Effect of optical brighteners on the insecticidal activity of a nucleopolyhedrovirus in three instars of *Spodoptera frugiperda*

Ana-Mabel Martínez, Oihane Simón, Trevor Williams & Primitivo Caballero*
Depto. de Producción Agraria, Universidad Pública de Navarra, 31006 Pamplona, Spain

Accepted: 19 August 2003

Key words: optical brighteners, *Spodoptera frugiperda*, fall armyworm, baculovirus, bioassay, LC_{50}, time to death, Lepidoptera, Noctuidae

Abstract

Certain optical brighteners are effective UV protectants, and can improve the insecticidal activity of baculoviruses. We evaluated the effect of 10 optical brighteners, from four chemically different groups, on the insecticidal activity of a nucleopolyhedrovirus (SfMNPV) in third instar *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). The most effective optical brighteners were Blankophor BBH and Calcofluor M2R, both of which are stilbenes. The distyryl-biphenyl derivative, Tinopal CBS, had no effect, whereas the stilbenes, Blankophor CLE and Leucophor SAC and the styrylbenzenic derivative, Blankophor ER, resulted in a decrease in virus induced mortality compared to larvae infected with SfMNPV alone. Mixtures of SfMNPV + 0.1% Calcofluor M2R had relative potencies of 2.7, 6.5, and 61.6 in the second, third, and fourth instars, respectively. The mean time to death differed with instar, but was not affected by the addition of 0.1% Calcofluor M2R. Analysis of published studies indicated that the concentration of Calcofluor M2R-related stilbenes was positively correlated with the relative potency observed in mixtures with homologous NPVs. The average magnitude of optical brightener activity did not differ significantly between early instars of 10 species of Lepidoptera. We conclude that virus formulations containing optical brighteners may be valuable for control of late instar lepidopteran pests.

Introduction

The fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is the most serious maize pest throughout Latin America (Andrews, 1988). Farmers usually make several applications of synthetic insecticides for the control of this pest during the growing season (Hruska & Gould, 1997). Repeated exposure to chemical insecticides has given cause for concern for the health of farmers in Latin America (McConnell & Hruska, 1993; Tinoco & Halperin, 1998). This has motivated studies focused on the development of a multicapsid nucleopolyhedrovirus (SfMNPV) (Baculoviridae) as a potential bioinsecticide of *S. frugiperda* (Williams et al., 1999).

Factors limiting the use of baculovirus insecticides include inactivation of the virus by UV solar radiation (Ignoffo et al., 1977) and a stage-dependent increase in resistance to virus infection (Payne et al., 1981; Briese, 1986; Sait et al., 1994). The incorporation of stilbene optical brighteners into baculovirus formulations can significantly improve UV protection and viral pathogenicity (Shapiro, 1992; Tamez-Guerra et al., 2000; McGuire et al., 2001; Okuno et al., 2003; Shapiro & Farrar, 2003). Shapiro (1992) reported that eight optical brighteners provided complete protection to a nucleopolyhedrovirus of *Lymantria dispar* (L.) (LdMNPV) after 60 min exposure to UV radiation in the laboratory. Studies in *S. frugiperda* showed that the brightener Tinopal LPW could enhance SfMNPV activity in early instars by a factor of more than 100 (Hamm & Shapiro, 1992; Martínez et al., 2000). Optical brighteners may also enhance the activity of a number of other insect viruses including granulovirus (Baculoviridae), cypovirus (Reoviridae), and entomopoxvirus (Poxviridae) (Shapiro & Dougherty, 1994; Dougherty et al., 1996; Hamm, 1999; Morales et al., 2000; Shapiro et al., 2002).

*Correspondence: Depto. de Producción Agraria, Universidad Pública de Navarra, 31006 Pamplona, Spain. Fax: +34 948 169 732; E-mail: pcm92@unavarra.es

Certain optical brighteners, for example Calcofluor M2R, bind strongly to β-glucans such as chitin and thereby affect chitin biosynthesis (Elorza et al., 1983; Roncero et al., 1988; Bartnicki-Garcia et al., 1994). The peritrophic membrane is a chitinous structure which represents a crucial barrier to baculovirus infection in lepidopteran larvae (Wang & Granados, 1998). Consumption of Calcofluor M2R causes the dissociation of proteins from the chitinous framework of the peritrophic membrane, resulting in a marked increase in its porosity (Wang & Granados, 2000). Moreover, Washburn et al. (1998) demonstrated that Calcofluor M2R enhanced the nucleopolyhedrovirus of *Autographa californica* (Speyer) (AcMNPV) infectivity by reducing the rate of sloughing of infected midgut cells in *Heliothis virescens* (Fabricius) and *Trichoplusia ni* (Hübner) larvae. When *H. virescens* was inoculated at various times during the fourth instar in the presence of Calcofluor M2R, the magnitude of viral enhancement was directly proportional to the intra-instar age of the host. High rates of sloughing of midgut cells in older larvae may well represent the principal cause of developmental resistance to infection by baculoviruses.

