# Enhancement in Activity of Homologous and Heterologous Baculoviruses Infectious to Fall Armyworm (Lepidoptera: Noctuidae) by Selected Optical Brighteners[1]

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**Abstract** The nuclear polyhedrosis virus (NPV) from *Spodoptera frugiperda* (J. E. Smith) (SfMNPV) was the most active virus tested against fall armyworm, larvae ($LC_{50}$ = 8.1 PIB per $mm^2$). No $LC_{50}$s could be obtained for the alfalfa looper, *Autographa californica* (Speyer), NPV (AcMNPV), the celery looper, *Anagrapha falcifera* (Kirby), NPV (AfMNPV), the wax moth, *Galleria mellonella* (L.), NPV (GmMNPV), or the bollworm, *Helicoverpa armigera* (Hűbner), NPV (HaMNPV). The addition of an optical brightener, Tinopal LPW® (1%), (Sigma Co., St. Louis, MO) significantly enhanced the activities of all NPVs. The most activie NPV/Tinopal LPW combination was SfMNPV, followed by AcMNPVm HaMNPV, AfMNPV, and GmMNPV. In terms of speed of kill, SfMNPV was the most active virus tested. When Tinopal LPW was added, the $LT_{50}$ was reduced by more than 35%. The addition of Tinopal LPW to the heterologous NPVs resulted in $LC_{50}$ and $LT_{50}$ values that were comparable to SfMNPV alone. Five of eight brighteners acted as activity enhancers for SfMNPV (i.e., Blankophor BBH, Blankophor HRS, Blankophor P167, Blankophor RKH, and Tinopal LPW), whereas Blankophor BSU, Blankophor DML, and Blankophor LPG did not enhance virus activity.

**Key Words** Fall Armyworm, *Spodoptera frugiperda,* NPV, optical brighteners.

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is a major lepidopterous pest on corn and sorghum in the United States (Luginbill 1928, Barfield and Jones 1979) and in Mexico and Latin America (Andrews 1988). A naturally-occurring nuclear polyhedrosis virus (SfMNPV) was isolated and has been tested for more than 30 years, using different delivery systems (Young and Hamm 1966, Hamm and Young 1971, Hamm and Hare 1982, Hamm and Styer 1985). While virus-caused mortality was obtained and epizootics could occur following SfMNPV treatment, mortality levels were usually not sufficient to control the population. Moreover, the use of such adjuvants as Coax® (Agro Solutions, San Marcos, CA) (e.g., feeding stimulants) did not increase virus-caused mortality (Hamm and Hare 1982). Shapiro and Robertson (1992) reported that selected stilbene optical brighteners acted as viral enhancers for the gypsy moth, *Lymantria dispar* (L.) nuclear polyhedrosis virus (LdMNPV). $LC_{50}$s were reduced up to 1800-fold and $LT_{50}$s were reduced up to 45%. Subsequently, it was demonstrated that viral enhancement also could occur with several viruses against the fall armyworm (Hamm and Shapiro 1992), the cotton bollworm (*Helico-*

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*verpa zea* Boddie) (Shapiro and Vaughn 1995) and the soybean looper (*Pseudoplusia includens* worker) (Zou and Young 1996). In the case of *S. frugiperda,* the activity of SfMNPV, as measured by the $LC_{50}$, was increased 164- to >1,000-fold by the addition of 0.1% Tinopal LPW (Hamm and Shapiro 1992). These results were so significant that Hamm and coworkers tested Tinopal LPW in the field against the fall armyworm (Hamm et al. 1994) and the beet armyworm, *Spodoptera exigua* (Hűbner) (Hamm et al. 1996). In both cases, the brightener enhanced the activities of the homologous viruses against their respective hosts. The purposes of our investigation were threefold: (1) determine whether other NPVs, which have been reported to infect lepidopterous insects from different genera, and even different families, were as infective as the specific, homologous NPV (SfMNPV) from *S. frugiperda;* (2) determine whether a stilbene optical brightener (Tinopal LPW) could also enhance the activities of these viruses against larvae of the fall armyworm; and (3) determine the activities of eight structurally-related stilbene stilbene optical brighteners as enhancers for the fall armyworm nuclear polyhedrosis virus (SfMNPV) against the fall armyworm. The overall purpose of this study was to obtain the most efficacious NPV-brightener combination against *S. frugiperda* which could be used in field control studies.

