Effect of Tinopal LPW on the Insecticidal Properties and Genetic Stability of the Nucleopolyhedrovirus of *Spodoptera exigua* (Lepidoptera: Noctuidae)

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ABSTRACT The influence of an optical brightener, Tinopal LPW, on the activity of a purified genotype of the nucleopolyhedrovirus (SeMNPV) of the beet armyworm, *Spodoptera exigua* (Hübner), was determined in second to fifth instar (L_2-L_5) *S. exigua*. When mixed with viral occlusion bodies (OB) 1% Tinopal LPW significantly reduced the median lethal dose (LD_{50}) of the virus in all instars compared with insects treated with SeMNPV alone. Levels of enhancement, as determined by LD_{50} values, ranged from 2.6- to 580-fold, depending on the instar. The greatest enhancement occurred on the two later instars, L_4 (70-fold) and L_5 (580-fold), which show a much higher resistance to SeMNPV infection than earlier instars. The median time to death (MTD) values were not significantly different in any instar among larvae treated with SeMNPV + Tinopal LPW and those treated with SeMNPV alone. Larval development in SeMNPV + Tinopal LPW treated larvae was retarded, in second and fourth instars, compared with controls or larvae treated with SeMNPV alone. The OB yields from SeMNPV treated larvae were almost 1.6-fold greater in second instars (9.3×10^6 OBs/larvae), and 1.9-fold greater in fourth instars (1.9×10^8 OBs/larvae), than those obtained in larvae treated with SeMNPV + Tinopal LPW. The addition of 1% Tinopal LPW to the virus suspension did not alter the genotypic composition of viral progeny during four successive passages of the virus.

KEY WORDS nucleopolyhedrovirus, *Spodoptera exigua*, insecticidal activity, optical brightener, synergism

BEET ARMYWORM, *Spodoptera exigua* (Hübner) (Lepidoptera; Noctuidae), is an economically important insect pest of many vegetable, ornamental and row crops in temperate and subtropical regions (Cayrol 1972). In Europe, this pest attacks both greenhouse and field crops (Sunderland et al. 1992), with the most serious crop damage resulting from the feeding of late instars on leaves and fruits (Smits 1987).

This noctuid pest has been difficult to control because of varying degrees of resistance to many chemical insecticides (Brewer and Trumble 1989, 1994), although new chemistries, such as spinosad, indoxacarb, methoxyfenozide, and others, are now available for control of *S. exigua* (Yee and Toscano 1998). Baculoviruses are safe and selective biological control agents and their use has been advocated in many parts of the world (Moscardi 1999). A multicapsid nucleopolyhedrovirus, specific to the beet armyworm (SeMNPV), has been used successfully as a bioinsecticide to control *S. exigua* populations in the field (Gelernter et al. 1986, Kolodny-Hirsch et al. 1993) and greenhouses (Smits and Vlak 1988). This virus has been produced commercially, registered in several countries, and marketed for use in crop protection under the name SPOD-X (Smits and Vlak 1994).

A range of compounds have been evaluated as potential adjuvants for inclusion in baculovirus formulations to overcome some of the limitations in the use of these pathogens as bioinsecticides. Over the last decade, several reports have shown that stilbene-derived optical brighteners may protect the virus from solar radiation (Shapiro 1992, Dougherty et al. 1996), increase the susceptibility of lepidopteran pests to viral infection (Hamm and Shapiro 1992, Shapiro and Robertson 1992, Hamm and Chandler 1996, Morales et al. 2001, Okuno et al. 2003), reduce larval survival time (Shapiro and Vaughn 1995, Vail et al. 1996, Shapiro 2000, Boughton et al. 2001), and extend the host range of the virus (Shapiro and Dougherty 1994).

The majority of studies with optical brighteners have been performed on early instars, except in the case of the gypsy moth, *Lymantria dispar* (L.), (Shapiro and Robertson 1992, Webb et al. 1994) and the soybean looper, *Pseudoplusia includens* (Walker), (Zou and Young 1994). These studies have reported stage-related differences in susceptibility to NPV-optical brightener mixtures. This is of particular rele-

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vance because for most baculovirus-lepidopteran host systems, including *S. exigua*, host larvae show increasing stage-dependent resistance to virus infection (Evans 1983, Briese 1986, Smits 1987, Escribano et al. 1999).

