Laboratory Evaluation of Selected Spray Adjuvants as Ultraviolet Light Protectants for the Nuclear Polyhedrosis Virus of the Celery Looper (Lepidoptera: Noctuidae)[1,2]

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Abstract Four spray adjuvants were tested in the laboratory as ultraviolet light (UV) protectants for the nuclear polyhedrosis virus of the celery looper, *Anagrapha falcifera* (Kirby) (AfMNPV), against the beet armyworm, *Spodoptera exigua* (Hübner). We tested a sodium lignin sulfonate (Lignosite AN®), two diaminostilbene disulfonic acid-derived fluorescent brighteners (Blankophor BBH® and Blankophor HRS®), and a nutrient-based feeding stimulant (Coax®). Lignosite AN was active as a UV protectant; Blankophor BBH, as both an enhancer and a UV protectant; Blankophor HRS, as an enhancer only, and; Coax, as a UV protectant only. Lack of an effect of Coax as a feeding stimulant may be due to the design of the bioassay, in which larvae were confined on small pieces of foliage. However, the practical utility of some, if not all, of these materials may be limited by the cost and/or bulk of the amounts required to achieve the desired effects.

Key Words Nuclear polyhedrosis virus, *Spodoptera exigua,* ultraviolet light, sodium lignin sulfonate, fluorescent brightener, feeding stimulant

Nuclear polyhedrosis viruses (NPVs) form a large group of viruses in the family Baculoviridae that includes many important pathogens of insect pests. Over the past three decades, much research has been conducted on NPVs with the aim of utilizing them as pest control agents (Maramorosch and Sherman 1985, Granados and Federici 1986, Adams and McClintock 1991). Recently, the development of NPVs that are genetically engineered to kill insects faster (Bonning and Hammock 1996) has prompted renewed interest in NPVs as microbial insecticides. While NPVs and other microbial control agents can provide control when applied to crops as simple mixtures of pathogens in water, they are usually formulated with various adjuvants to improve stability, handling, persistence, and activity (Jones et al. 1997, Burges and Jones 1998).

NPVs, including most, if not all, genetically modified NPVs developed to date, are highly susceptible to degradation by ultraviolet light (UV). Thus, extensive research has been done on materials that can protect NPVs and other microbial control agents from UV while they are on the plant surface, before ingestion by the target insect

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[2]Use of trade names does not imply endorsements of products named nor criticism of similar ones not mentioned.

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(reviewed by Jaques 1977, 1985, Shapiro 1995). Certain diaminostilbene disulfonic acid derivatives, known as fluorescent brighteners or optical brighteners, are effective UV protectants for NPVs (Shapiro 1992). These materials absorb energy in the form of UV and transmit it as visible light. Some of these materials are of particular interest because, independently of their UV protectant activity, they are also strong enhancers of the activity of many NPVs (Shapiro 1995, Dougherty et al. 1996, Farrar and Ridgway 1997). For example, in laboratory bioassays, median lethal concentrations (LC_{50}s) for the NPV of the gypsy moth, *Lymantria dispar* (L.), (LdMNPV) were reduced by as much as 1,837-fold by the addition of a fluorescent brightener (Shapiro and Robertson 1992, Shapiro et al. 1992). Washburn et al. (1998) reported that fluorescent brighteners may enhance viral activity by inhibiting the sloughing of infected midgut cells.

Because NPVs must be ingested by the target pest in order to infect, considerable work has also been done on adjuvants that act as feeding stimulants or phagostimulants, thereby increasing the amount of virus ingested (e.g., Andrews et al. 1975, Bell and Kanavel 1977, 1978, Bell and Romine 1980). Several materials of this type, based primarily on vegetable flours, oils, and sugars, have been marketed as adjuvants for microbial insecticides. At least one of these products, Coax® (*Agro*Solutions, San Marcos, CA), also acts as a UV protectant for viruses (Shapiro et al. 1983, Martignoni and Iwai 1985).

