Formulation with an Optical Brightener Does Not Increase Probability of Developing Resistance to *Spodoptera frugiperda* Nucleopolyhedrovirus in the Laboratory

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ABSTRACT Stilbene-derived optical brighteners can markedly enhance the insecticidal activity of certain baculoviruses. We evaluated the influence of an optical brightener on the rate at which *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) developed resistance to nucleopolyhedrovirus (SfMNPV). Two laboratory colonies of *S. frugiperda* were inoculated with an LC_{50} of SfMNPV, in the absence or presence of the optical brightener Tinopal LPW (0.1%), over a period of two and 11 generations, in the first and second experiment, respectively. Compared with the initial susceptibility of the insect colony, resistance ratios of 11- and 12-fold were observed after two generations of treatment with SfMNPV + Tinopal LPW and SfMNPV alone. Similar, but variable degrees of resistance were observed in the long-term experiment with resistance ratios of 8- to 35-fold after seven to 11 generations. The presence of Tinopal LPW alone, or in mixtures with SfMNPV, did not cause any systematic change in insect resistance in either experiment. At the end of the long-term experiment, debilitating effects on pupal weight, adult fecundity, and longevity were observed in the insects exposed to Tinopal LPW alone or in mixtures with SfMNPV, but the pattern of such effects among treatments differed in each generation. We conclude that optical brighteners are unlikely to affect the rate of development of resistance to nucleopolyhedroviruses applied as biological insecticides.

KEY WORDS optical brightener, *Spodoptera frugiperda*, baculovirus, resistance, sublethal effects

THE FALL ARMYWORM, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a major pest of maize and sorghum in the Americas. Baculoviruses are important pathogens of a wide range of insect pests (Hunter-Fujita et al. 1998). Field observations indicate that *S. frugiperda* is susceptible to a multicapsid nucleopolyhedrovirus (SfMNPV), which can cause natural epizootics of the disease (Fuxa 1982). This has motivated studies focused on the development of SfMNPV as a biological insecticide (Hamm and Young 1971, Williams et al. 1999).

To date, there have been no documented cases of insect resistance to baculoviruses applied as bioinsecticides in the field, although this may be a consequence of the small-scale use of most baculoviruses compared with that of synthetic insecticides (Fuxa 1993). Laboratory studies have demonstrated development of resistance to baculovirus infection in various lepidopteran pest species. Compared with susceptible insect lineages, the degree of resistance in these laboratory-selected lines typically range from 3- to 140-fold, or exceptionally up to ≈1000-fold in populations of *Anticarsia gemmatalis* (Hübner) continuously exposed to AgMNPV in the laboratory (Abot et al. 1996).

One method for managing pest resistance to virus involves periodically ceasing virus applications, thus relaxing selection for virus-resistant individuals. This would allow susceptible insects to reinvade virus-treated areas and mate with resistant insects, thus diluting the frequency of genes for virus resistance in the pest population (Fuxa and Richter 1989, 1998). Another possible method for resistance management involves formulating the virus with a stilbene-derived optical brightener, such as Tinopal LPW. By disruption of the larval peritrophic membrane, and the sloughing of midgut epithelial cells, the optical brightener greatly enhances the probability of infection by virus (Washburn et al. 1998, Wang and Granados 2000, Okuno et al. 2003).

Stilbene-derived optical brighteners have been demonstrated to markedly improve the insecticidal properties of nucleopolyhedroviruses (NPVs) of several species of Lepidoptera, most notably the lymantriid *Lymantria dispar* (L.) (Shapiro and Robertson 1992, Webb et al. 1996), and the noctuids *Agrotis ipsilon* (Hufnagel) (Boughton et al. 2001), *Helicoverpa zea* (Boddie) (Farrar et al. 2003), and several species

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of *Spodoptera*, including *S. frugiperda* (Hamm and Shapiro 1992; Vail et al. 1996; Shapiro 2000; Martínez et al. 2000; Murillo et al. 2003a,b; Okuno et al. 2003). These compounds have been patented for their ability to enhance the insecticidal capacity of baculoviruses (Shapiro et al. 1992), and their use in baculovirus formulations has generated considerable interest (Dougherty et al. 1996, Tamez-Guerra et al. 2000, McGuire et al. 2001). To this end, laboratory bioassays have demonstrated a 24-fold reduction in the lethal concentration (LC_{50}) of AgMNPV in a resistant *A. gemmatalis* population when administered in mixtures with 1% Tinopal LPW (Fuxa and Richter 1998).

