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Is It Feasible to Use Optical Brightener Technology with a Baculovirus Bioinsecticide for Resource-Poor Maize Farmers in Mesoamerica?

Ana Mabel Martínez,* Dave Goulson,† Jason W. Chapman,† Primitivo Caballero,‡ Ronald D. Cave,§ and Trevor Williams*

**ECOSUR, AP 36, 30700 Tapachula, Chiapas, Mexico; †Biological Sciences, University of Southampton, Southampton SO16 7PX, United Kingdom; ‡Depto. de Producción Agrária, Universidad Pública de Navarra, Pamplona, Spain; and §Escuela Agrícola Panamericana, El Zamorano, Honduras*

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Stilbene-derived optical brighteners greatly enhance the infectivity of a number of baculoviruses. This technology has been patented for use with insect pathogenic viruses in the United States and Canada. A baculovirus is currently being tested for its potential as a biological insecticide of *Spodoptera frugiperda* (Lepidoptera: Noctuidae), the principal insect pest of maize in Mesoamerica. A multiply embedded nucleopolyhedrovirus isolate originally from Nicaragua was bioassayed alone and in the presence of the optical brightener Tinopal LPW (1%), using second instar *S. frugiperda* larvae. The LC_{50} value of the virus alone was calculated at 82.1 polyhedral inclusion bodies (PIBs)/mm^2 of diet compared with 0.71 PIBs/mm^2 in the presence of Tinopal LPW. In contrast to other studies, the mean time to death of larvae exposed to virus and Tinopal LPW was significantly extended compared to larvae inoculated with virus alone. Analysis of the results of eight independent field trials in Mexico and Honduras revealed a significant positive relationship between log virus dose and percentage mortality observed in *S. frugiperda* larvae. Virus-induced mortality was approximately 50% at the highest application rate tested: 1000 larval equivalents (LE) of virus/ha. When the impact of parasitism was taken into account, larval mortality increased to 45.0–90.7% in plots treated with virus at 250 LE/ha or more. A cost analysis indicated that approximately 60% pest control can be achieved as a conservative estimate with virus application and the action of parasitoids for the price of a chemical insecticide. Formulating the virus with an optical brightener appears to be an attractive option based on laboratory findings but requires field testing. The use of optical brightener technology will probably be feasible for maize growers in Mesoamerica only if it is highly effective at very low concentrations (<0.1%) or the volume of the virus application can be reduced.

***Key Words: Spodoptera frugiperda;* nuclear polyhedrosis virus; synergism; Tinopal LPW; cost analysis.**

INTRODUCTION

The social and economic importance of maize as a basic grain in Mesoamerica[1] surpasses that of all other crops. Larvae of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), represent an important biological constraint on maize production in the region; infestation levels over 55% can result in a 15 to 73% reduction in crop yield (Hruska and Gould, 1997). The damage caused by the feeding of *S. frugiperda* larvae is highly apparent and growers often apply synthetic insecticides in granular formulations to control the pest. As such, insecticides represent an important economic input in maize production. Moreover, use of chemical insecticides by resource-poor rural growers carries with it health risks. A high incidence of chronic pesticide poisoning occurs in farm workers from southern Mexico and Nicaragua (McConnell and Hruska, 1993; Tinoco and Halperin, 1998). Clearly, Mesoamerican maize growers require safe, effective, cheap, and sustainable methods of insect pest control (Van Huis, 1981; Andrews *et al.*, 1992; Altieri and Masera, 1993). This need has prompted a collaborative international project focusing on the development of a multiply embedded nucleopolyhedrovirus (MNPV) (Baculoviridae) bioinsecticide of *S. frugiperda* for use by resource-poor maize growers in Mesoamerica. Preliminary studies in southern Mexico and Honduras confirmed that the virus has potential as a biological insecticide; the prevalence of infection at the highest application rate (6×10^{12} polyhedral inclusion bodies (PIBs)/ha) resulted in approximately 40% infection of *S. frugiperda* larvae collected 2 days postapplication.

[1] For this article, we consider Mesoamerica to be the region between the isthmus of Oaxaca, Mexico and the southern border of Panama with Colombia.