In the present study, we evaluated the UV-protectant properties of selected virus adjuvants, including two fluorescent brighteners, a nutrient-based feeding stimulant, and a sodium lignin sulfonate. The latter material is not known to have effects other than as a UV protectant, but it is inexpensive (see Discussion) and has been used successfully with LdMNPV (Reardon and Podgwaite 1994, Farrar et al. 1995).

For this study, we selected the NPV of the celery looper, *Anagrapha falcifera* (Kirby) (AfMNPV). This NPV is of particular interest as a biological control agent because it has a wider host range than most NPVs (at least 31 species of Lepidoptera in 10 families) (Hostetter and Puttler 1991, Vail et al. 1993). AfMNPV is infectious to a similar number of, and many of the same, species as the NPV of the alfalfa looper, *Autographa californica* (Speyer) (AcMNPV) (Gröner 1986, Adams and McClintock 1991). AfMNPV and AcMNPV are very closely related (Chen et al. 1996) but differ in their potency against important insect pests; in particular, AfMNPV is more potent than is AcMNPV against the corn earworm, *Helicoverpa zea* (Boddie) (Hostetter and Puttler 1991).

For our study, we applied the virus and adjuvants to foliage, with the concentrations of the adjuvants varied, then exposed them to UV. Many previous studies of UV protectants for microbial control agents included only a single concentration of each protectant, often with virus applied to artificial diets rather than to foliage (reviewed by Jaques 1977, 1985, Shapiro 1995). Therefore, evaluation of UV protectants at varying concentrations applied to foliage is needed. We selected the beet armyworm, *Spodoptera exigua* (Hübner), as the test insect because it is only moderately susceptible to AfMNPV (Farrar and Ridgway 1997). Thus, enhancement by fluorescent brighteners and increases in ingestion by feeding stimulants may be more readily detectable in the beet armyworm than in a more susceptible insect.

Materials and Methods

Insects, plants, and virus. We obtained all insects from stock cultures (Insect Biology and Population Management Research Laboratory, USDA-ARS, Tifton, GA,

or Gast Rearing, USDA-ARS, Starkville, MS). For tests of any given adjuvant, insects were from the same source. Larvae were reared, before and after virus treatment, on artificial diet (King and Hartley 1985).

We used collard, *Brassica oleracea* L. (*Acephala* group), cv. 'Vates,' as the host plant. Plants were grown in 10-cm diam pots, 2 to 4 plants per pot, in a greenhouse using a commercial potting medium (Pro Mix BX®, Premier Brands, Red Hill, PA). A temperature of 24 ± 3°C, photoperiod supplemented to 16:8 (L:D) h by low-pressure sodium vapor lamps, and weekly fertilization (Peters Professional 20-20-20®, Grace-Sierra, Milpitas, CA) were used. Plants were 5 to 6 wk old when used.

We obtained a sample of an aqueous suspension of AfMNPV (lot Af091295), labeled to contain 2.00×10^9 occlusion bodies (OB)/ml, from biosys (now Thermo Trilogy, Columbia, MD) on 22 April 1996.

UV source. The Blak Ray Model B 100 AP® lamp (UVP, Upland, CA) was used as the UV source. The lamp was mounted 38 cm above a flat surface. Treated leaves (see below) were placed on white paper on the perimeter of a circle, 18 cm diam, centered below the lamp. Halfway through the exposure period (see below), the leaves were turned over. On the perimeter of this circle, UV intensities, as measured with a UV light meter (model UVX®, UVP, San Gabriel, CA), were 179 and 1,408 $\mu W/cm^2$ at 310 (UV-B) and 365 (UV-A) nm, respectively. UV readings in the same room, 3 to 4 m from the lights, were 3 and 6 $\mu W/cm^2$ at the same wavelengths, respectively. For comparison, sunlight at midday in June at Beltsville, MD gave readings of 811 and 2820 $\mu W/cm^2$ at the same respective wavelengths. In tests in which two lamps were used, the lamps were spaced 1 m apart with a brown cardboard divider between them, so that areas illuminated would not overlap.