The objectives of the present study were to compare the effect of four chemically different groups of optical brighteners on SfMNPV activity and to determine the degree of synergism of the most active optical brightener against three instars of *S. frugiperda*. Finally, we reviewed published studies to identify factors affecting the degree of potentiation of selected optical brighteners on the infectivity of homologous nucleopolyhedroviruses.

Materials and methods

Insect rearing

A *Spodoptera frugiperda* colony was collected in the field at the Escuela Agrícola Panamericana, El Zamorano, Honduras. This colony was sent to the Universidad Pública de Navarra, Spain, where it was maintained in a growth chamber at 26 ± 2 °C, L16:D8, 70–80% r.h., using a wheatgerm-based semi-synthetic diet (Greene et al., 1976).

Virus production

The multicapsid nucleopolyhedrovirus of *Spodoptera frugiperda* (SfMNPV) used in this work had been previously characterized by Escribano et al. (1999). Occlusion bodies (OBs) were produced in fourth instar *S. frugiperda* reared on semi-synthetic diet. The virus-killed larvae were triturated in 0.1% (wt/v) sodium dodecyl sulphate (SDS) and centrifuged at 90 ***g*** for 5 min, and the supernatant was centrifuged at 3000 ***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 prior to use.

Optical brighteners

Ten optical brighteners were obtained as powders or liquids. All compounds were dissolved in sterile distilled water at a concentration of 0.1% (w/v or v/v). The optical brighteners were selected from four different chemical groups. These were seven stilbene acid derivatives: Blankophor BA, Blankophor CLE (Bayer, Barcelona, Spain), Blankophor BBH (Bayer, Pittsburgh, PA, USA), Calcofluor M2R (Sigma, St Louis, MO, USA), Leucophor AP, Leucophor SAC, and Leucophor UO (Croma, Guipuzcua, Spain); one biphenyl derivative: Tinopal CBS (Ciba, Mexico City); one styryl-benzenic derivative: Blankophor ER (Bayer, Barcelona, Spain); and one benzimidazole derivative: Uvitex BAC (Bayer, Barcelona, Spain).

Bioassays

To compare the effect of the optical brighteners on SfMNPV activity, third instar *S. frugiperda* were selected randomly and were inoculated with 1×10^5 OBs/ml suspended in water or a 0.1% solution of optical brightener using the droplet-feeding method (Hughes & Wood, 1981). The larvae were starved for approximately 12 h prior to inoculation. All suspensions contained 10% (w/v) sucrose and 0.001% (w/v) Fluorella Blue. Larvae that ingested the solution within 10 min were transferred to individual cells of a 25-compartment Petri dish and were provided with fresh diet. Larvae that failed to consume the droplet were discarded. Groups of 30 larvae were treated with virus alone or virus mixed with one of the optical brighteners. Additional groups of larvae were treated with water or 0.1% optical brightener solutions (including sucrose and Fluorella Blue) as controls. Larvae were held at constant temperatures of 25 ± 2 °C. The bioassay was performed three times. Virus induced mortality was assessed at 9 days post-inoculation. The prevalence of mortality in virus + optical brightener treatments was compared to that of the virus treatment by log-likelihood ratio test (G-test) using the SPSS program (SPSS Inc., Chicago, IL).

To compare the effect of virus + optical brightener mixtures in different instars, we selected Calcofluor M2R, which proved to be highly active in the single-concentration bioassays. For bioassays of virus alone, five concentrations between 1.1×10^3 and 7.2×10^5 OBs/ml for second instars, between 1.7×10^3 and 7.0×10^6 OBs/ml for third instars, and between 9.7×10^3, and 4.0×10^7 OBs/ml for fourth instars, were used to inoculate larvae using the droplet feeding method. For bioassays of OBs suspended in 0.1% Calcofluor M2R, five concentrations between 2.8×10^3

and 4.5×10^4 OBs/ml, 4.8×10^2 and 1.2×10^5 OBs/ml, and 4.9×10^2 and 4.0×10^4 OBs/ml were used for second, third, and fourth instars, respectively. Groups of 25 larvae were inoculated with each concentration and groups of 25 control larvae were treated with water or 0.1% Calcofluor M2R. The larvae were observed over 9 days post-inoculation at 8 h intervals to determine percentage mortality and time to death. Each bioassay was performed three times.