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Parasitism accounted for an additional 15–20% mortality of field-collected larvae (Williams *et al.*, 1999).

Two important limitations to the efficacy of this bioinsecticide relate, first, to the proportion of larvae that can be directly infected by the applied inoculum and, second, to the inactivation of the virus by UV solar radiation, a recognized problem for all biological insecticides (Ignoffo *et al.*, 1977; Griego *et al.*, 1985; Cohen *et al.*, 1991; Inglis *et al.*, 1995). To achieve a high prevalence of infection of larvae, it would be necessary to apply higher concentrations of virus, the costs of which would be prohibitive for a bioinsecticide targeted at impoverished maize growers. Protection of the virus from the detrimental effects of UV light can be achieved by incorporating reflectant, absorbent, or microencapsulating substances into the virus formulation, which has been the subject of considerable study, especially with baculoviruses.

Over the past 7 years, intriguing observations have been reported on the use of optical brighteners in virus formulations (Shapiro and Robertson, 1992; Hamm, 1999). Optical (fluorescent) brighteners are widely used in paints, fabrics, detergents, paper, and plastics, wherein they enhance the apparent whiteness of the product by absorbing UV radiation and emitting light in the blue portion of the visible spectrum. The types of optical brighteners of the greatest interest for their interactions with baculoviruses belong to a group of disulfonic acid-substituted stilbenes, of which the most studied is Tinopal LPW (Fluorescent brightener 28, Calcofluor white M2R) (Arguaer and Shapiro, 1997).

Initially, these substances were tested simply for their properties as UV protectants (e.g., Martignoni and Iwai, 1985), with highly promising results; 8 of 23 optical brighteners tested provided complete protection to nucleopolyhedrovirus exposed to a laboratory UV light source for 14 days (Shapiro, 1992). Subsequently, it was noticed that certain types of optical brightener markedly enhanced the infectivity of these viruses *in vivo*. Five different optical brighteners at a concentration of 1% reduced the LC_{50} value of *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) nucleopolyhedrovirus by a factor of between 400 and 1800 (Shapiro and Robertson, 1992). Almost simultaneously, Hamm and Shapiro (1992) reported a 164- to 303,000-fold reduction in the LC_{50} value of two isolates of *S. frugiperda* nucleopolyhedrovirus in the presence of 0.1% of the optical brightener Tinopal LPW.

The cells of the midgut epithelium are the site of primary baculovirus infection; the alkaline pH of the midgut region is crucial for the dissolution of viral PIBs and the liberation of virions. In the case of *Autographa californica* MNPV in *Trichoplusia ni* (Hübner) and *Heliothis virescens* F., it appears that the optical brightener M2R blocked the sloughing of primary infected midgut cells, leading to a higher probability of establishment of infection in larvae simultaneously fed virus + M2R compared to conspecifics fed virus alone (Washburn *et al.*, 1998). This technology was awarded a United States patent (Shapiro *et al.*, 1992) for the enhancement of the activity of insect pathogenic viruses.

Biological insecticides have unique and desirable properties as pest management agents, above all when used in combination with other natural enemies (Waage, 1997). The issue addressed in the present study is the potential of applying the optical brightener technology to the *S. frugiperda* virus bioinsecticide being developed for Mesoamerican maize growers. We present data confirming that an optical brightener can enhance the isolate currently being tested in Mexico and Honduras. We then address the problem of the efficacy of the MNPV as a biological insecticide and the cost of biological control compared with conventional chemical control measures. From a cost standpoint, we argue that using optical brighteners in virus formulations will probably only be feasible if the concentration of virus in spray applications can be significantly reduced, and/or if the optical brightener shows high synergistic activity in the field at low concentrations, and/or if the volume of water applied in spray application of the virus can be reduced yet remain effective in delivering it to the feeding site of the pest.