An issue that has not been addressed is that baculoviruses formulated with an optical brightener may affect the probability of developing resistance to viral infection. This is a matter of interest given that optical brighteners cause important changes in the function of the insect midgut and may themselves cause selection for individuals with altered gut characteristics, and thereby alter susceptibility to viral infection. We therefore aimed to determine the effect of an optical brightener on the rate at which two laboratory colonies of *S. frugiperda* developed resistance to SfMNPV. We subsequently evaluated the sublethal effects associated with exposure to SfMNPV and optical brightener alone, and in mixtures.

Materials and Methods

Insects and Rearing. Laboratory colonies were started independently of one another by using *S. frugiperda* larvae collected in November 1999 and March 2002 from maize fields within a 30-km radius of the town of Tapachula, Chiapas, Mexico. These insects were allowed to pupate and were sent directly to the Universidad Pública de Navarra, Pamplona, Spain, where they were maintained as two separate colonies in a growth chamber at 26 ± 2°C, 70–80% RH, and a photoperiod of 16:8 (L:D) h by using a wheat germ-based semisynthetic diet (Greene et al. 1976).

Virus Production. The multicapsid nucleopolyhedrovirus of *S. frugiperda* (SfMNPV) used in this work was characterized previously by Escribano et al. (1999). Virus was produced in fourth instars of *S. frugiperda* reared on semisynthetic diet. The virus-killed larvae were triturated in 0.1% (wt:vol) sodium dodecyl sulfate and centrifuged at 90 × *g* for 5 min, and the supernatant was centrifuged at 3,000 × *g* for 10 min. The resulting pellet, comprising viral occlusion bodies (OBs), was resuspended in sterile distilled water, counted using a bacterial counting chamber (Hunter-Fujita et al. 1998), and stored at 4°C before use.

Short-Term Selection for Resistance to SfMNPV. Throughout the study, all experimental procedures involving insects were performed at 25°C, 70–80% RH, and a photoperiod of 16:8 (L:D) h. After one generation in laboratory culture (colony started in 1999), groups of 300 second instars of *S. frugiperda* were selected at random and allocated to one of four treatments. The insects obtained from the laboratory colony were considered as generation 0. Each treatment involved inoculation by the droplet-feeding method (Hughes et al. 1986), in which all suspensions contained 10% (wt:vol) sucrose and 0.001% (wt:vol) Fluorella blue. The treatments were as follows: 1) SfMNPV alone (1.9×10^5 OBs/ml), 2) SfMNPV + Tinopal LPW [6.4×10^4 OBs/ml mixed with 0.1% (wt:vol) Tinopal LPW; Sigma, St. Louis, MO], 3) 0.1% Tinopal LPW alone, and 4) distilled water (control). The SfMNPV alone and SfMNPV + Tinopal LPW mixture were estimated to produce 50% mortality in the experimental population. The presence of Tinopal LPW decreased the LC_{50} of SfMNPV 2.9-fold. Previous studies indicated that second instars of *S. frugiperda* consumed a mean volume of 77 nl of virus suspension (O. Simón, unpublished data), which was sufficient to ensure that all insects in both virus and virus + Tinopal LPW treatments definitely consumed OBs. After inoculation, insects were reared to adulthood on semisynthetic diet. Adult female moths were allowed to mate with males from within their own treatment group. Of the resulting progeny, 300 larvae for the SfMNPV alone and SfMNPV + Tinopal LPW treatments and 150 larvae for the Tinopal LPW alone and control treatments were randomly assigned to the subsequent step of selection (identical to that mentioned above). The remaining larvae were subjected to bioassay by using SfMNPV alone (without optical brightener) to determine their susceptibility to virus infection.

For the bioassays, groups of 50 second instars were inoculated with one of five concentrations between 5.0×10^7 and 7.2×10^2 OBs/ml of virus alone using the inoculation technique mentioned above. Control insects were treated identically with solutions not containing virus. Larvae that failed to consume the droplets were discarded. Insects were reared on semisynthetic diet and were checked for virus mortality twice daily until 10 d postinoculation to determine the prevalence of virus-induced mortality. Each bioassay involved three replicates. The experiment was performed for two generations.

Long-Term Selection for Resistance to SfMNPV. A separate colony of *S. frugiperda*, started in 2002, was reared for two generations in the laboratory. To evaluate the effect of long-term exposure to virus in presence or absence of 0.1% Tinopal LPW, insects from this colony were subjected to virus inoculation over a period of 11 generations. Bioassay conditions and treatments were identical to that mentioned for the experiment on short-term selection, with exception that for the bioassay, groups of 75 second instars were used. Bioassays were performed in insects from generations 2 through 9. On occasions, there was an insufficient number of insects available to continue with the selection process and simultaneously perform bioassays of susceptibility. In such cases, priority was given to continuing the selection process, resulting in missing susceptibility values in certain treatments in some generations.