Mortality data were subjected to probit regression analysis against log [virus concentration] using the POLO PC program (LeOra Software, 1987). The mean time to death was calculated for the virus concentration needed to kill approximately 90% of the treated insects using the Generalised Linear Interactive Modelling (GLIM) program with a normal error distribution (Numerical Algorithms Group, 1993). The distribution of residuals and fitted values was examined using the model checking macros present in the GLIM program. Individuals that did not die due to virus infection were excluded from the analysis (Farrar & Ridgway, 1998).

Literature survey

To examine the relationships between the degree of potentiation of different nucleopolyhedrovirus isolates by certain optical brighteners and the larval stages of Lepidoptera, we searched the literature for published laboratory studies from peer-reviewed journals. The degree of virus potentiation by the optical brighteners (either as a relative potency value or as the ratio of LD_{50} or LC_{50} values of the virus, with or without optical brightener) was taken as the response variable with larval stage as a factor and brightener concentration as a continuous explanatory variable. The analysis was performed with the GLIM program (Numerical Algorithms Group, 1993). The behaviour of models was examined using the model checking macro present in the GLIM package. Only studies that involved the effect of the optical brighteners on the activity of homologous host-virus systems were included. We also limited the analysis to a group of structurally and chemically similar optical brighteners: Calcofluor M2R, Tinopal LPW, Tinopal UNPA-GX and Blankophor BBH.

Results

Third instar *S. frugiperda* treated with SfMNPV alone (1×10^5 OBs/ml) suffered 66.3% mortality (Table 1). At a concentration of 0.1%, several optical brighteners caused significant differences in the insecticidal activity of this virus ($G = 399.8$, d.f. = 10, $P < 0.001$) (Table 1). Five of the optical brighteners (Blankophor BBH, Calcofluor M2R, Leucophor AP, Leucophor SAC, and Leucophor UO) increased the prevalence of larval mortality, resulting in 87.7–100% infection. The most active brighteners were Blankophor BBH and Calcofluor M2R. In contrast, three compounds (Blankophor CLE, Leucophor SAC, and Uvitex BAC) had an adverse effect on the pathogenicity of the virus and two others (Tinopal CBS and Blankophor ER) had no significant effect (Table 1). No virus mortality or evidence of optical brightener toxicity was observed in control larvae.

Table 1 Effect of 10 optical brighteners on the activity of SfMNPV in third instar *Spodoptera frugiperda*

Treatments[a]	Type of brightener	Percentage mortality[b]
SfMNPV[c] alone		66.3
SfMNPV + Blankophor BA	Stilbene	91.1***
SfMNPV + Blankophor CLE	Stilbene	29.9***
SfMNPV + Blankophor BBH	Stilbene	100***
SfMNPV + Calcofluor M2R	Stilbene	100***
SfMNPV + Leucophor AP	Stilbene	94.4***
SfMNPV + Leucophor SAC	Stilbene	48.8*
SfMNPV + Leucophor UO	Stilbene	87.7*
SfMNPV + Tinopal CBS	Biphenyl	73.3
SfMNPV + Blankophor ER	Styryl-benzenic	61.3
SfMNPV + Uvitex BAC	Benzimidazole	20.4***

[a]All optical brighteners were used at a concentration of 0.1% (w/v or v/v).
[b]The prevalence of mortality in the presence of virus + optical brightener was compared to that of the virus alone (G-test, d.f. = 1, *$P < 0.05$, ***$P < 0.001$).
[c]SfMNPV inoculated at a single concentration of 1×10^5 occlusion bodies/ml.

Mixtures of 0.1% Calcofluor M2R resulted in a significant reduction in LC_{50} values in second, third, and fourth instar *S. frugiperda* (Table 2). Probit regression lines could not be fitted in parallel, and so the relative potencies were expressed as the ratio of LC_{50} values in the presence or absence of optical brightener (Robertson & Preisler, 1992). Relative potencies were 2.7, 6.5, and 61.6 for SfMNPV administered with Calcofluor M2R for second, third, and fourth instars, respectively (Table 2). The addition of Calcofluor M2R had the effect of maintaining the LC_{50} values of third and fourth instars similarly to those observed in second instars. In contrast, when the virus was administered in the absence of the optical brightener, LC_{50} values increased markedly with larval stage, especially in the fourth instar. Mortality never exceeded 3% in the control larvae.