Bioassays. We cut collard leaves from the plants, placed them in water-pics (water-filled, rubber-capped tubes; Aquapic®, Syndicate sales, Kokomo, IN), and held them overnight at about 24°C. Most leaves wilted after being cut; only those that regained their turgor overnight were used. Leaves were dipped in suspensions of virus and adjuvants (see below), held upright in the water pics, and allowed to dry. They were then laid flat, still in the water-pics, under the lamps, and removed after prescribed exposure periods (see below). Bioassays were always set up after all leaves had been removed from UV exposure.

Each leaf was cut into four pieces, each 2 to 4 cm^2. Each piece was placed individually in a 5.5 cm-diam Petri dish with moist filter paper. Six late first-to very early second-instar beet armyworm larvae (reared on artificial diet) were placed in each dish. Dishes were sealed with Parafilm® (American National Can, Greenwich, CT) and held at 27°C for 48 h. Larvae were then placed individually in cells of plastic bioassay trays (Bio-BA-128©, C-D International, Pitman, NJ) filled with artificial diet and held at 27°C. Mortality was recorded 9 d later. Data from the four pieces from each leaf (24 larvae) were pooled for calculation of percentage mortality.

Treatments. The initial test was designed to provide baseline data on the degradation of the virus in our system. Based on previous tests (Farrar and Ridgway 1997), we selected a rate of AfMNPV of 1000 OB/µl. We expected this rate to cause about 90% mortality in the absence of degradation or enhancers. A wetting agent, Triton X-155® (Union Carbide, Danbury, CT), at 0.01%, and a spreader, Kinetic® (Setre Chemical Co., Memphis, TN), at 0.125% were added to all treatments in all tests. All leaves were dipped in a suspension of virus, except for controls, which were dipped in water with Triton and Kinetic only.

Six treated leaves were placed in a circle under one lamp. Two other treated

leaves, and one control leaf, were kept unexposed. One leaf was removed from under the lamp at each hour from 1 to 6 h. Each exposed leaf was turned over halfway through its exposure period. The test was replicated six times.

We tested varying concentrations of a sodium lignin sulfonate, Lignosite AN® (Georgia Pacific, Bellingham, WA). Because this material was not expected to have any effects other than as a UV protectant, varying concentrations of it were not tested in the absence of UV. Based on results of the test described above (see Results), we selected an exposure time of 5 h, with the leaves turned over at 2.5 h. The same rate of virus, 1000 OB/µl, was used. Suspensions of virus with 0, 0.5, 1, 2, 5, and 10% (wt/vol) Lignosite AN were prepared. For each replicate, one leaf was dipped in each suspension, plus a control leaf (10% Lignosite AN only). All leaves, except the control and a second leaf treated with virus only, were exposed to the lamp. Two replicates were done together, each under a separate lamp, and the test was repeated four times over time (eight replicates total). Mean and standard error of mortality on the treatment with virus only and not exposed to UV were calculated for comparative purposes, but this treatment was not included in the statistical analysis.

Varying concentrations of two fluorescent brighteners, Blankophor® BBH (Burlington Chemical, Burlington, NC) and Blankophor® HRS (Bayer, Rock Hill, SC), were tested with virus, both on foliage exposed to UV and on foliage not exposed to UV. Because we expected these materials to enhance the virus, a lower rate of virus, 200 OB/µl, was used; we expected this rate to kill 25 to 50% of larvae in the absence of UV or enhancer. Rates of enhancer of 0.0, 0.05, 0.1, 0.25, and 0.5% (wt/vol.), plus a control (0.5% fluorescent brightener) were included. Two leaves were dipped in each suspension. One leaf of each treatment was exposed to UV; the other, held unexposed. The test was replicated as before, except with a total of ten replicates. Because of time limitations, the two brighteners were tested at different times; therefore, we made no statistical comparisons between the two fluorescent brighteners.