## Materials and Methods

**Insects, viral inoculum, and bioassays.** The colonized strain of *S. frugiperda,* established and maintained by Insect Pest Management and Pest Biology Research Laboratory, USDA-ARS, Tifton, GA, was used. The insects were reared on a wheat germ diet developed for the gypsy moth (Bell et al. 1981). Fall armyworm larvae were exposed to nuclear polyhedrosis viruses (NPVs) from several noctuids. The fall armyworm, nuclear polyhedrosis virus (SfMNPV) was obtained from USDA-ARS, Tifton, GA (J. J. Hamm), and was passed into *S. frugiperda* larvae (Hamm and Ware 1982, Hamm and Shapiro 1992). The bollworm, *H. armigera* (Hűbner), NPV (HaMNPV) also was obtained from USDA-ARS, Tifton, GA. The alfalfa looper, *Autographa californica* (Speyer), NPV (AcMNPV) was obtained from the Insect Biocontrol Laboratory, USDA-ARS, Beltsville, MD. The celery looper, *A. falcifera* (Kirby), NPV (AfMNPV) was obtained from D. L. Hostetter, USDA-ARS, Columbia, MO (now retired). The wax moth, *G. mellonella* (L.), NPV (GmMNPV) was obtained from G. G. Stairs, Ohio State (now retired). Each NPV but SfMNPV was passed into beet armyworm larvae before being assayed against fall armyworm larvae. Viral inclusion bodies (=polyhedral inclusion bodies, PIBs) from all NPVs were extracted from virus-killed larvae (Shapiro et al. 1981) and were diluted in distilled water or in an aqueous solution of optical brightener (1% w/w). Decimal dilutions were made, and PIB suspensions were applied to the diet (0.1 ml per cup; 1,338 $mm^2$ = surface area) at final concentrations ranging from 10 to 100,000 PIB per 30-ml cup. In addition, both untreated controls and test controls (1% brightener only) were used to determine effects upon fall armyworm larvae. Second instars (4 d old) were placed individually in each container (30 ml cup) and were reared for 14 d at 29°C, 50% RH, and a photoperiod of 12:12 (L:D) h. Tests were repeated seven times with 10 larvae per virus dilution per replicate; 10 untreated larvae, and 10 brightener-treated larvae per replicate. Mortality was assessed at day 3, then daily through day 7, and every 2 to 3 days thereafter until day 14.

**Optical brighteners.** Eight optical brighteners were obtained as powders. All materials were diluted in distilled water and were tested at 1% concentrations (wt:wt).

The brighteners used were Blankophor BBH (CAS #4404-43-7), Blankophor BSU (CAS #68971-49-3), Blankophor DML (CAS #16090-02-1) Blankophor HRS (CAS #61951-69-7), Blankophor LPG (CAS #133-66-4), Blankophor P167 (CAS #1670-24-9), Blankophor RKH (CAS #35632-99-6) (Bayer, Pittsburgh, PA) and Tinopal LPW (CAS #4404-43-7) (Sigma, St. Louis, MO). These brighteners were selected, because they are all bistriazinyl derivatives of 4,4′-diamino-2,2′-disulfonic acid (Argauer and Shapiro 1997).

**Statistical methods.** Concentration-mortality and time-mortality regressions were estimated by probit analysis (LeOra Software 1987) to monitor the biological activities of NPVs with and without brightener and SfMNPV with different brighteners. Failure of 95% CL to overlap was used as a criterion for significant differences at $LC_{50}$ or $LC_{90}$.