MATERIAL AND METHODS

Laboratory Bioassay

To determine the degree of synergism provided by an optical brightener and the Nicaraguan nucleopolyhedrovirus isolate (SfMNPV) being tested in Mexico and Honduras, a bioassay was performed based on the technique described by Del Rincón-Castro and Ibarra (1997). This isolate was previously characterized and showed high biological activity compared to other SfMNPV isolates from the United States and Argentina (Escribano *et al.*, 1999). Virus was produced in fourth instar *S. frugiperda* larvae maintained on semisynthetic diet based on soya and maize without formaldehyde (modified from Mihm, 1984). Virus-killed larvae were triturated in 0.1% (w/v) sodium dodecyl sulfate (SDS) and centrifuged at 90*g* for 5 min, and the supernatant was centrifuged at 3000*g* for 10 min. Pelleted PIBs were resuspended in sterile distilled water, counted using a bacterial counting chamber, and stored at 4°C for 24 h prior to use.

Sterile plastic Petri dishes (9 cm diameter) were half filled with a semisynthetic diet. The diet was allowed to solidify and the surface was inoculated with one of six concentrations of the Nicaraguan isolate: between 500 and 500,000 PIBs per Petri dish (equivalent to 0.09 to 90.22 PIBs/mm^2 of diet surface). For half of the inoculated Petri dishes, the virus was suspended in 0.1%

(w/v) SDS to reduce aggregation of viral PIBs; for the other half, virus was suspended in 1.0% (w/v) Tinopal LPW (Sigma Chemical Co.) and 0.1% SDS. Control Petri dishes were treated with identical concentrations of SDS or Tinopal in SDS solution.

A rectangular plastic grid 70 × 54 mm divided into 12 squares with an internal area of 15 × 15 mm was pressed into the diet to form 12 identical compartments into each of which was placed a second instar *S. frugiperda* larva taken from the laboratory culture maintained at El Colegio de la Frontera Sur (ECOSUR), Tapachula, Mexico. The grid was then covered with a thin glass slide and the lid of the Petri dish.

Larvae were held at 25 ± 1°C, 75–85% R.H. and 12h:12h L:D photoperiod. Larvae were checked twice daily for mortality until 14 days postinoculation by which time survivors had pupated. Viral deaths were confirmed by examination of Giemsa-stained smears of experimental insect cadavers. The bioassay was performed three times using 36 larvae (3 Petri dishes) at each viral concentration. Mortality data were subjected to logit analysis using GLIM. Scaling was performed as necessary to correct for minor overdispersion and the behavior of models was checked by examination of the distribution of residuals. Larvae that died of bacterial contamination were excluded from the analysis. The mean time to death was calculated using GLIM with a normal error distribution (Crawley, 1993); individuals that did not succumb to virus infection were excluded from the analysis (Farrar and Ridgway, 1998).

Field Experiments

To determine how effective this virus is for Mesoamerican maize growers, field data were obtained from eight independent field trials performed from June to September of 1997 and 1998 in Mexico, on the southern coastal plain of Chiapas State and in Honduras in the valley of El Zamorano, 30 km southeast of Tegucigalpa. Two of these eight studies have been published (Williams *et al.*, 1999); the rest are student theses that are to be published as individual papers. In all cases, data were collected in the same manner. Experimental blocks of maize were planted in 5 × 5 or 8 × 8 m plots and sprayed at 20 to 45 days postplanting with an aqueous suspension of the Nicaraguan SfMNPV isolate. Two days were allowed to elapse to permit larvae to become infected by feeding upon virus-contaminated foliage. At 2 days postspraying, 20 plants were sampled from each plot and *S. frugiperda* larvae were collected and taken to the laboratory, where they were individually reared on semisynthetic diet until death or pupation. The emergence of parasitoids from larvae and pupae was recorded. Death from virus infection was diagnosed by the typical liquefaction of the insect's body and confirmed by examination of Giemsa-stained larval smears for the presence of PIBs. Between one and three concentrations of virus were applied in each trial with a range of 50 larval equivalents (LE) (1 LE = 6×10^9 PIBs) to 1000 LE/ha in an aqueous suspension containing a commercial wetter-sticker (0.06% Adsee, Westrade, Guatemala or 0.2% AgralPlus, Zeneca, Mexico). Control plots were treated with wetter-sticker solution alone. For all trials four to six replicate plots received each treatment and virus suspension was applied in a volume of 811 liters water/ha using a manual knapsack sprayer with a cone nozzle. Additional treatments involving virus formulation or natural enemy manipulation were often included in the various field trials, but the results from plots that received such treatments were not included in the present analysis.