In the study described here, we have quantified mortality and time to death in beet armyworm L_2–L_5 instars after ingestion of SeMNPV mixed with Tinopal LPW compared with SeMNPV alone. We also assessed any potential effect of Tinopal LPW on the genotypic composition of the virus after serial passage in vivo and quantified the effects of the optical brightener on viral occlusion body production in L_2 and L_4 instars. We selected Tinopal LPW as this has proved to be among the most active optical brighteners for potentiation of the insecticidal capacity of baculoviruses (Hamm 1999, Shapiro and Argauer 2001).

Materials and Methods

Insects. *S. exigua* larvae were obtained from a colony maintained at the Universidad Pública de Navarra, Pamplona, Spain, where they were incubated at a constant temperature (25 ± 2°C), humidity (70 ± 5%), and photoperiod (16 h:8 h L:D) on an artificial diet.

Viruses. The wild-type SeMNPV isolate from Florida (SeMSeMNPV-US2) and the most abundant genotype in this population, named Se-US2A, were used for this study. Occlusion bodies (OBs) used in the bioassays were produced by a single amplification passage through L_4 *S. exigua.* Twenty-five starved larvae were inoculated orally with an occlusion body (OB) suspension and reared until death. SeMNPV-killed larvae were collected and OBs purified as described previously (Muñoz et al. 1998). OB titration was determined by three independent counts using a Neubauer chamber (Hawksley, Lancing, United Kingdom) under phase contrast microscopy at ×400.

Bioassays. The LD_{50} values of a mixture of SeMNPV and 1% Tinopal LPW (Sigma, St. Louis, MO) on L_2, L_3, L_4, and L_5 *S. exigua* were determined using a modified droplet feeding bioassay technique (Hughes et al. 1986). For each instar assayed, newly molted larvae were starved for 16–20 h and then allowed to drink from an aqueous suspension containing OBs, 1% (wt:vol) Tinopal LPW, 10% sucrose (wt:vol), and 0.001% (wt:vol) Fluorella Blue. An identical number of larvae were fed viral OBs suspended in a solution of sucrose and Fluorella Blue alone. Other groups of larvae were offered 1% Tinopal LPW only, and mock infections (water) as controls. A range of five doses of OBs previously calculated to produce mortalities between 5 and 95%, and an OB-free solution were supplied to groups of larvae. Twenty-five of the larvae that ingested the suspensions within 10 min were transferred to 25-well tissue culture plate containing fresh diet and reared at 25 ± 2°C. Bioassays were performed three times. Larvae were monitored three times a day for 7 d after inoculation. Mortality results were subjected to probit analysis (Finney 1971) using the POLO-PC program (Le Ora Software, 1987).

Time-mortality data were analyzed only from batches of L_2 and L_5 for which mortality was around 90%. The mean time to death (MTD) values were estimated using the Generalized Linear Interactive Modeling (GLIM) program with a Weibull distribution specified (Crawley 1993).

In Vivo Serial Passages. To determine the genetic stability and the relative proportions of the SeMNPV-US2 genotypic variants in the presence of Tinopal LPW, we performed four in vivo serial passages of the virus. Initially, four batches of 25 newly molted L_5 larvae were droplet fed on suspensions containing OB concentrations calculated to produce 90% mortality of the following inocula: (1) wild type Se-US2; (2) genotypic variant Se-US2A; (3) wild type Se-US2 + 1% Tinopal LPW; (4) genotypic variant Se-US2A + 1% Tinopal LPW. As controls, a cohort of larvae was fed with 1% Tinopal LPW alone and another one was mock-infected with water. SeMNPV-killed larvae were individually collected and OBs purified as indicated above. For each passage, 100 μl aliquots of OBs from each larva in a treatment were pooled, titred, and subjected to restriction endonuclease (REN) analysis. Pooled OBs were labeled as P1 and used as inocula for the second passage. The same procedure was used through three additional successive passages (P2, P3, P4) and in three replicate experiments.

Genotypic Analysis. Viral DNA from the P1, P2, P3 and P4 suspensions was isolated from 10^8 OBs in 300 μl of water and purified as described by Muñoz et al. (2000). Approximately 1 μg of viral DNA was incubated with the restriction enzyme *Pst*I. Reactions were stopped by addition of loading buffer (0.25% wt:vol bromophenol blue, 40% wt:vol sucrose in water), loaded in 0.7% TAE buffer (40 mM Tris-Acetate; 1 mM EDTA) agarose gels, and electrophoresed at 20–40 V for 6–12 h. Ethidium bromide stained gels were then photographed on a UV transilluminator using the Geldoc software (Bio-Rad, Madrid, Spain).

