Enhancement of Nucleopolyhedrovirus Activity in *Helicoverpa zea* (Boddie) and *Pseudoplusia includens* (Walker) Larvae with a Fluorescent Brightener[1]

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Abstract The enhancement of nucleopolyhedrovirus (NPV) activity by Tinopal® LPW (Tinopal), a stilbene fluorescent brightener, was compared in *Helicoverpa zea* (Boddie) and *Pseudoplusia includens* (Walker) using an on-diet bioassay method. Enhancement of the homologous NPV of each species was compared with three heterologous NPV that have a broad host range. In *H. zea,* the LC_{50} of *H. zea* NPV (HzSNPV) alone was 128 occlusion bodies (OBs)/cup, and the LC_{50} of it and *Heliothis armigera* NPV (HaMNPV) did not differ significantly. The activity of both viruses improved 18.6 fold when the OB suspension contained 1.0% Tinopal. The LC_{50}s of *Autographa californica* NPV (AcMNPV) and *Anagrapha falcifera* NPV (AfMNPV) without Tinopal in *H. zea* were greater than that of HzSNPV. However, the increase in activity of AcMNPV and AfMNPV at the highest concentrations of Tinopal was two to three fold greater than the increase in activity of HzSNPV. The LC_{50} of *P. includens* NPV (PiSNPV) (856 OBs/cup) alone in *P. includens* was similar to that of AfMNPV and AcMNPV, and much less than that of HaMNPV (19,947 OBs/cup). The addition of Tinopal to the treatment suspension of all four viruses resulted in significantly lower LC_{50}s at all Tinopal concentrations in *P. includens.* The highest concentration of Tinopal (1.0%) in the OB suspension lowered the LC_{50} of PiSNPV by 142.7 and AfMNPV by 89.7 fold. Tinopal in the OB suspension lowered the LC_{50} of AcMNPV and HaMNPV, but they remained less effective than PiSNPV with Tinopal. HaMNPV at all concentrations of Tinopal was much less active in *P. includens* than the other viruses with or without Tinopal.

Key Words Nucleopolyhedrosis, *Helicoverpa zea*, *Pseudoplusia includens*, fluorescent brighteners

Many nucleopolyhedroviruses (NPV) show promise as biological insecticides for control of susceptible lepidopteran larval pests of crops and forests. One factor that limits the usefulness of NPVs has been their efficacy, which is usualy lower than that of recommended chemical insecticides. Stilbene fluorescent brighteners (brighteners) are known to enhance activity of NPVs in lepidopteran larvae (Shapiro 1992, Hamm and Shapiro 1992, Shapiro and Robertson 1992, Zou and Young 1994, Hamm and Chandler 1994, Vail et al. 1996). These brighteners also improve the efficacy of NPV in the field when applied against lepidopterous larval pests (Vail et al. 1993, Webb et al. 1994, Hamm et al. 1994, Zou and Young 1996). The effectiveness of brighteners in increasing host susceptibility to NPV varies with the NPV and host. Tinopal® LPW increased activity of the homologous NPV by up to 1,670 fold in the gypsy moth, *Lymantria dispar* (L.) (Shapiro and Robertson 1992), 2,000 fold in the fall armyworm,

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Spodoptera frugiperda (J. E. Smith) (Hamm and Shapiro 1992), 1,500 fold in *Pseudoplusia includens* (Walker) (Zou and Young 1996), 13 fold in *S. exigua* (Hübner) (Hamm and Chandler 1996), and 9 fold in *Helicoverpa zea* (Boddie) (Shapiro and Vaughn 1995). Some NPVs with a broad host range for lepidopteran pests of forests and agriculture are among those viruses synergized by brighteners (Shapiro and Vaughn 1995, Vail et al. 1996), and this may increase their potential for use against secondary hosts. Results reported here compare the effectiveness of a brightener at an array of concentrations with three heterologous NPVs and the native NPVs of *H. zea* and *P. includens,* respectively. These are two species with greater than 100 fold difference in levels of responsiveness of their homologous NPV to brightener (Shapiro and Vaughn 1995, Zou and Young 1996).