The mean percentage virus-induced mortality or percentage parasitism observed in larvae from each treatment collected at 2 days postspraying in each trial was subjected to regression analysis using GLIM following arcsine transformation of percentage data. Model behavior was checked by examination of residuals. All reported means were backtransformed as were their asymmetrical standard errors or confidence limits. To examine the cost of biological control, simple regression curves were fitted to data points using Microsoft Excel.

RESULTS

Laboratory Bioassay

There was a marked reduction in the LC_{50} value of virus fed to *S. frugiperda* larvae in the presence of 1% Tinopal LPW. The LC_{50} of virus alone was calculated as 82.1 PIBs/mm^2 diet surface (range of 95% C.L.: 38.1–172) [regression equation: $y = 0.49\ln(x) - 2.16$; reduction in model deviance by fitting ln dose $\chi^2_1 = 145.6$, $P < 0.001$, scale parameter = 2.1] compared to 0.71 PIBs/mm^2, when virus was formulated with optical brightener (range of 95% C.L.: 0.46–1.03) [regression equation: $y = 1.02\ln(x) + 0.34$; reduction in model deviance due to fitting ln dose $\chi^2_1 = 133.5$, $P < 0.001$, scale parameter = 1.4]. The optical brightener was associated with a significant increase in the mean time to death ($F_{1,160} = 76.09$, $P < 0.001$) from 5.3 ± 0.4 days for virus alone to 7.6 ± 0.2 days for insect treated with virus plus Tinopal LPW (values are means ± 95% C.L.). No viral deaths occurred in any of the controls.

Field Experiments

Analysis of the results of spraying virus in eight independent field trials in Mexico and Honduras revealed a significant positive relationship between the logarithm of virus dose and percentage mortality observed in *S. frugiperda* larvae collected at 2 days postspraying and laboratory-reared until death or pupation ($F_{1,26} = 20.64$, $P < 0.001$). Virus-induced mortality ranged from 3.8 to 22% at the lowest application rate

(50 LE/ha) to approximately 50% at the highest application rate tested (1000 LE/ha), although it is obvious that within this range considerable variation was observed between trials (Fig. 1A). Virus-induced mortality in larvae from control plots was invariably low and never exceeded 2.5% in any sample. The prevalence of parasitoid emergence varied from zero to 47.5% in plots treated with virus. Parasitism was not significantly affected by the virus application rate ($F_{1,26} = 0.93$, N.S.), i.e., higher larval mortality due to virus infection did not adversely influence the emergence of larval and pupal parasitoids (Fig. 1B). The mean prevalence of parasitism in untreated control plots averaged over all studies was 31.1%. When mortality due to parasitoid emergence and virus infection were summed, the effect of log virus application rate was less pronounced but remained significant ($F_{1,26} = 5.08$, $P < 0.05$). The overall levels of mortality in field-collected larvae due to these two types of natural enemies varied between 45.0 and 90.7% in plots treated with virus at 250 LE/ha or more (Fig. 1C).