Calculation of OB Yield. Newly molted L_2 and L_4 *S. exigua* larvae were selected as determined by head capsule slippage. Batches of 50 larvae were inoculated with their corresponding LD_{90} values (148 OB or 56 OB + 1% Tinopal LPW for L_2; 6453 OB and 20 OB + 1% Tinopal LPW for L_4), as determined above. Similar numbers of larvae were treated with 1% Tinopal LPW or sterile distilled water as controls. Larvae that consumed the inocula were placed individually on artificial diet and maintained at 26 ± 2°C. The weight and molting time of individual larvae was registered daily until death to assess the influence of Tinopal LPW on the development of infected larvae and OB production. Weight measurements were first taken immediately before inoculation in L_4 larvae and 24 h postinoculation (h.p.i.) in L_2. Larvae were weighed daily until they died. When larvae became moribund and showed clear signs of advanced infection, they were individually transferred to clean Eppendorf tubes. At 8 h after death, cadavers were frozen and stored at −20°C. Three replicates were performed for each instar.

Table 1. LD_{50} values and relative potencies of Se-US2A without and with Tinopal LPW for second, third, fourth and fifth instar *S. exigua*

Treatment	Regression line		LD_{50} (OB/larva)	χ^2	Relative potency	C.L. (95%)	
	slope ± SE	intercept ± SE				Lower	Upper
L_2							
Se-US2A	1.36 ± 0.89	−1.27 ± 0.18	12.7	3.1	1.0	—	—
Se-US2A+T	1.07 ± 0.13	0.51 ± 0.53	5.0	0.6	2.6	1.7	3.9
L_3							
Se-US2A	0.71 ± 0.10	3.78 ± 0.21	52.3	1.5	1.0	—	—
Se-US2A+T	2.08 ± 0.33	2.88 ± 0.39	10.6	2.5	4.9	3.0	8.2
L_4							
Se-US2A	0.92 ± 0.10	2.82 ± 0.27	260.5	1.4	1.0	—	—
Se-US2A+T	1.72 ± 0.16	4.02 ± 0.14	3.7	1.4	69.9	45.4	107.5
L_5							
Se-US2A	1.19 ± 0.12	−0.11 ± 0.50	19656	1.7	1.0	—	—
Se-US2A+T	1.46 ± 0.18	1.75 ± 0.31	34.8	2.3	583.2	393.6	875.9

T, Tinopal LPW (1%). Parameters obtained from the POLO-PC program (LeOra Software 1987). Relative potencies were calculated after fitting regression lines with a common slope of 1.20 for second instar ($\chi^2 = 2.43$; df = 1; $P = 0.119$) and of 1.27 for fifth instar ($\chi^2 = 1.67$; df = 1; $P = 0.195$). For third and fourth instars the regression lines could not be fitted in parallel, and thus the relative potency is expressed as the ratio of LD_{50} values (Robertson and Preisler 1992).

The median increase in weight was calculated as the difference between the weight (mg) at harvest and the weight at inoculation for L_4, or at 24 h.p.i for L_2. For calculation of OB yields, 30 SeMNPV-killed larvae from each instar were randomly selected for OB purification as described above. Purified OBs were resuspended in 0.5 ml of bidistilled water and appropriately diluted for titration. OB yield per larva was quantified by counting triplicate samples of the diluted suspensions using a Neubauer chamber. OB production and weight gain data were not normally distributed. The Kruskal–Wallis nonparametric test was therefore used to compare differences among treatments, and the Mann–Whitney test was employed for comparisons involving two samples using SPSS v 10 (SPSS Inc., Chicago, IL).

Results

Effect of Tinopal LPW on the Pathogenicity and Virulence of Se-US2A. The addition of 1% Tinopal LPW to Se-US2A enhanced viral pathogenicity in all *S. exigua* instars tested, as indicated by a non-overlap of the 95% confidence limits of the relative potency (Table 1). The degree of potentiation of Tinopal LPW was much greater in L_4 (70-fold) and L_5 (583-fold) than in L_2 (2.6 fold) or L_3 (4.9 fold). Dose-mortality regression lines with and without Tinopal LPW for L_2 and L_5 were fitted in parallel with common slopes of 1.20 ($\chi^2 = 2.43$; df = 1; $P = 0.119$) and 1.27 ($\chi^2 = 1.67$; df = 1; $P = 0.195$), respectively. In L_3 and L_4, however, regression lines for SeMNPV + Tinopal LPW and SeMNPV alone could not be fitted in parallel. Relative potency was, therefore, calculated as the ratio of LC_{50} values (Robertson and Preisler 1992).