Materials and Methods

Helicoverpa zea and *P. includens* larvae were from colonies reared on semisynthetic diet (Burton 1969) in the Entomology Department, University of Arkansas, Fayetteville. Neonates were used in all tests. The *H. zea* NPV, a singularly embedded NPV, was produced in our laboratory from NPV-killed *H. zea* larvae that had been exposed to *H. zea* NPV (Elcar®, Sandoz Inc., San Diego, CA). The *P. includens* native NPV (PiSNPV), a singularly embedded NPV, was isolated from *P. includens* larvae collected from cotton in Guatemala (Livingston and Yearian 1972) and produced in *P. includens.* Three heterologous multiply embedded NPVs with a wide host range were used in the study. *Anagrapha falcifera* NPV (AfMNPV) was obtained from Sandoz Inc., San Diego, CA. The *Heliothis armigera* NPV (HaMNPV) isolate was obtained from J. J. Hamm, Insect Biology and Population Management Research Laboratory, USDA, ARS, Tifton, GA. The AcMNPV was obtained from P. V. Vail, Horticultural Crops Research Laboratory, Fresno, CA. All viruses were propagated in our laboratory in *H. virescens* larvae. The viruses were partially purified, and occluded virus (OB) quantified using an improved Neubauer hemacytometer (Young and Yearian 1979).

A diet-surface bioassay was used to determine viral activity (Ignoffo 1965). The desired concentration of OBs with or without brightener in 0.1 ml of distilled water was pipetted onto the surface (800 mm^2) of the semisynthetic diet (without formaldehyde) in a 28-ml plastic cup. The aliquot was spread by blowing a gentle stream of air over the cup surface and allowing it to dry. One neonate of the desired species (*P. includens* or *H. zea*) was transferred to each cup and maintained in a controlled-temperature chamber at 29 ± 1°C and a photoperiod of 14:10 (L:D) h. Larval mortality from NPV was recorded at 7 d and at pupation. Twenty-five larvae were used in each treatment with five replications per treatment. Larvae in each test were exposed to an array of NPV concentrations that would produce a wide range of mortality (between 10 and 90%) suitable for obtaining a dosage mortality curve. The OB concentration varied with insect species and brightener concentration. The brightener, Tinopal® LPW (Fluorescent Brightener 28) (Tinopal), was purchased from Sigma (St. Louis, MO). Each concentration of the brightener (0.03-1.0%) also was applied to the diet surface as a control.

Data were corrected for control mortality (Abbott 1925) and a concentration mortality response for each treatment was estimated by the probit procedure (SAS Institute 1988).

Results

The HzSNPV without brightener was highly virulent to *H. zea* larvae with a LC_{50} of 128 OBs/cup (Table 1). The addition of the lowest rates of Tinopal (0.03-.1%) decreased the LC_{50} of HzSNPV by 2.6 fold or less. The higher rates of Tinopal had a greater effect on viral activity with 1.0% Tinopal lowering the LC_{50} of HzSNPV by 18.6 fold. The LC_{50}s for HaMNPV alone and with Tinopal at all concentrations were similar to those of HzSNPV.

The LC_{50}s for AfMNPV and AcMNPV alone in *H. zea* were 716 and 557 OBs/cup, respectively, and much greater than that for HzSNPV (Table 1). The addition of Tinopal at all concentrations decreased the LC_{50} of AfMNPV and AcMNPV, and this decrease was more than 30 fold at the LC_{50} and LC_{90} doses when the Tinopal

Table 1. Infectivity of NPVs in first instar *Helicoverpa zea* bioassayed on diet with and without Tinopal

Tinopal (%) in NPV suspension	LC_{50} (fl)*,** (OBs/cup)	Relative activity	LC_{90} (fl)*,** (OBs/cup)	Relative activity	Slope
			HzSNPV		
0	128 (100-174)		1,232 (720-2,729)		1.3039
0.03	112 (87-150)	1.1	1,082 (643-2,324)	1.1	1.3015
0.1	50 (37-68)	2.6	1,103 (601-2,618)	1.1	0.9528
0.3	12 (9-16)	10.7	190 (127-329)	6.5	1.0811
1.0	6.9 (5.3-8.6)	18.6	47 (35-68)	26.2	1.5409
			HaMNPV		
0	179 (141-239)		1,177 (733-2355)		1.5644
0.03	75 (55-104)	2.4	1,481 (738-4520)	0.8	0.9873
0.1	117 (63-372)	1.5	10,812 (1,925-424,936)	0.1	0.6519
0.3	7.3 (4.8-11.8)	24.5	482 (151-4,618)	2.4	0.7042
1.0	9.6 (7.2-13.4)	18.6	144 (76-386)	8.2	1.0906
			AfMNPV		
0	716 (566-932)		7,867 (4,710-16,751)		1.2311
0.03	176 (131-230)	4.1	2,978 (1,894-5,519)	2.6	1.0423
0.1	72 (57-91)	9.9	915 (619-1,534)	8.6	1.1633
0.3	19 (13-26)	37.7	663 (365-1,581)	11.9	0.8287
1.0	20 (16-24)	35.8	199 (147-292)	39.5	1.2786
			AcMNPV		
0	557 (433-706)		4,536 (3,026-8,223)		1.4076
0.03	262 (187-361)	2.1	5,906 (3,127-15,894)	0.8	0.9474
0.1	251 (196-323)	2.2	2,229 (1,408-4,443)	2.0	1.3511
0.3	20 (15-27)	27.9	455 (269-950)	10.0	0.9475
1.0	16 (13-20)	34.8	143 (103-221)	31.7	1.3424

* fl = fiducial limit.