Sublethal Effects. Sublethal effects associated with SfMNPV and/or Tinopal LPW exposure were evalu-

ated on insects of generation 11, being the final generation subjected to selection procedures. Pupae were weighed 2 d after pupation and kept at 25 ± 1°C until adult emergence. On emerging, between 29 and 58 male + female pairs of adults from generation 11 were selected at random. To determine fecundity, each pair was placed in a separate oviposition container (11.5 cm in diameter by 6 cm in height) lined with tissue paper and provided with a 10% honey solution. The tissue paper was replaced every 2 d, and the number of eggs laid was counted until death of the female. Pairs that failed to reproduce were discarded.

The following generation (12) was reared without exposure to SfMNPV or Tinopal LPW. Fitness-related parameters, including pupal weight, mean fecundity, and adult longevity, were determined as described above. In this case, fecundity was evaluated in 39 pairs of adults for each treatment. Pairs that failed to mate were discarded. Egg hatch also was determined for between 25 and 30 females in each treatment. For this, a single randomly chosen egg mass was selected from the first bout of oviposition by each female. The egg mass was placed in a plastic cup, and the number of eggs that hatched was counted.

Statistical Analyses. Mortality data were subjected to probit regression analysis against log [virus concentration] by using the POLO PC program (LeOra Software 1987). Pupal weight and egg production results were analyzed by least significant difference (LSD), or where not normally distributed, by the nonparametric Mann–Whitney *U* test. Egg hatch was analyzed using contingency tables (χ^2 test). These analyses were performed using SPSS 8.0 (SPSS Inc., Chicago, IL).

Results

Short-Term Selection for Resistance to SfMNPV. Over a period of two generations, virus-induced mortality varied between 37 and 45% due to SfMNPV alone and between 37 and 46% due to SfMNPV + Tinopal LPW. No virus infections were observed in insects treated with Tinopal LPW alone or water controls. Selection for resistance to both treatments affected the susceptibility of *S. frugiperda* in a similar manner over two generations (Fig. 1a). The susceptibility of control insects did not change significantly during the course of the study, based on the overlap of 95% confidence intervals. In the second generation, LC_{50} values for the insects exposed to SfMNPV alone (6.2×10^6 OBs/ml [95% CL: 3.3×10^6–1.1×10^7]) and SfMNPV + Tinopal LPW (5.5×10^6 OBs/ml [95% CL: 3.7×10^6–8.1×10^6]) were 12- and 11-fold greater, respectively, compared with generation 0 (Fig. 1a). The LC_{50} values of insects treated with Tinopal LPW alone were significantly increased in both generations compared with generation 0, but these differences were not significant compared with the respective control groups of each generation (Fig. 1a).

Long-Term Selection for Resistance to SfMNPV. Similar to the previous experiment, the susceptibility of control insects did not change significantly during the course of this experiment, based on the overlap of 95% confidence intervals. Virus induced mortality varied between 34 and 67% due to SfMNPV alone and between 30 and 60% due to SfMNPV + Tinopal LPW, or exceptionally 81% mortality in generation 9 in the latter. In all treatments before generation 7, the resistance ratios (as indicated by relative potency values) were variable but did not exceed 4.5 in any treatment. However, in the final generations of the study (generations 7–9), there was a clear tendency to increase the degree of resistance to SfMNPV in all treatments except that of the control (Fig. 1b). In the SfMNPV treatment, the resistance ratio varied between 11 and 7.4 compared with the generation 0 (indicated by the axis at 1.0 in Fig. 1b). The greatest resistance ratios were seen in the SfMNPV + Tinopal LPW treatment, which varied from 23 to 33 in generations 7 and 8, respectively, and then fell to eight in generation 9. The resistance ratios in the Tinopal LPW treatment fluctuated from 9 in generation 7, 0.3 in generation 8, to 12 in generation 9.

Sublethal Effects. In generation 11, the final generation that was subjected to selection by treatment with SfMNPV with, or without Tinopal LPW, mean pupal weight was significantly reduced in treatments involving Tinopal LPW and SfMNPV alone, but significantly increased in the SfMNPV + Tinopal LPW treatment, compared with control insects (Table 1). Fecundity was highly variable and did not differ significantly from that of controls in any treatment. Longevity of adult females was 2–5 d longer in SfMNPV and SfMNPV + Tinopal LPW treatments, respectively, but reduced by ≈2.4 d in insects treated with Tinopal LPW alone, compared with controls (Table 1). Adult male longevity followed a similar pattern being 3.5 d longer in the SfMNPV treatment and 2.4 d longer in the SfMNPV + Tinopal LPW treatment, compared with controls, whereas the Tinopal LPW treatment was almost identical to that of control insects.