The addition of 0.1% Calcofluor M2R to the virus suspension resulted in an average increase of 4.5 h in the mean time to death (averaged across all instars), although this effect was not significant ($F_{1,27} = 1.20$, $P = 0.15$) (Figure 1).

Table 2 Probit analysis for SfMNPV alone and in mixtures with 0.1% Calcofluor M2R against second, third, and fourth instar *Spodoptera frugiperda*

Instar	Treatment	Slope ± SE	LC_{50} (OBs/ml)	Range of 95% CL	Relative potency[a] (Range of 95% CL)	χ^{2b}
2nd	SfMNPV	0.93 ± 0.38	2.6×10^4	1.3×10^4–5.1×10^4	1.0	3.65
	SfMNPV + M2R	1.92 ± 0.08	9.5×10^3	7.9×10^3–1.1×10^4	2.7 (1.8–4.0)	2.64
3rd	SfMNPV	0.77 ± 0.07	3.4×10^4	1.2×10^4–7.9×10^4	1.0	3.86
	SfMNPV + M2R	1.36 ± 0.13	5.2×10^3	3.8×10^3–6.9×10^3	6.5 (3.7–11.6)	0.91
4th	SfMNPV	0.72 ± 0.82	3.1×10^5	8.5×10^4–9.7×10^5	1.0	3.9
	SfMNPV + M2R	1.54 ± 0.16	5.1×10^3	3.8×10^3–6.8×10^3	61.6 (24.6–154)	1.74

Parameters obtained from the POLO-PC program (LeOra Software, 1987).

[a]The regression lines could not be fitted in parallel in any instar. Relative potency is therefore expressed as the ratio of LC_{50} values for each instar.

[b]Goodness-of-fit of probit model (χ^2 test, d.f. = 3).

In contrast, instar had a highly significant effect on the mean time to death, with average values of 99.0, 93.6, and 127.1 h for second, third, and fourth instars, respectively ($F_{2,26} = 138.6$, $P < 0.001$).

A total of 21 studies (including the present study) were identified involving homologous baculovirus bioassays in mixtures with Calcofluor M2R, Tinopal LPW, Tinopal UNPA-GX, or Blankophor BBH. These studies gave rise to 60 observations on the relative potency of the virus in the presence of optical brighteners at concentrations of between 0.01 and 1%, compared to the virus alone. Each observation represented a single concentration of brightener tested on a single species of insect. The majority of studies involved the gypsy moth *Lymantria dispar* (L.) or noctuid species, mainly *Spodoptera* spp. However, because only six observations involved third or fourth instar insects, the analysis was limited to the effects of optical brighteners in first and second instars (total sample n = 54). Linear regression of Ln [relative potency] against Ln [per cent optical brightener concentration] revealed a significant positive relationship ($F_{1,52} = 20.1$, $P < 0.001$) (Figure 2). Larval stage (first vs. second instar) had no significant effect on the degree of potentiation of virus activity by the optical brighteners ($F_{1,51} = 0.45$, $P = 0.51$).

Discussion

Optical brighteners belong to several chemical groups (e.g., stilbene, oxalone, pyrazoline lactone, coumarin), of which the greatest interest has been generated by the stilbene derivatives (Hamm, 1999). The most active compounds identified in our study were Calcofluor M2R and Blankophor BBH, both of which are stilbenes. However, this group also contained compounds (Blankophor CLE, Leucophor SAC) that adversely affected virus activity. The distyryl-biphenyl derivative (Tinopal CBS) had no effect,

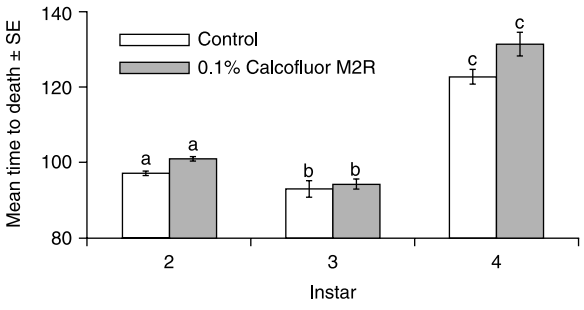

Figure 1 Mean time (± SE) to death hours calculated for second, third, and fourth instar *Spodoptera frugiperda* inoculated with SfMNPV alone or mixed with 0.1% Calcofluor M2R. Columns headed by identical letters are not significantly different between treatments at each instar (t-test in GLIM, $P > 0.05$).