** The control mortality was 5, 8, 11, 16 and 20% for 0, 0.03, 0.1, 0.3 and 1.0% Tinopal, respectively in the inoculum without NPV.

concentration in the treatment suspension was 1.0%. The improvement in activity of these two NPVs with the addition of Tinopal was approximately twice that of HzSNPV and HaMNPV in *H. zea*.

The LC_{50} and LC_{90} of PiSNPV alone were 856 and 10,698 OBs/cup, respectively, in *P. includens* (Table 2). The addition of Tinopal at only 0.03% to the viral suspension reduced the LC_{50} and LC_{90} of PiSNPV in *P. includens* by 6.2 and 35.5 fold, respectively. When the PiSNPV suspension contained 1.0% Tinopal, and LC_{50} was reduced by 142.7 fold. The AfMNPV and AcMNPV without Tinopal had comparable activity to PiSNPV with LC_{50}s of 538 and 1,573 OBs/cup, respectively. Tinopal at all concentrations decreased the LC_{50}s of AfMNPV and AcMNPV, but at Tinopal concentrations of 0.1% or greater the decreases were less than in PiSNPV alone. Furthermore, the decrease in the LC_{50} of AcMNPV with the addition of Tinopal did not exceed 24 fold,

Table 2. Infectivity of NPVs in first instar *Pseudoplusia includens* bioassayed on diet with and without Tinopal

Tinopal (%) in NPV suspension	LC_{50} (fl)*,** (OBs/cup)	Relative activity	LC_{90} (fl)*,** $\times 10^2$ (OBs/cup)	Relative activity	Slope
			PiSNPV		
0	856 (475-2,547)		1,069 (179-537)		0.6115
0.03	137 (97-184)	6.2	30.1 (18.5-59.4)	35.5	0.9544
0.1	20 (14-27)	42.8	5.3 (3.0-1.2)	202.4	0.8960
0.3	3.9 (1.8-6.6)	219.5	3.1 (1.6-9.1)	348.2	0.6769
1.0	6.0 (3.4-9.2)	142.7	3.0 (1.6-7.4)	362.3	0.7582
			AfMNPV		
0	538 (393-766)		164 (81-469)		0.8628
0.03	67 (45-98)	8.0	96 (44-284)	1.7	0.5935
0.1	14.8 (8.3-23.6)	36.4	26.4 (8.8-182)	6.2	0.5695
0.3	6.2 (4.5-8.2)	86.8	0.7 (0.5-1.0)	253.1	1.2628
1.0	6.0 (4.1-8.1)	89.7	0.9 (0.6-1.4)	193.6	1.109
			AcMNPV		
0	1,573 (1,145-2,168)		447 (248-1,003)		0.8819
0.03	520 (384-703)	3.0	256 (152-491)	1.7	0.7571
0.1	103 (80-135)	15.3	16.1 (10.0-31.2)	27.6	1.0733
0.3	66 (51-85)	23.8	9.4 (6.2-16.8)	47.3	1.1108
1.0	90 (68-118)	17.5	16.4 (9.8-33.4)	27.3	1.0157
			HaMNPV		
0	19,947 (11,755-44,049)		21,841 (5,319-249,701)		0.6284
0.03	5,226 (3,229-9,341)	3.8	12,243 (2,900-168,746)	1.8	0.5408
0.1	3,135 (2,116-4,675)	6.4	2,309 (919-10,055)	9.5	0.6863
0.3	3,729 (2,394-10,512)	5.3	5,222 (1,618-38,920)	4.2	0.5971
1.0	2,133 (1,363-3,197)	9.4	1,974 (780-8,960)	11.1	0.6517

* fl = fiducial limit.