Tinopal LPW did not alter the speed of kill of Se-US2A in L_2 or L_5. The MTD value (mean ± SE) on L_2 inoculated with SeMNPV + Tinopal LPW (108 ± 2.3 h) was similar to that obtained from larvae inoculated with SeMNPV alone (107 ± 1.6 h). The same pattern was observed in L_5 MTD values of the virus with (137 ± 3.2 h), and without (137 ± 3.6 h), Tinopal LPW.

Effect of Tinopal LPW on Development of Infected Larvae. Comparison of the median weight increase between treatments revealed that virus infection was the main factor resulting in reduced larval weight gain (Table 2). A weak but significant delay in the development of L_4 larvae treated with SeMNPV + Tinopal LPW was observed with respect to those treated with SeMNPV alone. L_4 were homogenous in weight at inoculation, whereas small but significant differences in weight were detected between treatments for L_2 at 24 h.p.i. ($\chi^2 = 26.7$; df = 3; $P < 0.001$), which was probably because of the inoculation process.

Molting at 48 h.p.i. was accomplished by 70% of mock-infected larvae, 57% of Tinopal LPW-treated larvae, 36% of larvae treated with SeMNPV alone, and only 12.5% of SeMNPV + Tinopal LPW inoculated

Table 2. Weight gain and occlusion body yields in L_2 and L_4 *S. exigua* following inoculation with SeMNPV alone or in mixtures with 1% Tinopal LPW

Treatment	Median initial weight (mg)[a,b]	Median increase in weight (mg)[b]	Median OB yield/larva ($\times 10^6$)[c]
L_2			
Water	1.31 a	9.94 a	—
Water + T	1.27 a	10.00 a	—
Se-US2A	1.15 b	0.69 b	9.32 a
Se-US2A + T	1.15 b	0.62 b	5.36 b
L_4			
Water	8.60 a	190.30 a	—
Water + T	8.40 a	170.05 b	—
Se-US2 A	8.50 a	41.65 c	187 a
Se-US2A + T	8.20 a	34.00 d	103 b

T, Tinopal LPW (1%).

[a] Weights were registered at 24 hours post inoculation for L_2 and at inoculation for L_4.

[b] Median values followed by the same letter are not significantly different (Kruskall-Wallis, $P > 0.05$) for comparisons within instar.

[c] Median values followed by the same letter are not significantly different (Mann-Whitney, $P > 0.05$) for comparisons within each pair of treatments.

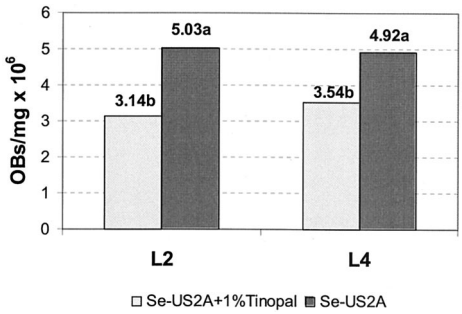

Fig. 1. Median yield values in millions of OBs/mg from *Spodoptera exigua* inoculated with Se-US2A and Se-US2A + 1% Tinopal LPW in the L_2 and L_4 stages. Columns headed the same letter are not significantly different for comparisons within each instar (Mann–Whitney test, $P > 0.05$).

larvae. Those larvae that consumed 1% Tinopal LPW either in water or together with SeMNPV showed a significantly retarded development ($\chi^2 = 188$, df = 3; $P < 0.001$). At the end of the bioassay, all of the non virus treated L_4 larvae had molted, while only 41% and 20% of those treated with SeMNPV alone or SeMNPV + Tinopal LPW, respectively, reached the fifth instar. Treatment with SeMNPV + Tinopal LPW significantly retarded larval development compared with the treatment of SeMNPV alone ($\chi^2 = 38.7$; df = 3; $P < 0.001$).

Effect of Tinopal LPW on OB Production. The addition of 1% Tinopal LPW significantly reduced the median number of OB/larva produced in larvae infected at L_2 and L_4 (Table 2). In addition, both instars showed significantly reduced yields in terms of OBs/mg of larva when they were inoculated with SeMNPV + Tinopal LPW compared with the respective treatments of SeMNPV alone (Mann–Whitney test, $P < 0.05$; Fig. 1). As expected, OB yields produced in later instars were higher than those in early instars, reflecting the marked differences in weight at death between instars.