## Results

The most active NPV against the fall armyworm was the homologous virus SfMNPV ($LC_{50}$ = 8.1 PIB per $mm^2$). No $LC_{50}$s could be obtained for AcMNPV, AfMNPV, GmMNPV, or HaMNPV against *S. frugiperda* (Table 1). The addition of Tinopal LPW (1%) significantly enhanced the activities of all NPVs, as evidenced by decreases in the $LC_{50}$s (Table 1). The $LC_{50}$ for the SfMNPV/LPW combination was reduced 113-fold to 0.07 PIB per $mm^2$. The increases of AcMNPV and HaMNPV were so great that the AcMNPV/LPW and HaMNPV/LPW combinations were 5.5 and 3.6-fold more active than SfMNPV alone (Table 1). Even though LPW increased the activity of GmNPV, this combination was still significantly ($P < 0.05$) less active than SfMNPV alone. In terms of $LC_{90}$s, the most active virus was SfMNPV (69.1 PIB per $mm^2$). The most active NPV/LPW combination was SfMNPV/LPW (2.2 PIB per $mm^2$). In terms of speed of kill. SfMNPV was the most active virus tested. $LT_{50}$s for AcMNPV, AfMNPV, GmMNPV, and HaMNPV could not be determined (Table 2). When Tinopal LPW was added to SfMNPV, the $LT_{50}$ and the $LT_{90}$ were reduced by more than 35% (i.e., 38.6% for the $LT_{50}$ and 37.3% for the $LT_{90}$). Moreover, the time required to increase virus-caused mortality was greatly decreased from 1.8d (SfMNPV) to 0.8d (SfMNPV/LPW) with the addition of Tinopal LPW. The addition of Tinopal LPW to AcMNPV, AfMNPV, GmMNPV, and HaMNPV resulted in $LT_{50}$ and $LT_{90}$ values that were similar to that of SfMNPV alone (Table 2). In all cases, the GmNPV/LPW combination resulted in the highest time of kill values. In a second series of tests, eight optical (=fluorescent) brighteners were used with SfMNPV and five of the eight brighteners acted as activity enhancers for SfMNPV, (i.e., Blankophor BBH, Blankophor HRS, Blankophor P167, Blankophor RKH, and Tinopal LPW). Both the virus concentration (i.e., $LC_{50}$, LC90) (Table 3) and speed of kill (i.e., $LT_{50}$, $LC_{90}$) (Table 4) were decreased. $LC_{50}$s were reduced from 20-fold (Blankophor HRS, Blankophor P167) to 140-fold (Tinopal LPW), and $LC_{90}$s were reduced from 10-fold (Blankophor HRS, Blankophor P167) to 158-fold (Tinopal LPW) (Table 3). The other two brighteners (i.e., Blankophor BBH and Blankophor RKH) were also excellent enhancers, as $LC_{50}$s were reduced by 40- and 106-fold, respectively, $LC_{90}$s were reduced by 25-fold (Blankophor BBH) and by 100-fold (Blankophor RKH). The optical brighteners that decreased $LC_{50}$s and $LT_{90}$s for SfMNPV also reduced $LT_{50}$s and $LT_{90}$s from 23 to 30% (i.e., insects in these groups died more quickly than those exposed to SfMNPV alone ($P < 0.05$) (Table 4).

Among these five brighteners (i.e., Blankophor BBH, Blankophor HRS, Blankophor

**Table 1. Effect of different nuclear polyhedrosis viruses and an optical brightener on $LC_{50}$ and $LC_{90}$ of SfMNPV**