A nutrient-based feeding stimulant, Coax, was tested in a manner similar to that for the fluorescent brightener. Coax is a liquid flowable material containing 65% water (Farrar and Ridgway 1994); this value was used to calculate rates in dry weight per unit volume. Rates of Coax of 0.0, 0.5, 1.0, 2.5, and 5.0% (dry wt/vol) with the virus at 200 OB/µl were included. The procedure was otherwise similar to that used for the fluorescent brighteners.

Data analysis. For each test, we calculated percentage mortality, normalized it by arcsine $\sqrt{\%}$ transformation, and used the PROC MIXED procedure of SAS (SAS Institute 1992) to perform analyses of variance (ANOVA). Treatments were included as fixed effects; replication, as a random effect. In the test of exposure times, linear and quadratic effects of exposure time on mortality were evaluated. In other tests, concentration of adjuvant, transformed logarithmically, was an independent variable. In tests with exposed and unexposed treatments, exposure, concentration of adjuvant, and their interaction were also independent variables. Where a significant interaction was found, data were also analyzed separately.

Results

Mortality of beet armyworm larvae on foliage initially treated with 1000 OB/µl of AfMNPV declined from 81.3% on foliage not exposed to UV to 24.4% after 5 h of exposure (Fig. 1). The linear effect of exposure time on mortality was significant (F = 15.49; df = 1, 40; P = 0.0003). Mortality did not decline further between 5 and 6 h of

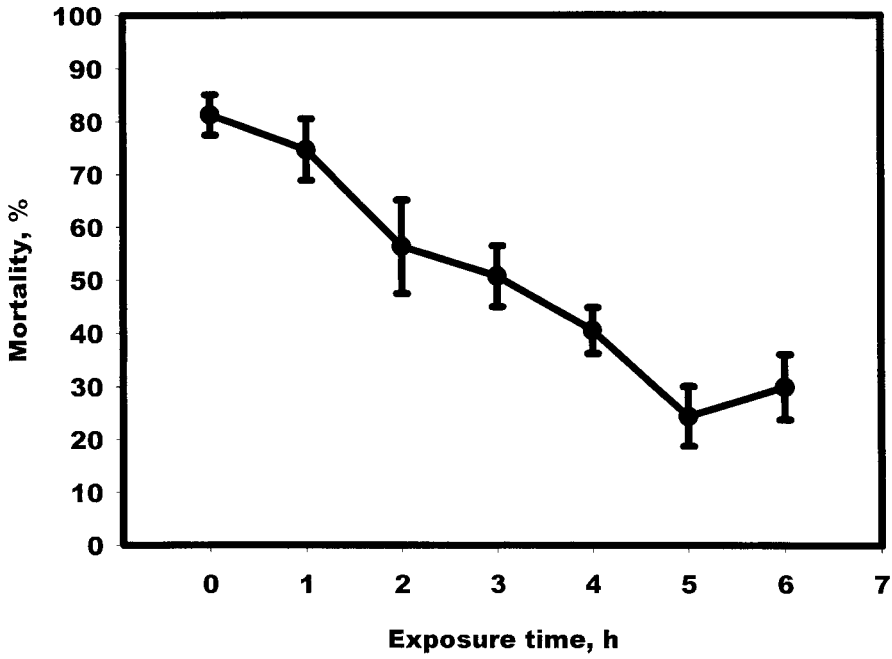

Fig. 1. Mean (±SE) mortality of beet armyworm larvae nine days after feeding as second instars on collard foliage dipped in a suspension of 1000 OB/µl AfMNPV and exposed to UV for varying intervals.

exposure, but this leveling off did not produce a significant quadratic effect of exposure time ($F = 1.75$; df = 1, 40; $P = 0.1936$), so within the 6-h exposure period, results indicate a linear effect of exposure on viral activity. No control mortality was seen in this or any other test in this study, so no adjustments were needed. Based on these results, we selected a 5-h exposure time, with the leaves turned over at 2.5 h, for subsequent tests.