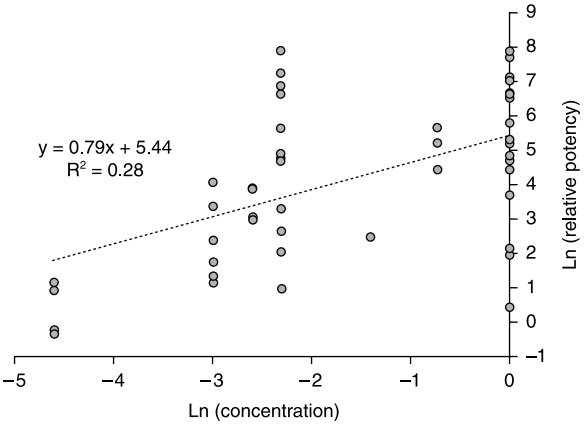

Figure 2 Linear regression of Ln [optical brightener concentration] against Ln [relative potency] for homologous nucleopolyhedroviruses assayed in first and second instars of 10 species of Lepidoptera. Data taken from 21 published studies in peer-reviewed journals.

whereas the styryl-benzenic derivative (Blankophor ER) resulted in a decrease in virus activity (Table 1). The negative effects on virus infection seen in some brighteners may be related to the anti-feedant properties of the brighteners, resulting in a reduced rate of feeding and consequently a lower probability of consuming a lethal dose of viral OBs (Sheppard & Shapiro, 1994; Martínez et al., 2003). However, the droplet feeding method used in our study should have minimized this effect by delivering a lethal dose of virus in a very brief period (10 min). Presumably, anti-feedant effects should not present a problem for the most active brighteners as they cause clear biological effects following the consumption of very small quantities or low concentrations (~0.1%) (Hamm, 1999).

Studies with *Lymantria dispar* (LdMNPV) (Shapiro & Robertson, 1992; Shapiro, 1992; Argauer & Shapiro, 1997), *Anticarsia gemmatalis* (Hübner) (AgMNPV) (Morales et al., 2000), and *Spodoptera litura* (Fabricius) (SpltNPV) (Okuno et al., 2003) have consistently reported that stilbene derivatives such as Tinopal LPW, Tinopal UNPA-GX, Blankophor RKH, and Blankophor BBH are the most effective synergists of nucleopolyhedrovirus infection. These studies have also identified stilbene compounds with no synergistic activity (Hostalux EBTN, Hostalux KS-N, Blankophor BSU) or a negative effect (Blankophor LPG). Similar to our study, distyryl-biphenyl derivative optical brighteners (Tinopal CBS and Uvitex NFW) had no significantly effect on the activity of AgMNPV, which is used to control the soya pest *A. gemmatalis* in Brazil (Morales et al., 2000).

Argauer & Shapiro (1997) reported that the ability of optical brighteners to synergize the activity of LdMNPV was positively correlated with the intensity of fluorescence of the compound; in this respect, the most fluorescent compounds were the stilbenes. The spatial arrangement of alkoxy, amino, hydroxyl, sulphonic acid, and halogen groups contribute to the fluorescent properties of these compounds (Villaume, 1958) although which component, or set of components, are responsible for enhancing viral activity remains unclear (Shapiro & Argauer, 1997). Recently, Okuno et al. (2003) reported that the activity of SpltNPV was significantly enhanced by replacing the diethyl amino group of Tinopal UNPA-GX with other groups, suggesting that the chemical groups in this position may be involved in the enhancing activity.

In our study, the degree of virus potentiation for second instar *S. frugiperda* was 2.7-fold, which is markedly lower than the 164-fold decrease in LC_{50} values in first instars observed by Hamm & Shapiro (1992) or the 115-fold decrease in second instars reported by Martínez et al. (2000), in the presence of 0.1% and 1% Tinopal LPW, respectively. Such differences in activity may be due to differences in the methodology used for the bioassays. The previous studies used the diet surface contamination technique to deliver inoculum and this involved extended periods of consumption of optical brightener, whereas we employed the droplet feeding technique, involving a very brief exposure to optical brightener. Extended periods of exposure to optical brighteners are likely to have had greater effects on the integrity of the peritrophic membrane and sloughing of infected gut cells, compared to brief dosing exposure.