| Treatment | Slope (±SEM) | $LC_{50}$* (95%CL) | AR** | $LC_{90}$+ (95%CL) | AR |
|---|---|---|---|---|---|
| SfMNPV | 1.38 | 8.14 | 1.0 | 69.19 | 1.00 |
| | (0.02) | 5.90–11.23 | | 40.64–117.79 | |
| FAWMNPV+ | 1.26 | 0.07 | 113.4 | 2–18 | 31.7 |
| Tinopal LPW | (0.01) | 0.05–0.11 | | 1.08–4.42 | |
| AfMNPV | | ** | | ** | |
| AfMNPV+ | 1.37 | 2.01 | 4.0 | 46.97 | 1.5 |
| Tinopal LPW | 0.01 | 1.35–3.01 | | 23.99–91.95 | |
| AcMNPV | | ** | | ** | |
| AcMNPV+ | 1.26 | 0.70 | 11.6 | 98.36 | 5.5 |
| Tinopal LPW | 0.01 | 0.50–0.98 | | 4.21–12.39 | |
| GmMNPV | | | | | |
| GmMNPV+ | 1.19 | 39.250 | 0.2 | 464.30 | 0.1 |
| Tinopal LPW | 0.01 | 28.43–57.92 | | 215.09–1002.26 | |
| HaMNPV | | ** | | ** | |
| HaMNPV+ | 1.05 | 2.27 | 3.6 | 37.56 | 1.8 |
| Tinopal LPW | 0.01 | 1.37–3.28 | | 20.80–67.81 | |

* $LC_{50}$s and $LC_{90}$s are expressed as PIBs per $mm^2$ five concentrations per virus (.007 to 74.7PIB per $mm^2$) 7 replicates; 10 untreated larvae and 10 Tinopal LPW-treated larvae per replicate. No mortality was observed among any untreated or brightener-larvae.

** $LC_{50}$s and $LC_{90}$s could not be determined at 74.7PIB/$mm^2$

Activity ratio is calculated by dividing the $LC_{50}$ or $LC_{90}$ for SfMNPV (Standard) by the $LC_{50}$ $LC_{90}$s for SfMNPV/Tinopal LPW. The greater the AR, the greater is the enhancement provided by the brightener.

P167, Blankophor RKH, and Tinopal LPW), few differences were found ($P > 0.05$) in $LC_{50}$s, $LC_{90}$s and the times required for mortality to increase from 50% to 90%. Three optical brighteners (i.e., Blankophor BSU, Blankophor DML, Blankophor LPG) did not act as enhancers. The $LC_{50}$ value for SfMNPV/LPG was less than that of SfMNPV alone, but the difference was non-significant ($P > 0.05$). From the standpoint of $LT_{50}$s and $LT_{90}$s there were no differences found ($P > 0.05$) among these and the virus standard (=SfMNPV alone).

## Discussion

During the past 30 years SfMNPV has been tested against the fall armyworm. While limited control has been obtained in small field tests (Hamm and Hare 1982), this virus is generally considered to have low virulence for *S. frugiperda*. In our study we took three approaches: (1) would other NPVs be infective for the fall armyworm?

**Table 2. Effect of different NPVs and and optical brightener upon speed of kill of SfMNPV: $LT_{50}$ and $LT_{90}$**

| Treatment | $LT_{50}$* (95%CL) | $LT_{90}$* (95%CL) | Difference between+ $LC_{50}$ and $LC_{90}$ |
|---|---|---|---|
| SfMNPV | 5.7 | 7.5 | 1.8 |
| | (4.8–6.4) | (6.6–10.0) | |
| SfMNPV + Tinopal LPW | 3.5 | 4.7 | 0.8 |
| | (3.0–3.6) | (4.0–6.5) | |
| AfMNPV | ** | ** | |
| AfMNPV + Tinopal LPW | | | |
| HaMNPV | ** | ** | |
| HaMNPV + Tinopal LPW | 5.1 | 7.3 | 2.2 |
| | (4.9–6.3) | (6.5–9.2) | |
| GmMNPV | ** | ** | |
| GmMNPV + Tinopal LPW | 6.5 | 9.4 | 2.9 |
| | (5.5–8.0) | (7.8–13.1) | |
| AcMNPV | ** | ** | |
| AcMNPV + Tinopal LPW | 5.3 | 6.6 | 1.3 |
| | (4.6–5.9) | (5.1–9.3) | |

* $LT_{50}$s and $LT_{90}$s are expressed in days and are determined for a concentration of 74.7 PIBs/mm$^2$ 7 replicates.