In generation 12, the first generation in which all selection procedures were relaxed, mean pupal weight was reduced in all treatments compared with controls (Table 1). Fecundity was also clearly reduced in all treatments compared with controls, especially so in the SfMNPV treatment in which fecundity values were one-half that of the control insects. Moreover, the fertility of eggs was reduced in the SfMNPV treatment (82% egg hatch, $n = 4142$) and the SfMNPV + Tinopal LPW treatment (88%, $n = 5622$) compared with the control (93%, $n = 4843$) and Tinopal LPW treatment (94%, $n = 5584$) ($\chi^2 = 299$, df = 3, $P < 0.001$). In contrast to the previous generation, no differences in adult longevity were detected in any treatment for either sex, compared with the controls (Table 1).

Discussion

Two separate laboratory colonies of *S. frugiperda* were artificially selected for resistance to SfMNPV, in the absence or presence of 0.1% Tinopal LPW, over a period of two and 11 generations. In both experiments, the selection procedure was based on an LC_{50} con-

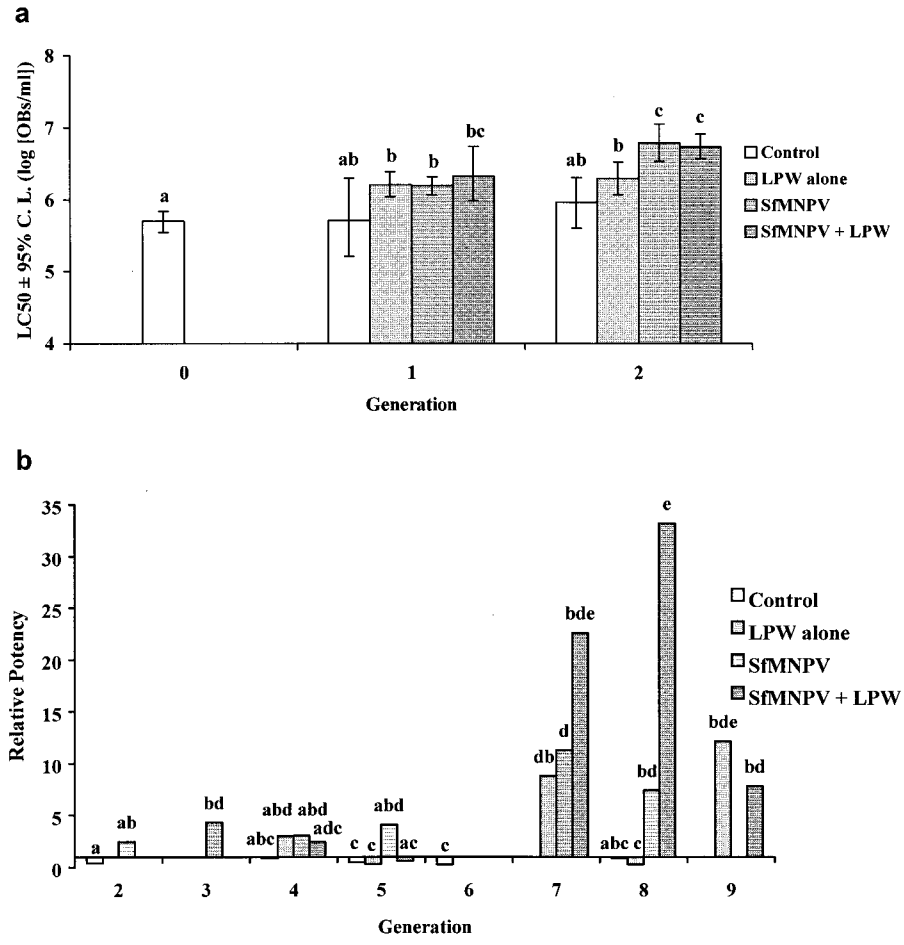

Fig. 1. (a) LC_{50} (log [OBs/ml] ± 95% CL) values for *S. frugiperda* exposed to SfMNPV alone, mixed with 0.1% Tinopal LPW, or 0.1% Tinopal LPW alone over a period of two generations. (b) Relative potency, calculated as the ratio of effective concentrations compared with the initial generation 0 (representing a potency value of 1.0, shown as the x-axis at 1.0), for *S. frugiperda* larvae subjected to selection over a period of nine generations. Missing values indicate that insufficient insects were available to perform bioassays. In both graphs, columns headed by identical letters are not significantly different, based on the nonoverlap of 95% confidence intervals.

centration of SfMNPV alone or the equivalent mortality response by using SfMNPV + 0.1% Tinopal LPW. Treatment with 0.1% Tinopal LPW alone resulted in minimal mortality of larvae (<5%) but was included to control for possible effects of selection after exposure to the optical brightener. At the end of