Mortality of larvae on foliage initially treated with 1000 OB/µl of AfMNPV without Lignosite AN was 88.3% and 12.1% on unexposed and exposed foliage, respectively. On exposed foliage, mortality increased with the addition of Lignosite AN, up to 52.1% with 10% Lignosite AN ($F = 25.83$; df = 1, 28; $P = 0.0001$) (Fig. 2).

In the test of Blankophor BBH, mortality of larvae on foliage initially treated with 200 OB/µl of AfMNPV without the fluorescent brightener was 39.9% and 5.6% on unexposed and exposed foliage, respectively. Mortality of larvae increased with increasing rates of Blankophor BBH both in the presence of UV and in its absence, with the greatest increase being in the presence of UV (Fig. 3). Mortality was significantly affected by UV ($F = 70.89$; df = 1, 87; $P = 0.0001$), rate of Blankophor BBH ($F = 39.31$; df = 1, 87; $P = 0.0001$), and the interaction thereof ($F = 5.57$; df = 1, 87; $P = 0.0205$). Analyzed separately, the effect of rate of Blankophor BBH was significant both in the absence of UV ($F = 56.60$; df = 1, 41; $P = 0.0001$) and in its presence ($F = 76.62$; df = 1, 41; $P = 0.0001$).

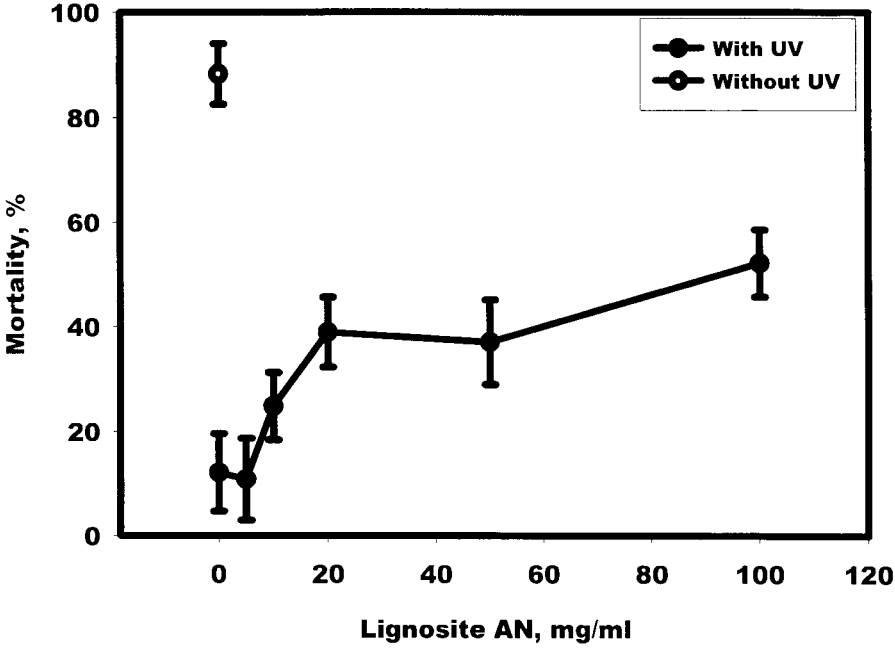

Fig. 2. Mean (±SE) mortality of beet armyworm larvae nine days after feeding as second instars on collard foliage dipped in a suspension of 1000 OB/µl AfMNPV with varying concentrations of Lignosite AN and exposed to UV for 5 h, and on foliage treated with virus only and not exposed to UV.

Results for Blankophor HRS were similar to those for Blankophor BBH, except that the increase in mortality in the presence of UV was similar to that in the absence of UV (Fig. 4). Mortality was thus affected by UV ($F = 49.80$; df = 1, 91; $P = 0.0001$) and rate of Blankophor HRS ($F = 35.34$; df = 1, 91; $P = 0.0001$), but not by the interaction thereof ($F = 0.03$; df = 1, 91; $P = 0.8624$).

