.27	12,143	0.2452

* Source and corrected total degrees of freedom, respectively.
** Significantly different at the 0.05 ([a]) and the 0.01 ([b]) level using ANOVA (PROC GLM, SAS Institute 1988) and LSD.

The corrected percentage of mortality from virus in larvae collected from the virus treatments ranged from 15.1 to 72.8% for those collected on day 4 and 5.0 to 70.0% on day 7 (Table 6). On day 4, larval mortality was significantly higher in 6×10^{11} PIB/ha HzNPV and HaNPV treatments (72.8%) than in all other treatments except 3×10^{11} PIB/ha of HzNPV ($P < 0.05$). On day 7, larval mortality was significantly higher in 3 and 6×10^{11} PIB/ha HzNPV treatments than in all but 1.5×10^{11} PIB/ha of HzNPV and 6×10^{11} PIB/ha of HaNPV ($P < 0.05$). On both dates, mortality was significantly lower in each AgNPV treatment than in treatments of the other viruses at the same rate ($P < 0.05$) (Table 6). Examination of the group effects of virus treatments (virus, rate, time) revealed a significant effect for virus, rate, and time and a significant interaction only between virus and time (Table 7). This significant interaction revealed that across all rates the mean percentage of larval mortality did not differ significantly on either date between HzNPV and HaNPV. The mean percentage of mortality was higher for both viruses on day 4 and for HzNPV on day 7 than for AcNPV, but did not differ significantly between HaNPV and AcNPV on day 7. Across all rates, the mean percentage of larval mortality for AgNPV was significantly lower than for the other viruses on days 4 and 7.

Effectiveness of HzNPV against corn earworm in this test was comparable to that reported previously. Ignoffo et al. (1965) obtained 88% reduction in larval numbers when HzNPV was applied to grain sorghum at a rate higher (25×10^{11} PIB/ha) than was applied in this test. Although mortality was high in this test, it also was delayed, and larval development and crop damage occurred during this period. Furthermore, Roome (1975) reported that HaNPV at high rates ($1.5\text{-}3.0 \times 10^{12}$ PIB/ha) controlled *H. armigera* on heading grain sorghum, but population reduction occurred primarily between 7 and 21 days after application.

HzNPV more effectively controlled larvae on heading grain sorghum than the multiply-embedded viruses. HaNPV and AcNPV resulted in a high level of mortality at the highest rates, and also showed potential for use. Greater control with the HzNPV over HaNPV or AcNPV is inconsistent with bioassay data on grain sorghum heads presented in Table 1, although laboratory bioassays (Table 2) showed the HzNPV was more virulent than the multiply-enveloped viruses. The greater effectiveness of HzNPV than the other viruses for corn earworm larvae in grain sorghum heads may result in more rapid kill of primary-infected larvae and (or) production of greater numbers of polyhedra. This could have resulted in more intense epizootics developing from secondary inoculum than with AcNPV or HaNPV. The one to two week delay in achieving control in the HzNPV treatments would also indicate that secondary spread of the virus was an important factor in achieving larval population suppression. Although our results suggest that the virus should be timed against very small larvae, Teakle et al. (1985) concluded that timing of the HaNPV application against *H. armigera* was not critical, so long as most of the population was third instar or smaller at treatment.

In summary, persistence of all virus preparations sprayed on heading grain sorghum was short. Elcar exhibited greater initial activity against corn earworm larvae and persistence on grain sorghum than unformulated virus preparations. Initial activity of unformulated HzNPV, however, was similar to that of the multiply-embedded viruses, HaNPV and AcNPV. In the small plot

Table 6. Corrected percentage of mortality from nuclear polyhedrosis virus in corn earworm larvae collected from heading grain sorghum after application of the viruses.

Treatment**	Treatment Rate/ha ($x10^{11}$ PIB/ha)	Percentage of corrected larval mortality*	
		4 days	7 days
AgNPV	1.5	15.1 e	5.0 g
	3.0	24.2 de	17.3 fg
	6.0	36.3 cd	24.8 ef
HaNPV	1.5	49.5 bc	51.3 bc
	3.0	56.6 b	48.0 bc
	6.0	72.8 a	60.1 ab
AcNPV	1.5	39.5 c	31.0 de
	3.0	38.3 cd	31.3 de
	6.0	46.4 bc	41.4 cd
HzNPV	1.5	49.4 bc	59.6 ab
	3.0	59.6 ab	70.0 a
	6.0	72.8 a	69.2 a

* Larval mortality in controls was 1.0 and 11.0% in 4 and 7 day collections, respectively. Nonviral mortality was low in all treatments and did not differ between treatments in either collection. Larval numbers in Sevin XLR-Plus plots at 4 and 7 days after treatment were too few for collection. Means within a column not followed by the same letters(s) are significantly different (5% level LSD).

** Ac, *Au. californica;* Ag, *An. gemmatalis;* Ha, *H. armigera;* Hz, *H. zea.*

Table 7. Results of Analysis of Variance main effects and interactions for Table 5.

Source	f ratio	df*	p**
Virus (V)	14.43	1,95	0.0001^{b}
Rate (R)	2.99	2,95	0.0001^{b}
Date (D)	216.40	1,95	0.0216^{a}
V x R	1.58	6,95	0.1657
V x D	2.94	3,95	0.0391^{a}
R x D	0.49	2,95	0.6123
V x R x D	0.68	6,95	0.6666

* Source and corrected total degrees of freedom, respectively.

** Significantly different at the 0.05 (^{a}) and the 0.01 (^{b}) level using ANOVA (PROC GLM, SAS Institute 1988) and LSD.

efficacy test, HzNPV provided larval mortality and population reduction superior to that of all multiply embedded NPV preparations. HaNPV and AcNPV did provide a high level of larval mortality and a reduction in population density, however, and may be useful at higher rates than those used in this test. The best virus treatments required one week or longer to provide a significant population reduction of corn earworm larvae.

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