Table 1. Effects of SfMNPV, Tinopal LPW, and SfMNPV + Tinopal LPW on the pupal weight, fecundity, and adult longevity of *S. frugiperda* in the generation 11 (after 11 generations of selection) and generation 12 (in which no selection procedures were performed)

Treatments	Mean pupal weight[a] (mg ± SE) (*n*)	Fecundity (eggs/female ± SE) (*n*)	Adult longevity[a] (d ± SE) (*n*)	
			Female	Male
Generation 11				
Control	206.0 ± 3.6a (187)	749.5 ± 75.0a (39)	12.7 ± 0.7a (37)	12.9 ± 0.6a (38)
SfMNPV	171.3 ± 3.4b (71)	663.1 ± 121.7a (20)	14.7 ± 0.9b (21)	16.4 ± 0.8b (21)
LPW alone	184.3 ± 4.3c (90)	998.6 ± 106.7a (27)	10.3 ± 0.6c (30)	13.0 ± 1.1a (30)
SfMNPV + LPW	213.5 ± 2.5d (158)	775.4 ± 71.7a (44)	19.9 ± 0.7d (34)	15.3 ± 0.9b (34)
Generation 12				
Control	205.4 ± 1.1a (971)	1101.3 ± 80.0a (32)	14.7 ± 0.6a (39)	16.8 ± 0.8a (39)
SfMNPV	164.3 ± 1.4b (507)	504.8 ± 56.0b (32)	15.3 ± 0.8a (39)	15.7 ± 0.9a (39)
LPW alone	200.6 ± 1.4c (668)	865.2 ± 60.2c (34)	16.0 ± 0.7a (39)	15.8 ± 0.5a (39)
SfMNPV + LPW	194.8 ± 1.4d (547)	701.6 ± 74.3c (33)	16.2 ± 0.8a (31)	16.4 ± 0.9a (31)

Numbers followed by identical letters are not significantly different ($P < 0.05$) for comparisons between treatments within each generation.
[a] Pupal weight and adult longevity were analyzed by Mann–Whitney *U* test (generation 11) and by Students *t*-test (generation 12).

the first study, resistance ratios of 11- and 12-fold were detected for insects exposed to SfMNPV + Tinopal LPW and SfMNPV alone, respectively, by the second generation (Fig. 1a). Similar levels of resistance were observed for the insects exposed to SfMNPV alone and SfMNPV + Tinopal LPW in the long-term experiment, but in this case, elevated resistance ratios (8–35-fold) were observed after seven generations (Fig. 1b). All resistance ratios were based on comparison with the susceptibility of the initial generation (0) to infection by SfMNPV.

The question posed in this study, of whether inoculation of virus in the presence of optical brighteners altered the rate or probability of developing resistance to SfMNPV infection, was an intriguing one, given that these compounds can dramatically affect the function of the lepidopteran midgut (Wang and Granados 2000). Certain stilbene-derived optical brighteners, including Tinopal LPW, bind strongly to chitin and thereby affect chitin biosynthesis (Elorza et al. 1983, Roncero et al. 1988, Bartnicki-García et al. 1994). These compounds solubilize proteins from the chitinous structure of the peritrophic membrane present in lepidopteran larvae (Wang and Granados 2000). The peritrophic membrane protects midgut epithelial cells from physical abrasion and microbial agents ingested during feeding (Tellam 1996, Wang and Granados 1998). As a result, degradation of the peritrophic membrane disrupts insect feeding (Sheppard and Shapiro 1994) and reduces developmental rate and insect survival (Martínez et al. 2004).

The results of the two experiments indicate that Tinopal LPW did not systematically increase the probability or rate of development of resistance to SfMNPV, although the magnitude of resistance ratios observed in the long-term experiment was the highest seen in any treatment (23- to 33-fold) (Fig. 1b). Similarly, treatment with optical brightener alone did not result in any systematic change in insect resistance to SfMNPV infection in either experiment.

The degree of resistance detected in our study was greater than that reported by Fuxa et al. (1988) for a population of *S. frugiperda* collected in Louisiana, which developed 4.5-fold after eight generations of treatment with an LD_{80} dose of SfMNPV at the neonate stage. In other species, resistance ratios observed after laboratory selection procedures were 140-fold in *Phthorimaea operculella* (Zeller) (Briese and Mende 1983), 4.4- to 22-fold in *Trichoplusia ni* (Hübner) (Milks and Myers 2000, 2003), and most notably >1000-fold in *A. gemmatalis* (Abot et al. 1996). For logistical reasons, it is necessary to balance the severity of the selection process with the requirement for a sufficient number of survivors to be able to continue the experiment, and periodically bioassay the susceptibility of the experimental population. Consequently, selection procedures typically use doses or concentrations designed to induce 50 or 80% mortality in treated insects, as was the case in our study. This procedure reflects the high prevalence of virus-induced mortality expected after application of elevated concentrations of OBs as biological insecticides in field crop situations.