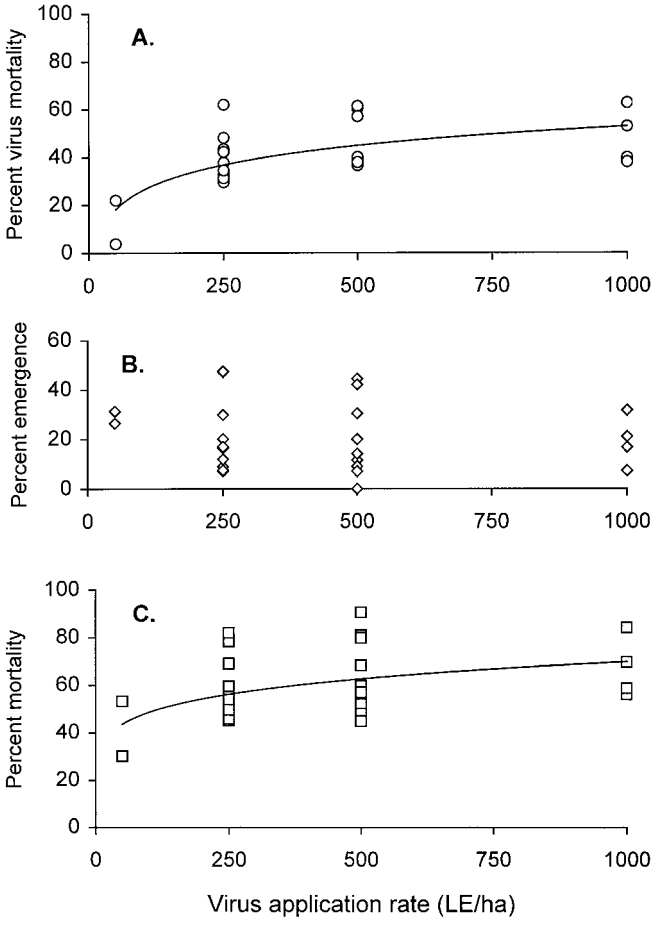

FIG. 1. Results of eight field trials in Mexico and Honduras involving the application of 50–1000 larval equivalents (LE)/ha of SfMNPV to maize at 20–45 days postplanting. *Spodoptera frugiperda* larvae were collected at 2 days postapplication and reared in the laboratory until pupation or death. (A) Significant positive relationship between virus application rate and percentage virus mortality of *S. frugiperda* larvae. (B) Percentage parasitoid emergence from *S. frugiperda* larvae collected in virus-treated plots. There was no significant reduction in parasitoid emergence in plots treated with high concentrations of virus. (C) Significant positive relationship between overall mortality of *S. frugiperda* larvae (virus infection + parasitism) and virus application rate. (1 LE = 6×10^9 viral PIBs).

Cost Analysis

A breakdown of the costs involved in producing the *S. frugiperda* MNPV showed that to produce, purify, quantify, and formulate 1000 LE of virus cost US$ 48.43 in Mexico and US$ 34.98 in Honduras, the cost difference being mainly due to higher labor costs in Mexico (Williams *et al.*, 1999). When the percentage pest control achieved by application of virus (data from regression analysis shown in Fig. 1A) is plotted against the cost of the virus, it can be appreciated that the virus compares poorly to chemical control in terms of pest-induced mortality; for the price of a chemical insecticide,[2] the virus only achieves 35–40% pest mortality (Fig. 2A). However, when the impact of parasitoid mortality is included in the analysis (data taken from Fig. 1C), the picture changes, with approximately 60% pest mortality achievable for the same price as a chemical insecticide (Fig. 2B).

DISCUSSION

Laboratory bioassay using a *S. frugiperda* MNPV isolate from Nicaragua confirmed previous observations that Tinopal LPW greatly enhanced the infectivity of the fall armyworm nucleopolyhedrovirus (Hamm and Shapiro, 1992). The degree of enhancement (LC_{50} ratio) observed in the present study was 115-fold, which is somewhat less than that reported by Hamm and Shapiro (1992) for two isolates from the United States, wherein at least a 164-fold decrease in the LC_{50} value was recorded in the presence of the optical brightener. The 42% increase in mean time to death observed in larvae infected with virus and optical brightener was not expected; Tinopal LPW caused significant decreases in the median LT_{50} values of various heterologous nucleopolyhedroviruses in four species of noctuid larvae (Shapiro and Vaughn, 1995; Vail *et al.*, 1996) and also in *L. dispar* larvae infected with their homologous virus (Shapiro and Robertson, 1992; Shapiro and Dougherty, 1994). The reason for this difference is not immediately apparent, although it may be related to reductions in rate of development and feeding behavior reported for larvae inoculated with virus in the presence of Tinopal LPW (Sheppard and Shapiro, 1994) or

[2] For example, chlorpyrifos costs US$ 15.35 per liter in Mexico and US$ 12.25 in Honduras.