** $LT_{50}$s and $LT_{90}$s could not be determined at 74.7PIB/mm$^2$

+ The difference (in days) from an $LT_{50}$ to an $LT_{90}$ indicates the time required to increase virus-caused mortality from 50% to 90%.

(2) would an optical brightener (Tinopal LPW), which acts as a viral enhancer, for LDMNPV enhance the activity of the other virus, and (3) would other optical brighteners that enhance other NPVs also as a viral enhancer for SfMNPV?

SfMNPV is specific and does not infect a closely related insect, *S. exigua* (Hűbner) (Hamm and Styer 1985). In instances where *S. frugiperda* coexists with *H. zea* or *Prodenia unipuncta* (Haworth) in sweet corn ecosystems (Young and Hamm 1965, Weber and Ferro 1991), SfMNPV would only infect the fall armyworm. In such an instance, the use of a specific virus might be undesirable as a control strategy. Two or more viruses (one for each pest insect species) or a less specific virus could be considered.

More than 20 years ago the alfalfa looper, *A. californica* (Speyer), NPV (AcMNPV) was shown by Vail and colleagues to have a wide host range (Vail et al. 1971a,b, 1978). More recently, a variant of AcMNPV was isolated from the celery looper, *A. falcifera* (Kirby) (AfMNPV), which also has a wide host range (Hostetter and Puttler 1990, Vail et al. 1992). These two discoveries opened the possibilities of using a single NPV against more than one insect pest species for population control. Hamm

**Table 3. The effect of different optical brighteners upon the activity of SfMNPV: $LC_{50}$ and $LC_{90}$**

| Treatment | Slope (±SEM) | $LC_{50}$* (95% CL) | AR* | $LC_{90}$* (95% CL) | AR** |
|---|---|---|---|---|---|
| SfMNPV | 1.17 | 13.12 | 1.0 | 79.60 | 1.0 |
| | (0.01) | 8.41–20.23 | | 48.65–150.82 | |
| SfMNPV + DML | 1.04 | 20.70 | 0.6 | 163.53 | 0.5 |
| | (0.01) | 12.86–33.33 | | 93.50–344.84 | |
| HRS | 1.03 | 0.63 | 21.0 | 15.17 | 10.7 |
| | (0.01) | 0.39–1.05 | | 7.03–16.34 | |
| P167 | 1.26 | 0.66 | 20.0 | 8.49 | 9.3 |
| | 0.02 | 0.43–1.02 | | 12.08–20.25 | |
| BBH | 1.30 | 0.33 | 40.0 | 3.19 | 24.7 |
| | (0.02) | 0.22–0.49 | | 1.88–6.58 | |
| LPG | 1.52 | 9.23 | 1.4 | 64.42 | 1.2 |
| | (0.03) | 6.44–13.34 | | 39.46–129.90 | |
| BSU | 1.23 | 26.08 | 0.5 | 286.92 | 0.2 |
| | (0.02) | 17.49–39.09 | | 166.33–607.80 | |
| RKH | 1.52 | 0.12 | 105.8 | 0.86 | 101.1 |
| | (0.03) | 0.09–0.18 | | 0.54–1.64 | |
| LPW | 1.42 | 0.09 | 140.5 | 0.50 | 158.0 |
| | (0.03) | 0.07–0.12 | | 0.31–0.80 | |

* $LC_{50}$s and $LC_{90}$s are expressed as PIBs per $mm^2$; 5 concentrations per virus (.007 to 74.7 PIBs per $mm^2$); 6 replicates; 10 untreated larvae and 10 larvae per brightener per replicate. No mortality was observed among any untreated or brightener treated larvae.