Effect of Tinopal LPW on Genetic Stability. Tinopal LPW did not affect the genotypic composition of either clone (Se-US2A) or wild-type (Se-US2wt) at any of the four successive in vivo passages. *Pst*I profiles of viral DNA, obtained from each of the four passages and from the three replicates of Se-US2A and Se-US2 with 1% Tinopal LPW, were identical to those of the parental viruses (results not shown).

Discussion

In the present work, four different *S. exigua* instars were used to study the influence of Tinopal LPW on the insecticidal properties of Se-US2A. In addition, we analyzed the genetic stability of the virus through four successive passages in larvae in the presence and absence of the optical brightener.

The LD_{50} of Se-US2A was significantly reduced by Tinopal LPW in all beet armyworm instars (L_2 through L_5). This synergistic effect had already been observed in L_1 and L_2 *S. exigua* (Hamm and Chandler 1996, Shapiro 2000, Shapiro and Argauer 2001) but no enhancement was detected for later instars (Zou and Young 1994). In other host-virus systems, such as those of *P. includens* (Zou and Young 1994) and *L. dispar* and their homologous NPVs (Shapiro and Robertson 1992), the addition of Tinopal LPW also enhanced NPV mortality in the late instars to a similar degree to that of early instars. However, our results clearly demonstrate that for SeMNPV, the more developed the larvae at the time of infection, the stronger was the virus enhancing effect provided by Tinopal LPW.

In general, the synergistic effect of optical brighteners increases as larval susceptibility to the infection decreases, independent of the host-virus system (Hamm 1999). For SeMNPV, this was demonstrated in several *S. exigua* biotypes with different susceptibilities to infection by this virus (Hamm and Chandler 1996). Because host resistance to baculoviruses increases with host age (Briese 1986), an effect especially evident in the *S. exigua*-SeMNPV system (Smits 1987), the tendency of optical brighteners to effectively eliminate stage-dependent resistance to SeMNPV is of the greatest importance (compare L_2 LD_{50} of 5.0 OBs with L_4 LD_{50} of 35 OBs in the presence of Tinopal LPW). In the field, where the presence of different larval stages at the same time and place is the normal situation, the formulation of SeMNPV with an optical brightener may allow effective control of damaging late instars, permit the use of lower OB concentrations in each application and may enhance the persistence of OBs through solar UV protection.

The effect of Tinopal LPW on the midgut peritrophic membrane (PM) may explain the similar dose–responses observed in the different instars. Stilbene optical brighteners degrade the chitin structure that forms the PM (Wang and Granados 2000). Specifically, Tinopal LPW increases the permeability of the midgut PM allowing for a higher number of virions to infect the midgut epithelial cells, thus, increasing the probability of systemic infection (Wang and Granados 2000). Recently, it has been demonstrated that the viral-enhancing activity of the optical brighteners was correlated with their ability to degrade the PM structure (Okuno et al. 2003). The occurrence of several *Anticarsia gemmatalis* biotypes showing resistance to their homologous nucleopolyhedrovirus and bearing a thicker PM than nonresistant conspecifics, supports the hypothesis that the PM represents a key barrier to baculovirus infection (Levy et al. 2002).

Tinopal LPW had no direct effect on MTD estimates, which is contrary to most previous studies. Approximately, 30–40% reductions in lethal time values (LT_{50}) have been recorded for SeMNPV when this virus was administered with Tinopal LPW (Shapiro and Argauer 2001). However, many of these reductions result from comparing treatments that cause significantly different levels of mortality. Because optical brighteners greatly reduce NPV lethal doses, NPV-Tinopal treatments constitute a greater relative viral dose than treatments involving NPV alone and a reduction of speed of kill would thus be anticipated. For this reason, we compared the MTDs of SeMNPV

+ Tinopal LPW and SeMNPV alone that produced similar levels of mortality. Results analogous to these have been previously obtained with the nucleopolyhedroviruses of *Agrotis ipsilon* (Boughton et al. 2001), *Spodoptera littoralis* (Murillo et al. 2003), and *Spodoptera frugiperda* (Martínez et al. 2000), for which similar experimental approaches and data analyses were employed.