Only two other studies have compared optical brightener performance across different instars. Both of these studies reported a stage-dependent increase in the degree of potentiation by optical brighteners in different instars. In *L. dispar*, in which LC_{50} values for LdMNPV decreased 185-fold in the second instar and 1670-fold in the fourth instar, when mixed with 1% Tinopal LPW (Shapiro & Robertson, 1992). Similarly, when administered with 0.1% Calcofluor M2R, LC_{50} values of PiNPV decreased 14.8-fold in the first instar and 1584-fold in second instar *Pseudoplusia includens* (Walker) (Zou & Young, 1996).

Calcofluor M2R had the effect of maintaining the LC_{50} values of SfMNPV in third and fourth instar *S. frugiperda*, similar to those observed in the second instar (Table 2). This highlights the importance of studies such as ours, especially as older instars are the principal cause of economic losses in crops and are the hardest to control by baculovirus insecticides.

Analysis of the published literature indicated that the magnitude of optical brightener potentiation did not differ significantly between first and second instars, and drew attention to the paucity of studies involving late instars. By contrast, the concentration of optical brightener used in virus + brightener mixtures was identified as significantly affecting the degree of potentiation of homologous nucleopolyhedroviruses. This confirms the results of specific tests in second instar *P. includens* and *L. dispar*, in which a positive relationship between brightener concentration and the magnitude of viral synergism was evident (Zou & Young, 1996; Shapiro & Argauer, 1997; Thorpe et al., 1999). This probably reflects a simple dose effect, the quantity of optical brightener consumed exerting a greater effect on peritrophic membrane permeability and gut cell turnover at high concentrations, and a comparatively reduced biological effect at low concentrations. However, at very high concentrations (~5%), optical brighteners may not increase viral activity beyond that seen at lower concentrations (0.1–1%), possibly due to the anti-feedant effect of these compounds (Li & Otvos, 1999). Similar effects have been reported for optical brighteners in mixtures with heterologous viruses (Shapiro & Dougherty, 1994; Vail et al., 1996).

The mean time to death increased from 99.0 h in the second instar to 127.1 h in the fourth instar, although this parameter was not affected by droplet feeding of SfMNPV + 0.1% Calcofluor M2R in any of the instars tested. Other studies involving diet contamination techniques have detected significant reductions in the time to kill of homologous and heterologous viruses in the presence of optical brighteners (Shapiro & Robertson, 1992; Shapiro & Dougherty, 1994; Shapiro & Vaughn, 1995; Vail et al., 1996; Morales et al., 2000). The reason for these differences may again be due to bioassay methods. For example, mixtures of Calcofluor M2R and the nucleopolyhedrovirus of *Agrotis ipsilon* (Hufnagel) had no effect on survival times (ST_{50}) when administered by droplet feeding (Boughton et al., 2001).

We conclude that baculovirus formulations containing optical brighteners should be of particular value for the control of late instar lepidopteran pests. The high cost of some optical brighteners could be an important limitation to their use in biopesticide formulations (Martínez et al., 2000), as well as their persistence in the environment (Webb et al., 1994), their effect on the behaviour of pollinators, and the rate of growth of crops treated with sprays containing optical brighteners (Goulson et al., 2000, 2003). Studies on the environmental impact of these substances are merited prior to their use as viral adjuvants on a wide scale.

Acknowledgements

We thank Ignacio Barbería Fernández for technical assistance. A.M. Martínez received financial support from the Agencia Española de Cooperación Internacional.

References

Andrews KL (1988) Latin American research on *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Florida Entomologist 71: 631–653.

Argauer R & Shapiro M (1997) Fluorescence and relative activities of stilbene optical brighteners as enhancers for the gypsy moth (Lepidoptera: Lymantriidae) baculovirus. Journal of Economic Entomology 90: 416–420.

Bartnicki-Garcia S, Persson J & Chanzy H (1994) An electron microscope and electron diffraction study of the effect of calcofluor and Congo red on the biosynthesis of chitin *in vitro*. Archives of Biochemistry and Biophysics 310: 6–15.

Boughton AJ, Lewis LC & Bonning BC (2001) Potential of *Agrotis ipsilon* nucleopolyhedrovirus for suppression of the black cutworm (Lepidoptera: Noctuidae) and effect of an optical brightener on virus efficacy. Journal of Economic Entomology 94: 1045–1052.

Briese DT (1986) Insect resistance to baculoviruses. The Biology of Baculoviruses, Vol. II (ed. by RR Granados & BA Federici), pp. 238–259. CRC Press, Boca Raton, FL.

Dougherty E, Guthrie KP & Shapiro M (1996) Optical brighteners provide baculovirus activity enhancement and UV radiation protection. Biological Control 7: 71–74.