In the test of Coax, mortality of larvae on foliage initially treated with 200 OB/µl of AfMNPV without Coax was 50.2% and 7.7% on unexposed and exposed foliage, respectively. With the addition of Coax, larval mortality increased primarily in the presence of UV (Fig. 5). Mortality was significantly affected by UV ($F = 238.12$; df = 1, 89; $P = 0.0001$) and the interaction of UV and rate of Coax ($F = 14.07$; df = 1, 89; $P = 0.0003$), but not by rate of Coax itself ($F = 1.14$; df = 1, 89; $P = 0.2892$). Analyzed separately, the effect of rate of Coax was significant in the presence of UV ($F = 49.07$; df = 1, 41; $P = 0.0001$), and nonsignificant in the absence of UV ($F = 1.03$; df = 1, 43; $P = 0.3154$).

Discussion

In our system, Lignosite AN gave good UV protection for AfMNPV, but fairly high rates were required to achieve this protection. The data indicate that 2% (wt/vol) or

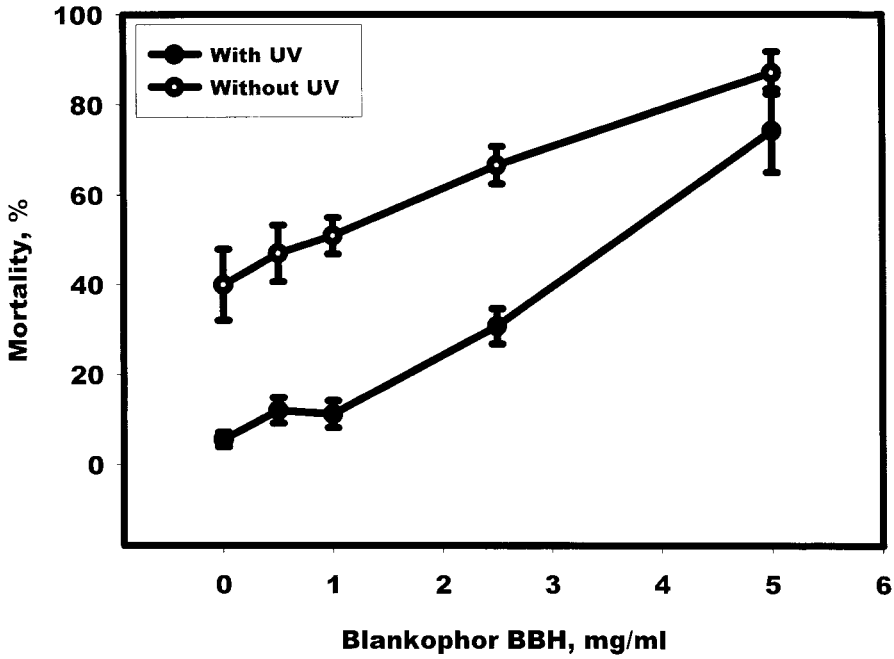

Fig. 3. Mean (±SE) mortality of beet armyworm larvae nine days after feeding as second instars on collard foliage dipped in a suspension of 200 OB/µl AfMNPV with varying concentrations of Blankophor BBH with or without exposure to UV for 5 h.

greater would probably be necessary. While rates of 6% Lignosite AN have been used in sprays of LdMNPV against the gypsy moth (Reardon and Podgwaite 1994), these treatments are low volume aerial applications. On crops such as vegetables, where higher spray volumes are frequently used, these rates may be impractical. For example, at a volume of 20 gal/acre (187 liters/ha), 2% would equate to 3.2 lb. Lignosite AN/acre (3.59 kg/ha).