** Activity Ratio is calculated by dividing the $LC_{50}$ for SfMNPV (Standard) by the $LC_{50}$ for SfMNPV/brightener.

(1982) tested an isolate of HaMNPV from Russia and found that *H. zea, H. virescens, S. exigua* and *S. frugiperda* larvae were infected. *Helicoverpa zea* larvae were the most susceptible, whereas *S. frugiperda* larvae were least susceptible. Tompkins et al. (1988) showed that HaMNPV had a wider host range than the SNPV of *H. zea.* Fraser and Hink (1982) reported that an MNPV from the greater wax moth, *G. mellonella* (L.), (GmMNPV) also infected cabbage loopers, *T. ni* (Hűbner), and corn earworms (Noctuidae), as well as tobacco hornworms, *Manduca sexta* (L.) (Sphingidae). Subsequent *in vitro* studies demonstrated that GmMNPV was also infective for larvae (Witt and Janus 1977) and cell cultures of the cabbage looper and fall armyworm (Witt and Janus 1977, Fraser and Hink 1982). With the exception of SfMNPV, none of the NPVs tested were highly infectious for the fall armyworm. These data are very instructive and may be typical for the use of wide-s pectrum NPVs such as AcMNPV and AfMNPV. While these NPVs infect insects from different species,

**Table 4. The effect of optical brighteners upon speed of kill of SfMNPV: $LT_{50}$ and $LT_{90}$**

| Treatment | $LT_{50}$* (95% CL) | $LT_{90}$* (95% CL) | Difference between** $LT_{50}$ and $LT_{90}$ |
|---|---|---|---|
| SfMNPV | 4.8 | 6.1 | 1.3 |
| | (4.3–5.4) | (5.4–8.4) | |
| SfMNPV + DML | 3.9 | 6.9 | 3.0 |
| | (3.4–4.8) | (4.9–9.0) | |
| HRS | 3.3 | 4.3 | 1.0 |
| | (2.8–3.7) | (3.8–5.9) | |
| P167 | 3.7 | 4.7 | 1.0 |
| | (3.2–4.1) | (4.2–6.4) | |
| BBH | 3.4 | 4.5 | 1.1 |
| | (3.0–3.9) | (4.0–6.2) | |
| LPG | 4.8 | 6.2 | 1.4 |
| | (4.2–5.4) | (5.5–9.0) | |
| BSU | 4.6 | 5.9 | 1.3 |
| | (3.9–5.2) | (4.4–8.7) | |
| RKH | 3.5 | 4.6 | 1.1 |
| | (3.1–4.0) | (4.1–5.3) | |
| LPW | 3.5 | 4.5 | 1.0 |
| | (3.0–4.0) | (3.4–6.2) | |

* $LT_{50}$s and $LT_{90}$s are expressed in days and are determined for a concentration of 74.7 PIB per $mm^2$; 6 replicates.

** The difference (in days) from an $LT_{50}$ to a $LT_{90}$ indicates the time required to increase virus-caused mortality from 50% to 90%.

genera, and even families, activities vary widely from insect to insect. Shapiro and Vaughn (1995) demonstrated that the most virulent NPV for *H. zea* larvae was the homologous NPV HzSNPV, whereas NPVs as AcMNPV, AfMNPV GmMNPV and HaMNPV were much less virulent. In the case of the beet armyworm, the most virulent NPV was the homologous NPV SeMNPV (90 PIB per cup), whereas AcMNPV, AfMNPV, GmMNPV, and HaMNPV had low infectivity (Shapiro, unpub. data). The lesson from this research is that, in general, the homologous virus has the most biological activity against the homologous insect.

Regardless of the NPV tested, Tinopal LPW increased the activities of the NPVs tested and resulted in $LC_{50}$s for AcMNPV, AfMNPV, and HaMNPV that were 11.6-, 4.0-, and 3.6-fold lower that than of SfMNPV alone (Table 1). These results were similar to those obtained for *H. zea* (Shapiro and Vaughn 1995) and for *S. exigua* (Shapiro, unpub. data). For these three host-virus systems, three conclusions may be