Elorza MV, Rico H & Sentandreu R (1983) Calcofluor white alters the assembly of chitin fibrils in *Saccharomyces cerevisae* and *Candida albicans* cells. Journal of General Microbiology 129: 1577–1582.

Escribano A, Williams T, Goulson D, Cave RD, Chapman JW & Caballero P (1999) Selection of a nucleopolyhedrovirus for control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae): structural, genetic, and biological comparison of four isolates from the Americas. Journal of Economic Entomology 92: 1079–1085.

Farrar RR & Ridgway RL (1998) Quantifying time-mortality relationships for nuclear polyhedrosis viruses when survivors are present. Environmental Entomology 27: 1289–1296.

Goulson D, Derwent LC, Penagos DI & Williams T (2003) Effects of optical brighteners included in biopesticide formulations on the growth of crops. Agriculture Ecosystems and Environment 95: 235–240.

Goulson D, Martínez AM, Hughes OH & Williams T (2000) Effects of optical brighteners used in biopesticide formulations on the behavior of pollinators. Biological Control 19: 232–236.

Greene GL, Leppla NC & Dickerson WA (1976) Velvetbean caterpillar: a rearing procedure and artificial medium. Journal of Economic Entomology 69: 478–488.

Hamm JJ (1999) Interactions in entomology; enhanced infectivity of entomopathogenic viruses by fluorescent brighteners. Journal of Entomological Science 34: 8–16.

Hamm JJ & Shapiro M (1992) Infectivity of fall armyworm (Lepidoptera: Noctuidae) nuclear polyhedrosis virus enhanced by a fluorescent brightener. Journal of Economic Entomology 85: 2149–2152.

Hruska AJ & Gould F (1997) Fall armyworm (Lepidoptera: Noctuidae) and *Diatrea lineolata* (Lepidoptera: Pyralidae): Impact of larval population level and temporal occurrence on maize yield in Nicaragua. Journal of Economic Entomology 90: 611–622.

Hughes PR & Wood HA (1981) A synchronous peroral technique for the bioassay of insect viruses. Journal of Invertebrate Pathology 37: 154–159.

Hunter-Fujita FR, Entwistle PF, Evans HF & Crook NE (1998) Insect Viruses and Pest Management. John Wiley, Chichester, UK.

Ignoffo CM, Hostteter DL, Sikorowski PP, Sutter G & Brooks WM (1977) Inactivation of representative species of entomopathogenic viruses, a bacterium, fungus and protozoan by an ultraviolet light source. Environmental Entomology 6: 441–445.

LeOra Software (1987) POLO-PC. A User's Guide to Probit or Logit Analysis. LeOra Software, Berkeley, CA.

Li SY & Otvos IS (1999) Optical brighteners enhance activity of nuclear polyhedrosis virus against western spruce budworm (Lepidoptera: Tortricidae). Journal of Economic Entomology 92: 335–339.

Martínez AM, Caballero P & Williams T (2003) Effects of an optical

brightener on the development, body weight and sex ratio of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Biocontrol Science & Technology, in press.

Martínez AM, Goulson D, Chapman JW, Caballero P, Cave RD & Williams T (2000) Is it feasible to use optical brightener technology with a baculovirus bioinsecticide for resource-poor maize farmers in Mesoamerica? Biological Control 17: 174–181.

McConnell R & Hruska A (1993) An epidemic of pesticide poisoning in Nicaragua: Implications for prevention in developing countries. American Journal of Public Health 83: 1559–1562.

McGuire MR, Tamez-Guerra P, Behle RW & Streett DA (2001) Comparative field stability of selected entomopathogenic virus formulations. Journal of Economic Entomology 94: 1037–1044.

Morales L, Moscardi F, Sosa-Gómez DR, Paro FR & Soldorio IL (2000) Fluorescent brighteners improve *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) nucleopolyhedrovirus (AgMNPV) activity on AgMNPV-susceptible and resistant strains of the insect. Biological Control 20: 247–253.

Numerical Algorithms Group (1993) The GLIM System: Release 4 Manual (ed. by Francis B, Green M & Payne C). Clarendon Press, Oxford, UK.

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

Payne CC, Tatchell GM & Williams CF (1981) The comparative susceptibilities of the *Pieris brassicae* and *P. rapae* to a granulosis virus from *P. brassicae*. Journal of Invertebrate Pathology 38: 273–280.

Robertson JL & Preisler HK (1992) Pesticide Bioassays with Arthropods. CRC Press, Boca Raton, FL.