** The control mortality was 2, 4, 9, 12 and 23% for 0, 0.03, 0.1, 0.3 and 1.0% Tinopal, respectively in the inoculum without NPV.

and this decrease was 2.8 to 9.2 fold less than that for PiSNPV. The HaMNPV had only a low level of activity against *P. includens* larvae with a LC_{50} of 19,947 OBs/cup. The addition of Tinopal to the HaMNPV suspension also resulted in relatively less reduction in LC_{50} against *P. includens* than with the other viruses, and this reduction did not exceed 9.4 fold at the LC_{50} level of mortality (Table 2).

Discussion

The brightener, Tinopal, enhanced the activity of all four NPVs in larvae of *H. zea* and *P. includens.* Enhancement of viral activity in *P. includens* was much greater than that in *H. zea* for their respective homologous NPV. Enhancement of activity in *H. zea* generally increased with the Tinopal concentration for all four viruses tested. These viruses in *P. includens* were enhanced only by Tinopal concentrations up to 0.3% with the exception of HaMNPV, which demonstrated only a low level of activity in this host.

Shapiro and Vaughn (1995) compared the activity of HzSNPV, HaMNPV, AfMNPV and AcMNPV with and without 1.0% Tinopal in *H. zea.* They reported a lower LC_{50} value for HzSNPV than for the heterologous viruses. However, enhancement of the Plusinae NPVs, AcMNPV and AfMNPV in *H. zea* by 1.0% Tinopal was approximately two to five fold greater than that of the heliothine viruses. In our test 1.0% Tinopal enhanced the less active AcMNPV and AfMNPV approximately twice that of HzSNPV and HaMNPV. Zou and Young (1994) reported that Tinopal at only 0.01% provided a low level of enhancement of both HzSNPV and PiSNPV in the first instar of their homologous host but not in later instars. However, Tinopal at 1.0% did enhance the activity of both viruses through the fourth instar of their respective host.

Although Tinopal enhanced all four nucleopolyhedroviruses in *H. zea* in this study, it was much less than the level of enhancement others have obtained with viruses infecting some hosts (Hamm and Shapiro 1992, Shapiro 1992, Hamm and Chandler 1996, Zou and Young 1996). Other lepidopteran hosts in which NPVs appear to be enhanced at a relatively low level such as *H. zea* are *S. exigua* (Hamm and Chandler 1996) and *Choristoneura occidentalis* (Clemens) (Li and Otvos 1999). The higher response of NPVs in *P. includens* to Tinopal are closer to that of *L. dispar* (Shapiro and Robertson 1992) and *S. frugiperda* (Hamm and Chandler 1996) of their homologous NPV. The NPVs of these hosts have relatively low activity, which may suggest that NPVs with less activity are more responsive to a brightener.

The PiSNPV in *P. includens* was approximately seven fold less virulent than was HzSNPV in *H. zea.* However, enhancement of PiSNPV in *P. includens* by Tinopal over its range of concentrations averaged approximately 12 fold greater than for HzSNPV in *H. zea.* For example, 0.1% Tinopal increased activity of PiSNPV in *P. includens* by 42.8 fold, but increased activity of HzSNPV in *H. zea* by only 2.6 fold. Zou and Young (1996) reported that Tinopal at 0.1% in the inoculum increased activity of PiSNPV in *P. includens* by 14.8 fold. At the highest rates of Tinopal, activity of PiSNPV for *P. includens* was comparable to that of HzSNPV in *H. zea.* Enhancement of heterologous viral activity also differed for the two insects. Enhancement of AfMNPV in *P. includens* and HaMNPV in *H. zea* by Tinopal were similar to that of the hosts respective homologous NPV. At the highest two Tinopal concentrations, enhancement of the remaining heterologous NPVs were approximately two fold less in *H. zea,* but at least 8.0 fold less in *P. includens.*

These results also demonstrate that Tinopal will not necessarily enhance a het-

erologous NPV of low virulence more than the more virulent homologous virus. As with PiSNPV in *P. includens,* the addition of Tinopal to a viral formulation or the spray tank mixture may not improve the performance of a heterologous NPV as much as expected, based on its performance with the homologous virus. Enhancement by Tinopal appears to differ with insect host, virus and brightener concentration; and each combination should be tested before one can anticipate Tinopal effectiveness. Enhancement of NPVs in the field has been less than in laboratory bioassays, but better than the NPV alone (Webb et al. 1994, Hamm et al. 1994, Zou and Young, 1996). Results of these tests suggest that the brightener will increase performance of the heterologous NPVs in the field. This should improve their usefulness against a mixture of lepidopterous larval pests on a crop. These data suggest that when the brightener is used at highest rates, a heterologous NPV can be more effective in the field than the homologous NPV without brightener.

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