Blankophor BBH appeared to act as both an enhancer and a UV protectant. The significant effect of rate of Blankophor BBH in the absence of UV indicates an effect as an enhancer. The greater effect of Blankophor BBH in the presence of UV, as indicated by the significant interaction of UV and rate of Blankophor BBH, is consistent with activity as a UV protectant as well as an enhancer. These results are consistent with previous reports of activity of fluorescent brighteners as UV protectants (Shapiro 1992), as enhancers (Shapiro and Robertson 1992, Shapiro et al. 1992), and as both an enhancer and a UV protectant (Dougherty et al. 1996). Vail et al. (1996) reported a similar material to enhance AfMNPV against the beet armyworm on artificial diet. Rates required to achieve increased mortality in the presence of UV were lower than those required for Lignosite AN. However, Blankophor BBH at 1% of a dip was a moderate feeding deterrent, at least to larvae of the gypsy moth (Farrar et al. 1995) and corn earworm (R.R.F., unpubl. data), and a related material was

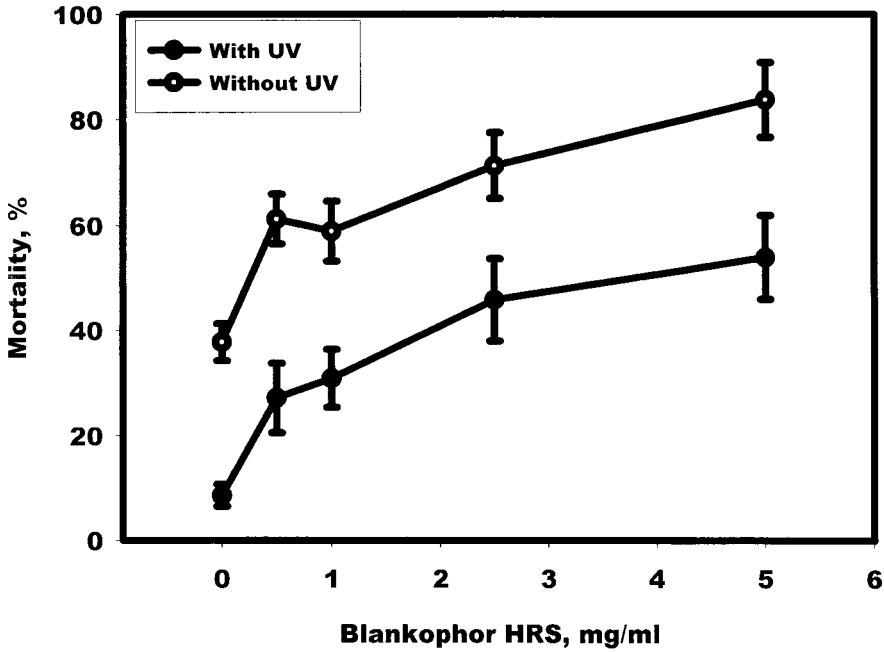

Fig. 4. Mean (±SE) mortality of beet armyworm larvae nine days after feeding as second instars on collard foliage dipped in a suspension of 200 OB/µl AfMNPV with varying concentrations of Blankophor HRS with or without exposure to UV for 5 h.

deterrent to the beet armyworm (Vail et al. 1996). Farrar et al. (1999) found indications that against beet armyworm larvae on whole plants, Blankophor BBH was less effective as an enhancer than against larvae on single leaves, possibly because of feeding deterrence. In the present study, confinement of larvae on small pieces of leaves would have limited the opportunity for larvae to avoid the enhancer.

Blankophor HRS, in contrast, appeared to act primarily, if not entirely, as an enhancer rather than as a UV protectant. The significant effect of rate of Blankophor HRS on mortality indicates enhancement. The lack of a significant interaction between rate of enhancer and UV indicates no significant activity as a UV protectant; Blankophor HRS had similar effects in the presence and absence of UV. Because we tested Blankophor BBH and Blankophor HRS at different times, direct statistical comparisons of the two materials should not be made. However, at least in the absence of UV, no great differences between the two enhancers are apparent. The apparent difference in activity as UV protectants is not surprising; Shapiro (1992) tested 23 fluorescent brighteners (not including Blankophor BBH or HRS, however) as UV protectants for LdMNPV and obtained rates of protection of the virus ranging from <10% to >90%. The effect of Blankophor HRS on the feeding behavior of beet armyworm larvae is unknown, but it does not appear to deter feeding by corn earworm larvae (R.R.F., unpubl. data).