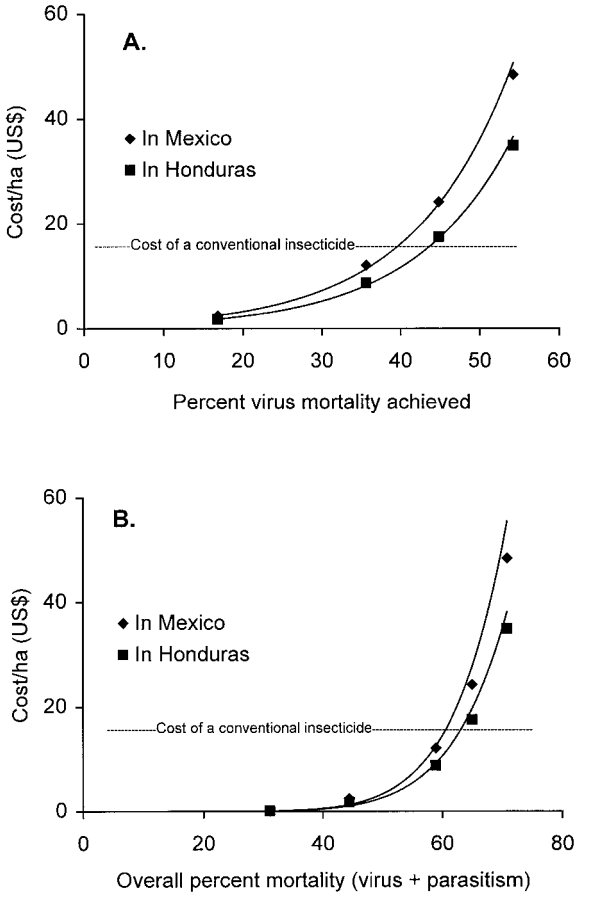

FIG. 2. Mortality of *Spodoptera frugiperda* larvae collected at 2 days following the application of 50–1000 larval equivalents (LE)/ha SfMNPV and reared in the laboratory until death or pupation (data taken from regressions shown in Figs. 1A and 1B) plotted against the cost of the virus application (1000 LE = US$ 48.43 in Mexico and US$ 34.98 in Honduras). (A) Relationship between percentage virus-induced mortality of *S. frugiperda* larvae and the cost of virus application in Mexico ($R^2 = 0.9985$) and Honduras ($R^2 = 0.9985$). Dashed line indicates the cost of a conventional insecticide (1 liter/ha chlorpyrifos, approx US$ 15 in Mexico). (B) Relationship between overall *S. frugiperda* larval mortality from virus infection plus parasitoid emergence and the cost of virus application over 1 ha in Mexico ($R^2 = 0.9809$) and Honduras ($R^2 = 0.9863$). Dashed line indicates the cost of a conventional insecticide (1 liter/ha chlorpyrifos, approx US$ 15 in Mexico).

related to the behavior of the isolate selected for our study. Microscopic examination of Giemsa-stained smears gave no evidence of infection by granuloviruses or other pathogens that may have affected the time to death of experimental insects. The possibility of an interaction between SDS, used to reduce aggregation of viral PIBs, and the activity of the optical brightener cannot be excluded. SDS may have also increased the antifeedant effect of Tinopal LPW; lower feeding rates in the presence of SDS and optical brightener may have resulted in reduced ingestion of virus, leading to longer times to death. For application in the field, however, virus would almost always be mixed with an ionic or nonionic detergent or a commercial wetter-sticker to enhance virus delivery to the pest feeding site, irrespective of whether or not an optical brightener was present.

The feasibility of incorporating optical brighteners in a bioinsecticide for use in Mesoamerica depends on a number of biological, economic, and social factors that relate to the degree of improvement in pest mortality derived from the novel virus formulation, the cost of biological control compared to that of chemical pesticides, and the human and environmental safety of biological insecticide formulated with stilbene optical brighteners.

It is essential to confirm that the synergistic and UV-protection properties of these substances observed in laboratory bioassay experiments are reflected in improved levels of target pest control in field studies. Webb *et al.* (1994) reported that gypsy moth larval mortality increased from 85–88% with the standard virus formulation to 97–98% with the optical brightener Phorwite AR. There were generally high levels of natural mortality from virus in control plots; spraying the optical brightener alone increased natural virus mortality from about 64% in untreated plots to 90% with 1% Phorwite AR alone.