Roncero C, Valdivieso MH, Ribas JC & Duran A (1988) Effect of Calcofluor white on chitin synthases from *Saccharomyces cerevisae*. Journal of Bacteriology 170: 1945–1949.

Sait SM, Begon M & Thompson DJ (1994) The influence of larval age on the response of *Plodia interpunctella* to a granulosis virus. Journal of Animal Ecology 63: 541–550.

Shapiro M (1992) Use of optical brighteners as radiation protectants for gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus. Journal of Economic Entomology 85: 1682–1686.

Shapiro M & Argauer R (1997) Components of the stilbene optical brightener Tinopal LPW as enhancers for the gypsy moth (Lepidoptera: Lymantriidae) baculovirus. Journal of Economic Entomology 90: 899–904.

Shapiro M & Dougherty EM (1994) Enhancement in activity of homologous and heterologous viruses against the gypsy moth (Lepidoptera: Lymantriidae) by an optical brightener. Journal of Economic Entomology 87: 361–365.

Shapiro M & Farrar Jr RR (2003) Fluorescent brighteners affect feeding rates of the corn earworm (Lepidoptera: Noctuidae) and act as enhancers and sunlight protectants for its nucleopolyhedrovirus. Journal of Entomological Science 38: 286–299.

Shapiro M, Farrar Jr RR, Domek J & Javaid I (2002) Effects of virus concentration and ultraviolet irradiation on the activity of corn earworm and beet armyworm (Lepidoptera: Noctuidae) nucleopolyhedrosis. Journal of Economic Entomology 95: 243–249.

Shapiro M & Robertson JL (1992) Enhancement of gypsy moth (Lepidoptera: Lymantriidae) baculoviruses activity by optical brighteners. Journal of Economic Entomology 85: 1120–1124.

Shapiro M & Vaughn JL (1995) Enhancement in activity of homologous and heterologous baculoviruses infections to cotton bollworm (Lepidoptera: Noctuidae) by an optical brightener. Journal of Economic Entomology 88: 265–269.

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

Tamez-Guerra P, McGuire MR, Behle RW, Hamm JJ, Sumner HR & Shasha BS (2000) Sunlight persistence and rainfastness of spray-died formulations of baculoviruses isolated from *Anagrapha falcifera* (Lepidoptera: Noctuidae). Journal of Economic Entomology 93: 210–218.

Thorpe KW, Cook SP, Webb RE, Podgwaite JD & Reardon RC (1999) Aerial application of the viral enhancer Blankophor BBH with reduced rates of gypsy moth (Lepidoptera: Lymantriidae) nucleopolyhedrovirus. Biological Control 16: 209–216.

Tinoco R & Halperin D (1998) Poverty, production and health: inhibition of erythrocyte cholinesterase through occupational exposure to organophosphate insecticides in Chiapas, Mexico. Archives of Environmental Health 53: 29–35.

Vail PV, Hoffmann DF & Tebbets JS (1996) Effects of a fluorescent brightener on the activity of *Anagrapha falcifera* (Lepidoptera: Noctuidae) nuclear polyhedrosis virus to four noctuid pests. Biological Control 7: 121–125.

Villaume FG (1958) Optical brighteners in soaps and detergents. Journal of the American Oil Chemist's Society 35: 558–566.

Wang P & Granados RR (1998) Observations on the presence of the peritrophic membrane in larval *Trichoplusia ni and* its role in limiting baculovirus infection. Journal of Invertebrate Pathology 72: 57–62.

Wang P & Granados RR (2000) Calcofluor disrupts the midgut defense system in insects. Insect Biochemistry and Molecular Biology 30: 135–143.

Washburn JO, Kirkpatrick BA, Hass-Stapleton E & Volkman LE (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. Biological Control 11: 58–69.

Webb RE, Shapiro M, Podwaite JD, Ridgway RL, Venables L, White GB, Argauer RJ, Cohen DL, Witcosky J, Kester KM & Thorpe KW (1994) Effect of optical brighteners on the efficacy of gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus in forest plots with high or low levels of natural virus. Journal of Economic Entomology 87: 134–143.

Williams T, Goulson D, Caballero P, Cisneros J, Martínez AM, Chapman JW, Roman DX & Cave RD (1999) Evaluation of a baculovirus bioinsecticide for small-scale maize growers in Latin America. Biological Control 14: 67–75.

Zou Y & Young SY (1996) Use of a fluorescent brightener to improve *Pseudoplusia includens* (Lepidoptera: Noctuidae) nuclear polyhedrosis virus activity in the laboratory and field. Journal of Economic Entomology 89: 92–96.