The reduction of OB yield that we observed in larvae treated with SeMNPV + Tinopal LPW compared with larvae inoculated with SeMNPV alone is consistent with similar observations on *L. dispar* (Shapiro and Robertson 1992). These authors found that larvae exposed to LdMNPV + Tinopal LPW mixtures were about one-half the weight of larvae exposed to LdMNPV alone, although no correlation between OB yield and larval weight was established. Previous studies indicated that Tinopal LPW retarded development and reduced larval weight gain either when administered in combination with an NPV (Sheppard and Shapiro 1994, Wang and Granados 2000) or alone (Martínez et al. 2003). Therefore, we expected a reduction in weight gain for those larvae that had been inoculated with the brightener, which indeed occurred in L_4 larvae. Yet, the large reduction in OB yield cannot be accounted for by the reduction in larval weight gain alone, because clear differences were also observed in the number of OBs/mg of larva.

The fact that Tinopal LPW reduces OB yield has several practical consequences. First, this brightener should not be considered for baculovirus in vivo mass production. Second, Tinopal LPW could theoretically affect pest management programs where baculoviruses are applied according to an inoculative strategy because the transmission process, and therefore, the bimodal temporal pattern of mortality caused by the virus in field populations, relies on the in situ amplification and liberation of viral progeny (Woods and Elkinton 1987, Sterling et al. 1988). However, field studies on *L. dispar* tend to contradict this prediction (Webb et al. 1994). This type of strategy is not as common in crop ecosystems as the inundative strategy, in which control is essentially based on the application of high rates of virus and therefore, baculovirus field performance would not be impaired by the use of Tinopal LPW.

Viral DNA restriction endonuclease profiles remained unchanged throughout four in vivo passages, indicating that Tinopal LPW did not affect the genotypic composition of the wild type Se-US2 viral inoculum or the purified Se-US2A genotype. Noticeable changes in the genome such as large deletions, insertions, or reorganizations were also not seen. More subtle changes such as point mutations or small deletions or insertions, which might have been produced when virions infected gut cells in the presence of the brightener, could not be detected by the means employed here and have not been reported for any other host-virus systems to date. If such minor changes occurred, they did not alter the most important biological features of the virus as a bioinsecticide.

Despite the positive aspects that favor optical brightener + NPV mixtures, studies on field efficacy under commercial growing conditions are required to demonstrate that the potentiation observed in the laboratory translates to the field. The cost of these substances as viral adjuvants (Martínez et al. 2000), their effect on pollinators (Goulson et al. 2000) and on crop growth (Goulson et al. 2003) are also issues of concern for which further environmental evaluations are required before optical brighteners can be included in commercial baculovirus formulations.

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References Cited

Boughton, A. J., L. C. Lewis, and B. C. Bonning. 2001. Potential of *Agrotis ipsilon* nucleopolyhedrovirus for suppression of the black cutworm (Lepidoptera: Noctuidae) and effect of an optical brightener on virus efficacy. J. Econ. Entomol. 94: 1045–1052.

Brewer, M. J., and J. T. Trumble. 1989. Field monitoring for insecticide resistance in beet armyworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 82: 1520–1526.

Brewer, M. J., and J. T. Trumble. 1994. Beet armyworm resistance to fenvalerate and methomyl: resistance variation and insecticide synergism. J. Agric. Entomol. 11: 291–300.

Briese, D. T. 1986. Insect resistance to baculovirus, pp. 237–263. *In* R. R. Granados and B. A. Federici [eds.], The biology of baculoviruses. CRC, Boca Raton, FL.

Cayrol, R. A. 1972. Famille des Noctuidae. *In* Balachowsky [ed.], Entomologie appliquée à l'agriculture. Masson et Cie, Paris, France.

Crawley, M. J. 1993. GLIM for ecologists. Blackwell Science, Oxford, United Kingdom.

Dougherty, E. M., K. Guthrie, and M. Shapiro. 1996. Optical brighteners provide baculovirus activity enhancement and UV radiation protection. Biol. Contr. 7: 71–77.

Escribano, A., T. Williams, D. Goulson, R. D. Cave, J. W. Chapman, and P. Caballero. 1999. Selection of a nucleopolyhedrovirus for control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae): structural, genetic, and biological comparison of four isolates from the Americas. J. Econ. Entomol. 92: 1079–1085.

Evans, H. F. 1983. The influence of larval maturation on mortality on responses of *Mamestra brassicae* L. (Lepidoptera: Noctuidae) to nuclear polyhedrosis virus infection. Arch. Virol. 75: 163–169.

Finney, D. J. 1971. Probit analysis. Cambridge University Press, Cambridge, United Kingdom.