S. frugiperda MNPV formulated with Tinopal LPW did not generally improve the overall level of virus-induced mortality of this pest in maize plots (Hamm *et al.*, 1994). No improvement in *S. frugiperda* virus mortality was observed with Tinopal LPW concentrations greater than 1% and, in general, the volume of water used for applying the virus was influential; high volume applications were more effective in delivering the virus into the whorl feeding site of *S. frugiperda* larvae. Hamm *et al.* (1994) concluded that Tinopal LPW increased the mortality due to virus, but that mortality due to parasitism was reduced in plots treated with virus and optical brightener, resulting in marginal improvements in pest mortality averaging 5–15%. Factors such as virus delivery may be more important limitations to the degree of pest control achievable by baculovirus bioinsecticides than virus infectivity or persistence of PIBs on plant surfaces, but field studies of delivery methods and virus infection rates in the presence of optical brighteners are needed to elucidate such issues.

Resource-poor maize growers will never adopt a biological insecticide if the cost exceeds that of commercial chemical products; the price of biological control, therefore, is of key importance. The virus is produced in the Mexican and Honduran laboratories on a small or very small scale, essentially for experimental purposes only. We would, therefore, expect considerable economies of scale if virus was produced in large quantities for commercial purposes. Moreover, a prerequisite for

farmer acceptance of virus-based pest control is that the grain harvested from virus-treated maize would have to compare favorably in both yield and quality to that achieved by chemical control. In general, it appears that maize plants can tolerate a considerable degree of defoliation with little loss of grain yield, leading to high economic infestation thresholds (Hruska and Gould, 1997), which are compatible with virus-based control practices that give a substantial but not absolute degree of pest control.

In addition to virus and parasitoid-induced mortality, the level of pest control would probably be enhanced by the action of predators in virus-treated maize because insect predators are not adversely affected by baculoviruses but are markedly reduced following pesticide treatment. Nevertheless, for a bioinsecticide, levels of pest mortality exceeding 80% are desirable to convince growers of the efficacy of the product. This is the principal motivation for evaluating virus formulations with optical brighteners. These substances are not particularly cheap, however. In Mexico, the cheapest stilbene optical brightener offered by Ciba Corp. (Mexico City), Tinopal CBS, costs US$ 44/kg. As the virus is usually applied in large volumes of water (811 liters/ha), even concentrations of 0.5% would require 4.06 kg of optical brightener costing US$ 179. By reducing the volume of water used to apply the virus or using lower concentrations (<0.1%) of the optical brightener, the cost of the formulation may be reduced to more realistic levels (Fig. 3). An alternative option may be to offer the virus together with an optical brightener in a granular formulation for manual application directly to the whorl, as is already common in the region. These issues cannot be resolved until the effects of application volume, optical brightener concentration, and type of formulation have been field tested. Of course these simple cost analyses place no value on environment or health issues or on the probability of pest resurgence in crops treated with conventional synthetic insecticides, which are not readily quantifiable in direct economic terms.

Turning to social issues, one of the principal concerns of pesticide use in Mesoamerica is the risk of exposure of agricultural workers to these noxious chemicals. The source of the problem is apparently twofold: (i) getting the message across to farmers concerning the dangers of chronic exposure to pesticides is not given high priority by health services, agricultural extensionists, or pesticide companies; and (ii) the hot, humid climate of the region makes use of protective clothing, face masks, or other safety equipment extremely uncomfortable. While highly pathogenic to their insect hosts, baculoviruses are extremely host-specific and safe to humans, making them particularly appropriate for programs of biological control in developing countries (Jones *et al.*, 1993). Given that optical brighteners are ubiquitous additives in washing powders, paper, and fabrics, the health risks posed by exposure to optical brighteners in a virus formulation are probably small. Because of the traditionally relaxed attitude to pesticide handling in Mesoamerica, it seems likely that