The development of resistance of *S. frugiperda* to virus in the laboratory presumably indicates the presence of resistance genes in natural populations. *S. frugiperda* is a very mobile pest (Fuxa 1987) and is found year-round in Neotropical regions such as the coastal plain of Chiapas State, Mexico. Variation in the genetic composition of natural populations, possibly influenced by the differential fitness of resistant and susceptible phenotypes, may be highly influential in determining the likelihood and rate of developing resistance to viral infection (Fuxa et al. 1988). Indeed, virus resistance studies are contingent on population differences in genetic heterogeneity (Milks and Myers 2000) and/or the genetic composition of the individuals used to initiate the colonies (Briese 1986). Briese and Mende (1983) argued that where resistance genes are present in inbreeding laboratory populations, a change in their frequencies could occur rapidly, even under moderate selection. It is perhaps not surprising then, that laboratory colonies collected in the same area but at different times of the year (at the end of the dry season in March and at the end of the rainy season in November), and in different years, showed markedly different responses to selection for virus resistance.

Sublethal effects were observed in the final generation (11) of insects subjected to selection procedures, and in the following generation (12), which was not subjected to any selection procedures. However, the pattern of sublethal effects among treatments differed in each generation. Among the most interesting of the sublethal effects was the reduced pupal weight in SfMNPV-treated insects, although mixtures of SfMNPV + Tinopal LPW did not behave similarly. There was also a clear decrease in fecundity in all treatments compared with the control in generation 12 but not in generation 11. This implies that the individuals that survived selection in generation 11 were those with a much higher reproductive capacity than the population mean, which only became apparent in generation 12. There seemed to be no clear correlations among sublethal effects such as pupal weight and fecundity or adult longevity in either generation. Egg fertility was slightly reduced in the SfMNPV alone and SfMNPV + Tinopal LPW treatments, whereas the Tinopal LPW and control treatments were almost identical in this respect.

Debilitating effects observed in the survivors of virus inoculum have been widely documented in lepidopteran baculovirus systems (Burand and Park 1992, Goulson and Cory 1995, Rothman and Myers 1996, Myers et al. 2000, Matthews et al. 2002). Moreover, the sublethal effects of baculovirus infection can play an influential role in the population dynamics of their hosts (Boots and Norman 2000). Such debilitating effects may be due to diversion of host energy reserves to combat the disease (Bong and Sikorowski 1991) and/or to hormonal changes induced in the sublethally infected host (O'Reilly and Miller 1989). However, until recently (Burden et al. 2002), few studies

have presented evidence for the presence of the virus in the survivors of sublethal doses of inoculum.

Significant reductions in adult fecundity and egg fertility were detected in *S. frugiperda* (Fuxa and Richter 1989) and *A. gemmatalis* (Fuxa and Richter 1998) after selection for resistance in the laboratory. The diminished reproduction seen in these cases, however, was attributed to a trade-off between investment in progeny production and the expression of resistance genes (Fuxa and Richter 1989). Sublethal viral diseases in Lepidoptera often result in reductions in insect weight and adult longevity (Rothman and Myers 1996). In our study, the reason for the increase in adult longevity in insects of generation 11 exposed to SfMNPV (with or without Tinopal LPW) is unclear; it was evidently not related to differences in fecundity or body weight at the pupal stage. Previously, Fuxa and Richter (1991) demonstrated the presence of OBs in 3–10% of the adult progeny of sublethally infected *S. frugiperda*, although some of the OBs were not infectious and did not contained virions (Fuxa et al. 1992). We assume that the sublethal effects observed in the current study were due, at least partially, to the persistence of SfMNPV in a proportion of the insects of generations 10 and 11.

In conclusion, the presence of Tinopal LPW did not affect the rate at which *S. frugiperda* developed resistance to an homologous nucleopolyhedrovirus in short-term or long-term laboratory experiments. However, treatment with Tinopal LPW alone or in mixtures with SfMNPV resulted in certain debilitating effects on the pupal weight, fecundity, and longevity of *S. frugiperda*, but not in a systematic manner. Use of optical brighteners in baculovirus formulations seems not to increase the risk of developing residence to infection by these viruses when used as biological insecticides.

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References Cited

Abot, A. R., F. M., J. R. Fuxa, D. R. Sosa-Gómez, and A. R. Richter. 1996. Development of resistance by *Anticarsia gemmatalis* from Brazil and the United States to a nuclear polyhedrosis virus under laboratory selection pressure. Biol. Control 7: 126–130.

Bartnicki-García, S., J. Persson, and H. Chanzy. 1994. An electron microscope and electron diffraction study of the effect of calcofluor and Congo red on the biosynthesis of chitin *in vitro.* Arch. Biochem. Biophys. 310: 6–15.