drawn: (1) the homologous NPV was the most virulent; (2) Tinopal LPW increased the activity of the heterologous NPVs (as measured by $LC_{50}$s) so that the heterologous NPV/Tinopal LPW combinations had activities similar to those of the homologous NPVs alone; and (3) the most active NPV/LPW combination was that of the homologous NPV/Tinopal LPW combination. In terms of $LT_{50}$s, the addition of Tinopal LPW to SfMNPV reduced the $LT_{50}$ by almost 40% and significantly reduced the time required from the $LT_{50}$ to the $LT_{90}$ (i.e., it required fewer days to increase the virus-caused mortality from 50% to 90%) (Table 2). For the heterologous NPVs, the addition of Tinopal LPW resulted in $LT_{50}$s that were comparable to that for the homologous NPV alone (SfMNPV) (Table 2).

Concerning speed of kill, these generalizations appear to be valid: (1) the homologous virus is faster acting than the heterologous NPVs; (2) Tinopal LPW increased the speed of kill of the heterologous viruses, which was now comparable to that of the homologous NPV alone; and (3) the fastest speed of kill was obtained with a combination of homologous NPV/Tinopal LPW. The eight brighteners used in this study SfMNPV were used with LdMNPV (Argauer and Shapiro 1997) (HzSNPV) (Shapiro, unpub. data), and SeMNPV against their respective hosts. (Shapiro, unpub. results) (Table 5). In general, the results were quite similar among the four virus-host systems and can be summarized: (1) Blankophor BBH, Blankophor HRS, Blankophor P167, Blankophor RKH, and Tinopal LPW acted as viral enhancers (2) Blankophor BSU, Blankophor DML, and Blankophor LPG did not act as enhancers for LdMNPV, SeMNPV, and SfMNPV. (Blankophor BSU and Blankophor DML enhanced the ac-

**Table 5. Relative activities of eight optical brighteners as viral enhancers for LdMNPV, SfMNPV, SeMNPV, and HzSNPV**

| | NPV tested** | | | |
|---|---|---|---|---|
| Optical Brightener* | LdMNPV† | SfMNPV‡ | SeMNPV§ | HzSNPV§ |
| Blankophor BBH | 830 | 40 | 140 | 4 |
| Blankophor BSU | 1.1 | 0.5 | 1.4 | 6.1 |
| Blankophor DML | 0.8 | 0.6 | 0.9 | 4.9 |
| Blankophor HRS | 90 | 21 | 420 | 10 |
| Blankophor LPG | 0.4 | 1.4 | 0.7 | 1.0 |
| Blankophor P167 | 410 | 20 | 11 | 4 |
| Blankophor RKH | 1000 | 106 | 50 | 10 |
| Tinopal LPW | 1300 | 140 | 90 | 18 |

* Optical brighteners were tested at 1% (wt:wt). Relative activity of standard NPV (=NPV only) = 1.0.

** Values listed indicate the Activity Ratio, which is the ratio of $LC_{50}$ of homologous NPV vs. homologous NPV/Tinopal LPW combination. Values greater than 1.0 (=value for the homologous NPV alone) indicate activity greater than that of the virus alone; values less than 1.0 indicate activity less than that of the virus alone.

† Data from Argauer and Shapiro (1997).

‡ Data from the present study.

§ Data from Shapiro (unpublished).

tivity of HzSNPV, but Blankophor LPG did not). In general, Tinopal LPW was the best enhancer, followed by Blankophor RKH, Blankophor HRS, and Blankophor BBH.

These brighteners represent an intriguing challenge to entomologists dealing with basic and applied issues. At this time, brighteners appear to be the only materials that can influence both the virus concentration required to kill ($LC_{50}$) and the time required to kill ($LT_{50}$), as well as influencing the host range of a given virus. Good progress has been made in elucidating the mode of action of these materials (Adams et al. 1994, Dougherty et al. 1995, Sheppard and Shapiro 1994, Sheppard et al. 1994, Washburn et al. 1998), but the complete picture has yet to be obtained. Once the mode (or modes) of action are understood, these brighteners (or future generations of like materials) will play an important role in insect control using entomopathogenic viruses.

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