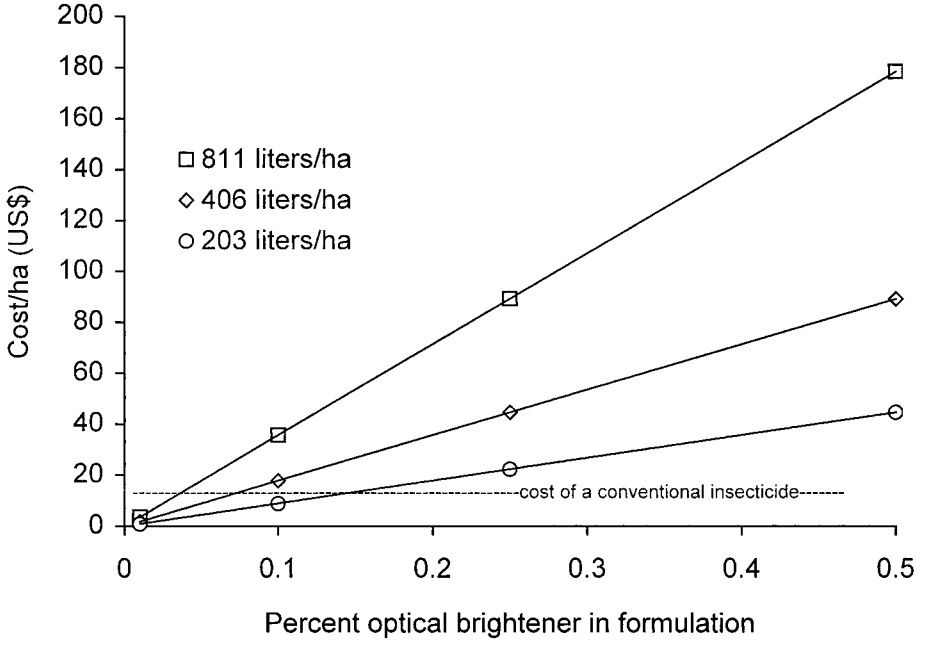

FIG. 3. Cost of incorporating different concentrations of optical brightener in the virus formulation when applied in volumes of 203, 406, or 811 liters water/ha. The cost shown represents the cost of the optical brightener alone and does not include the cost of the virus. Dashed line indicates the cost of a conventional insecticide (1 liter/ha chlorpyrifos, approx US$ 15 in Mexico).

farmers would indeed be exposed to optical brightener during spraying of a formulated virus product.

Stilbene-derived compounds are reported to be fairly persistent in the environment. No decrease in the concentration of Blankophor BBH was detected 1 month following application to oak trees in United States forests, whereas fluorescence assays performed 6 months later showed that 28–40% of the substance persisted on the foliage (Webb *et al.*, 1994). The rapid growth dilution shown by maize plants means that optical brighteners applied to maize will probably be rapidly diluted and the burning of crop residues may result in little accumulation of optical brighteners in the soil, although, for the moment this remains speculation that requires support from field studies.

Optical brighteners have been demonstrated to increase the host range of these viruses and may even be useful tools for the study of host specificity mechanisms (Shapiro and Vaughn, 1995; Shapiro and Dougherty, 1994). The effect of extending the host range of baculovirus to include nontarget lepidopteran hosts may not be of serious concern because the effect is temporary in situations in which virus and optical brightener are simultaneously inoculated to a heterologous host larva. Opportunities for the transmission of the progeny virus from such infections are not likely to be enhanced in the absence of optical brightener.

In conclusion, laboratory studies indicate that stilbene-derived optical brighteners can have a dramatic effect on the quantity of baculovirus needed to cause a lethal infection in lepidopteran larvae. The cost and efficacy of virus insecticides may be crucial factors in determining the adoption of biorational products by maize growers. Formulation of virus with optical brighteners appears to offer a valuable means of improving the efficacy of the bioinsecticide. Field trials are clearly needed to determine whether or not virus-induced pest mortality is greatly enhanced by such formulations and whether the degree of enhancement of pest control, or the reduction in the quantity of virus needed to achieve effective control, justify the additional cost of incorporating an optical brightener.

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