Bong, C.F.J., and P. P. Sikorowski. 1991. Effects of cytoplasmic polyhedrosis virus and bacterial contamination on growth and development of the corn earworm, *Helicoverpa zea* (Lepidoptera: Noctuidae). J. Invertebr. Pathol. 57: 406–412.

Boots, M., and R. Norman. 2000. Sublethal infection and the populations dynamics of host-microparasite interactions. J. Anim. Ecol. 69: 517–524.

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.

Briese, D. T. 1986. Insect resistance to baculoviruses, pp. 238–259. *In* R. R. Granados and B. A. Federici [eds.], The biology of baculoviruses, vol. II. CRC. Boca Raton, FL.

Briese, D. T., and H. A. Mende. 1983. Selection for increased resistance to a granulosis virus to the potato moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). Bull. Entomol. Res. 73: 1–9.

Burden, J. P., C. M. Griffiths, J. S. Cory, P. Smith, and S. M. Sait. 2002. Vertical transmission of sublethal granulovirus infection in the Indian meal moth, *Plodia interpunctella.* Mol. Ecol. 194: 608–615.

Burand, J. P., and E. J. Park. 1992. Effect of nuclear polyhedrosis virus infection on the development and pupation of gypsy moth larvae. J. Invertebr. Pathol. 60: 171–175.

Dougherty, E., K. P. Guthrie, and M. Shapiro. 1996. Optical brighteners provide baculovirus activity enhancement and UV radiation protection. Biol. Control 7: 71–74.

Elorza, M. V., H. Rico, and R. Sentandreu. 1983. Calcofluor white alters the assembly of chitin fibrils in *Saccharomyces cerevasiae* and *Candida albicans* cells. J. Gen. Microbiol. 129: 1577–1582.

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.

Farrar, R. R. Jr., M. Shapiro, and I. Javaid. 2003. Photostabilized titanium dioxide and a fluorescent brightener as adjuvants for a nucleopolyhedrovirus. Biocontrol 48: 543–560.

Fuxa, J. R. 1982. Prevalence of viral infections in populations of fall armyworm, *Spodoptera frugiperda*, in southeastern Louisiana. Environ. Entomol. 11: 239–242.

Fuxa, J. R. 1987. *Spodoptera frugiperda* susceptibility to nuclear polyhedrosis virus isolates with reference to insect migration. Environ. Entomol. 16: 218–223.

Fuxa, J. R. 1993. Insect resistance to viruses, pp. 197–209. *In* N. E. Beckage, S. N. Thompson, and B. A. Federici [eds.], Parasites and pathogens of insects, vol. II. Academic, San Diego, CA.

Fuxa, J. R., and A. R. Richter. 1989. Reversion of resistance by *Spodoptera frugiperda* to a nuclear polyhedrosis virus. J. Invertebr. Pathol. 53: 52–56.

Fuxa, J. R., and A. R. Richter. 1991. Selection for an increased rate of vertical transmission of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) nuclear polyhedrosis virus. Environ. Entomol. 20: 603–609.

Fuxa, J. R., and A. R. Richter. 1998. Repeated reversion of resistance to nucleopolyhedrovirus by *Anticarsia gemmatalis.* J. Invertebr. Pathol. 71: 159–164.

Fuxa, J. R., F. L. Mitchell, and A. R. Richter. 1988. Resistance of *Spodoptera frugiperda* (Lep.: Noctuidae) to a nuclear polyhedrosis virus in the field and laboratory. Entomophaga 33: 55–63.

Fuxa, J. R., H. E. Weidner, and A. R. Richter. 1992. Polyhedra without virions in a vertically transmitted nuclear polyhedrosis virus. J. Invertebr. Pathol. 60: 53–58.

Greene, G. L., N. C. Leppla, and W. A. Dickerson. 1976. Velvetbean caterpillar: a rearing procedure and artificial medium. J. Econ. Entomol. 69: 478–488.

Goulson, D., and J. S. Cory. 1995. Sublethal effects of baculovirus in the cabbage moth *Mamestra brassicae.* Biol. Control 5: 361–367.

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 J. R. Young. 1971. Value of virus presilk treatment for corn earworm and fall armyworm in sweet corn. J. Econ. Entomol. 64: 144–146.

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.

Hunter-Fujita, F. R., P. F. Entwistle, H. F. Evans, and N. E. Crook. 1998. Insect Viruses and pest management. Wiley, Chichester, United Kingdom.

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

Martínez, A. M., P. Caballero, and T. Williams. 2004. Effects of an optical brightener on the development, body weight and sex ratio of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Biocontrol Sci. Technol. 14: 193–200.

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. Control 17: 174–181.