Gelernter, W. D., N. C. Toscano, K. Kido, and B. A. Federici. 1986. Comparison of a nuclear polyhedrosis virus and chemical insecticides for control of the beet armyworm (Lepidoptera: Noctuidae) on head lettuce. J. Econ. Entomol. 79: 714–717.

Goulson, D., A. M. Martínez, W. O. Hughes, and T. Williams. 2000. Effects of optical brighteners used in biopesticide formulations on the behavior of pollinators. Biol. Contr. 19: 232–236.

Goulson, D., L. C. Derwent, D. I. Penagos, and T. Williams. 2003. Effects of optical brighteners included in biopesticide formulations on the growth of crops. Agric. Ecosyst. Environ. 95: 235–240.

Hamm, J. J. 1999. Interactions in entomology: enhanced infectivity of entomopathogenic viruses by fluorescent brighteners. J. Entomol. Sci. 34: 8–16.

Hamm, J. J., and M. Shapiro. 1992. Infectivity of fall armyworm (Lepidoptera: Noctuidae) nuclear polyhedrosis virus enhanced by a fluorescent brightener. J. Econ. Entomol. 85: 2149–2152.

Hamm, J. J., and L. D. Chandler. 1996. Effects of a nuclear polyhedrosis virus and a fluorescent brightener on colonies of *Spodoptera exigua* (Hübner). J. Entomol. Sci. 31: 355–362.

Hughes, P. R., N.A.M. Van Beek, and H. A. Wood. 1986. A modified droplet feeding method for rapid assay of *Bacillus thuringiensis* and baculoviruses in noctuid larvae. J. Invertebr. Pathol. 48: 187–192.

Kolodny-Hirsch, D. M., D. L. Warkentin, B. Alvarado-Rodríguez, and R. Kirkland. 1993. *Spodoptera exigua* nuclear polyhedrosis virus as a candidate viral insecticide for the beet armyworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 86: 314–321.

LeOra Software. 1987. POLO-PC. A user's guide to probit or logit analysis. LeOra Software, Berkeley, CA.

Levy, S. M., A.M.F. Falleiros, F. Moscardi, and E. A. Gregório. 2002. Differences in the peritrophic membrane of susceptible and resistant *Anticarsia gemmatalis* larvae to the insect nucleopolyhedrovirus (AgMNPV). Thirty-Fifth Annual Meeting of the Society for Invertebrate Pathology, Foz do Iguassu, Brazil.

Martínez, A. M., D. Goulson, J. W. Chapman, P. Caballero, R. D. Cave, and T. Williams. 2000. Is it feasible to use optical brightener technology with a baculovirus bioinsecticide for resource-poor maize farmers in Mesoamerica? Biol. Contr 17: 174–181.

Martínez, A. M., P. Caballero, and T. Williams. 2003. Effects of an optical brightener on the development, body weight and sex ratio of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Biocontr. Sci. Technol. (in press).

Morales, L., F. Moscardi, D. R. Sosa-Gómez, F. E. Paro, and I. L. Soldorio. 2001. Fluorescent brighteners improve *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) nucleopolyhedrovirus (AgMNPV) activity on AgMNPV-susceptible and resistant strains of the insect. Biol. Contr. 20: 247–253.

Moscardi, F. 1999. Assessment of the application of baculoviruses for control of Lepidoptera. Annu. Rev. Entomol. 44: 257–289.

Muñoz, D., J. I. Castillejo, and P. Caballero. 1998. Naturally occurring deletion mutants are parasitic genotypes in a wild- type nucleopolyhedrovirus population of *Spodoptera exigua.* Appl. Environ. Microbiol. 64: 4372–4377.

Muñoz, D., I. Ruiz de Escudero, and P. Caballero. 2000. Phenotypic characteristics and relative proportions of three genotypic variants isolated from a nucleopolyhedrovirus of *Spodoptera exigua.* Entomol. Exp. Appl. 97: 257–282.

Murillo, R., D. Muñoz, and P. Caballero. 2003. Host range and biological activity of three *Spodoptera* nucleopolyhedrovirus genotypic variants and the effect of Tinopal LPW on the most active variant. Int. J. Pest Management 49: 155–161.

Okuno, S., J. Takatsuka, M. Nakai, S. Ototake, A. Masui, and Y. Kunimi. 2003. Viral-enhancing activity of various stilbene-derived brighteners for a *Spodoptera litura* (Lepidoptera: Noctuidae) nucleopolyhedrovirus. Biol. Contr. 26: 146–152.