Matthews, H. J., I. Smith, and J. P. Edwards. 2002. Lethal and sublethal effects of a granulovirus on the tomato moth *Lacanobia oleracea.* J. Invertebr. Pathol. 80: 73–80.

McGuire, M. R., P. Tamez-Guerra, R. W. Behle, and D. A. Streett. 2001. Comparative field stability of selected entomopathogenic virus formulations. J. Econ. Entomol. 94: 1037–3902.

Milks, M. L., and J. H. Myers. 2000. The development of larval resistance to a nucleopolyhedrovirus is not accompanied by an increased virulence in the virus. Evol. Ecol. 14: 645–664.

Milks, M. L., and J. H. Myers. 2003. Cabbage looper resistance to a nucleopolyhedrovirus confers cross-resistance to two granulovirus. Environ. Entomol. 32: 286–289.

Murillo, R., D. Muñoz, and P. Caballero. 2003a. 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 Manage. 49: 147–153.

Murillo, R., R. Lasa, D. Goulson, T. Williams, D. Muñoz, and P. Caballero. 2003b. Effect of Tinopal LPW on the insecticidal properties and genetic stability of the nucleopolyhedrovirus of *Spodoptera exigua* (Lepidoptera: Noctuidae). J. Econ. Entomol. 96: 1668–1674.

Myers, J. H., R. Malakar, and J. S. Cory. 2000. Sublethal nucleopolyhedrosis infection effects on female pupal weight, egg mass size, and vertical transmission in gypsy moth (Lepidoptera: Lymantriidae). Environ. Entomol. 29: 1268–1272.

O'Reilly, D. R., and L. K. Miller. 1989. A baculovirus blocks insect molting by producing ecdysteriod UDP-glucosyl transferase. Science (Wash. DC) 245: 1110–1112.

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. Control 26: 146–152.

Roncero, C., M. H. Valdivieso, J. C. Ribas, and A. Duran. 1988. Effect of calcofluor white on chitin syntheses from *Saccharomyces cerevasiae.* J. Bacteriol. 170: 1945–1949.

Rothman, L. D., and J. M. Myers. 1996. Debilitating effects of viral diseases on host Lepidoptera. J. Invertebr. Pathol. 67: 1–10.

Shapiro, M. 2000. Enhancement in activity of homologous and heterologous baculoviruses infections 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: Lymantriidae) baculoviruses activity by optical brighteners. J. Econ. Entomol. 85: 1120–1124.

Shapiro, M., E. M. Dougherty, and J. J. Hamm. 1992. Compositions and methods for biocontrol using fluorescent brighteners. U.S. Patent No. 5. 124: 149.

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

Tamez-Guerra, P., M. R. McGuire, R. W. Behle, J. J. Hamm, H. R. Sumner, and B. S. Shasha. 2000. Sunlight persistence and rainfastness of spray-died formulations of baculoviruses isolated from *Anagrapha falcifera* (Lepidoptera: Noctuidae). J. Econ. Entomol. 93: 210–218.

Tellam, R. L. 1996. The peritrophic matrix, pp. 86–114. *In* M. J. Lehane and P. F. Billingsley [eds.], Biology of the insect midgut, Chapman & Hall, London, United Kingdom.

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. Control 7: 121–125.

Wang, P., and R. R. Granados. 1998. Observations on the presence of the peritrophic membrane in larval *Trichoplusia ni* and its role in limiting baculovirus infection. J. Invertebr. Pathol. 72: 57–62.

Wang, P., and R. R. Granados. 2000. Calcofluor disrupts the midgut defense system in insects. Insect Biochem. Mol. Biol. 30: 135–143.

Washburn, J., O., B. A. Kirkpatrick, E. Hass-Stapleton, and L. E. Volkman. 1998. Evidence that the stilbene-derived optical brightener M2R enhances *Autographa californica* M nucleopolyhedrovirus infection of *Trichoplusia ni* and *Heliothis virescens* by preventing sloughing of infected midgut epithelial cells. Biol. Control 11: 58–69.

Webb, R. E., N. H. Dill, J. M. Mclaughlin, L. S. Kershaw, J. D. Podwaite, S. P. Cook, K. W. Thorpe, R. R. Farrar, R. L. Ridgway, R. W. Fuester, et al. 1996. Blankophor BBH as an enhancer of nuclear polyhedrosis virus in arborist treatments against the gypsy moth (Lepidoptera: Lymantriidae). J. Econ. Entomol. 89: 957–962.

Williams, T., D. Goulson, P. Caballero, J. Cisneros, A. M. Martínez, J. W. Chapman, D. X. Roman, and R. D. Cave. 1999. Evaluation of a baculovirus bioinsecticide for small-scale maize growers in Latin America. Biol. Control 14: 67–75.

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