Robertson, J. L., and H. Preisler. 1992. Pesticide bioassays with arthropods. CRC, Boca Raton, FL.

Shapiro, M. 1992. Use of optical brighteners as radiation protectants for gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus. J. Econ. Entomol. 85: 1682–1686.

Shapiro, M. 2000. Enhancement in activity of homologous and heterologous baculoviruses infectious to beet armyworm (Lepidoptera: Noctuidae) by an optical brightener. J. Econ. Entomol. 93: 572–576.

Shapiro, M., and J. L. Robertson. 1992. Enhancement of gypsy moth (Lepidoptera: Noctuidae) baculovirus activity by optical brighteners. J. Econ. Entomol. 85: 1120–1124.

Shapiro, M., and E. M. Dougherty. 1994. Enhancement in activity of homologous and heterologous viruses against the gypsy moth (Lepidoptera: Lymantriidae) by an optical brightener. J. Econ. Entomol. 87: 361–365.

Shapiro, M., and J. L. Vaughn. 1995. Enhancement in activity of homologous and heterologous baculoviruses infectious to cotton bollworm (Lepidoptera: Lymantriidae) by an optical brightener. J. Econ. Entomol. 88: 265–269.

Shapiro, M., and R. Argauer. 2001. Relative effectiveness of selected stilbene optical brighteners as enhancers of the beet armyworm (Lepidoptera: Noctuidae) nuclear polyhedrosis virus. J. Econ. Entomol. 94: 339–343.

Sheppard, C. A., and M. Shapiro. 1994. Physiological and nutritional effect of a fluorescent brightener on nuclear polyhedrosis virus-infected *Lymantria dispar* (L.) larvae (Lepidoptera: Lymantriidae). Biol. Contr. 4: 404–411.

Smits, P. H. 1987. Nuclear polyhedrosis virus as biological control agent of *Spodoptera exigua.* Ph.D. thesis, Wageningen Agricultural University, Wageningen, Netherlands.

Smits, P. H., and J. M. Vlak. 1988. Biological activity of *Spodoptera exigua* nuclear polyhedrosis virus against *S. exigua* larvae. J. Invertebr. Pathol. 51: 107–114.

Smits, P. H., and J. M. Vlak. 1994. Registration of the first viral insecticide in The Netherlands: the development of Spod-X, based on *Spodoptera exigua* nuclear polyhedrosis virus. Med. Fac. Landbouw. Univ. Gent. 59: 385–392.

Sterling, P. H., P. M. Kelly, M. R. Speight, and P. F. Entwistle. 1988. The generation of secondary infection cycles following the introduction of nuclear polyhedrosis virus to a population of the brown-tail moth, *Euproctis chrysorrhoea* L. (Lep., Lymantriidae). J. Appl. Entomol. 106: 302–311.

Sunderland, K. D., R. J. Chambers, N. L. Helyer, and P. I. Sopp. 1992. Integrated pest management of greenhouse crops in northern Europe. Horticult. Rev. 13: 1–66.

Vail, P. V., D. F. Hoffmann, and J. S. Tebbets. 1996. Effects of a fluorescent brightener on the activity of *Anagrapha falcifera* (Lepidoptera: Noctuidae) nuclear polyhedrosis virus to four noctuid pests. Biol. Contr. 7: 121–125.

Wang, P., and R. R. Granados. 2000. Calcofluor disrupts the midgut defense system in insects. Insect Biochem. Mol. Biol. 38: 135–143.

Webb, R. E., M. Shapiro, J. D. Podgwaite, R. L. Ridgway, L. Venables, G. B. White, R. J. Argauer, D. L. Cohen, J. Witcosky, K. M. Kester, and K. W. Thorpe. 1994. Effect of optical brighteners on the efficacy of gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus in forest plots with high and low levels of natural virus. J. Econ. Entomol. 87: 134–143.

Woods, S. A., and J. S. Elkinton. 1987. Bimodal patterns of mortality from nuclear polyhedrosis virus in gypsy moth (*Lymantria dispar*) populations. J. Invertebr. Pathol. 50: 151–157.

Yee, W. L., and N. C. Toscano. 1998. Laboratory evaluations of synthetic and natural insecticides on beet armyworm (Lepidoptera: Noctuidae) damage and survival on lettuce. J. Econ. Entomol. 91:56–63.

Zou, Y., and Y. Young. 1994. Enhancement of nuclear polyhedrosis virus activity in larval pests of Lepidoptera by a stilbene fluorescent brightener. J. Entomol. Sci. 29: 130–133.

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