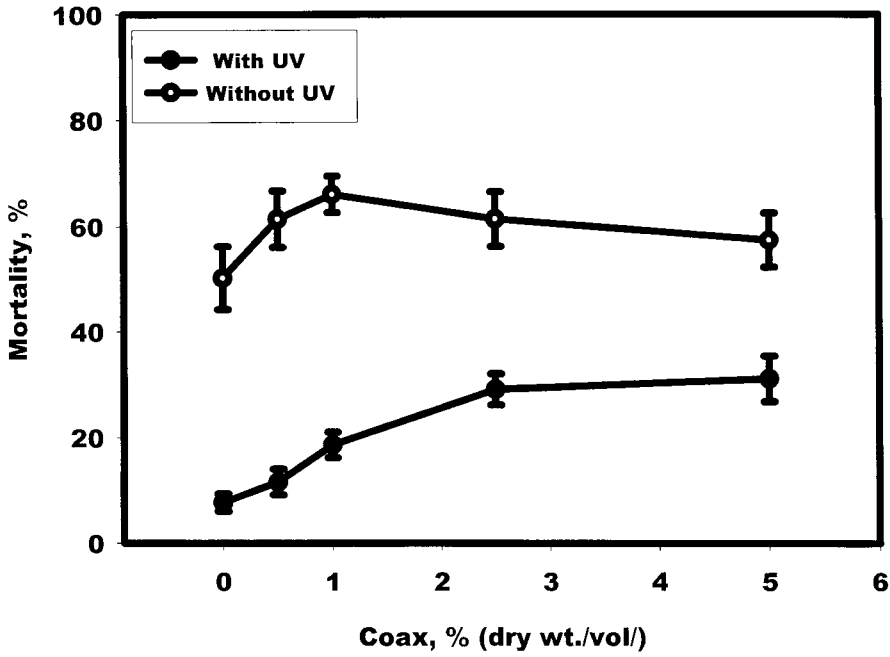

Fig. 5. Mean (±SE) mortality of beet armyworm larvae nine days after feeding as second instars on collard foliage dipped in a suspension of 200 OB/µl AfMNPV with varying concentrations of Coax with or without exposure to UV for 5 h.

In this study, Coax appeared to act primarily, if not entirely, as a UV protectant rather than as a feeding stimulant. The lack of a significant effect of Coax on mortality in the absence of UV indicates a lack of activity as a feeding stimulant. The significant effect of Coax in the presence of UV, and the significant interaction of rate of Coax and UV, are consistent with activity as a UV protectant. These results are apparently in contrast with previous studies showing similar feeding stimulants to increase the efficacy of the NPV of the corn earworm (Andrews et al. 1975, Bell and Kanavel 1977, 1978, Bell and Romine 1980, Luttrell et al. 1983). Ridgway and Farrar (1999) found that Coax applied to whole bean plants acted as both a feeding stimulant and as a UV protectant for *Bacillus thuringiensis* Berliner against the corn earworm. In the present study, however, as with Blankophor BBH, confinement of larvae on small pieces of leaves may have limited the effects of the adjuvant on feeding behavior. Farrar and Ridgway (1995a) obtained increased mortality of several insects using a similar bioassay for feeding stimulants with *B. thuringiensis* which is known to be a feeding deterrent to many insects (reviewed by Farrar and Ridgway 1995b). The significant effect of Coax as a UV protectant is consistent with reports of Shapiro et al. (1983) and Martignoni and Iwai (1985). However, fairly high rates, 1 to 5% dry wt/vol, which equate to about 3 to 15% vol/vol, were required to achieve this effect.

Results of the present study indicate that all of the adjuvants included have at least the technical potential to improve the efficacy of NPVs. However, the bulk and/or cost

of the amount of material required may limit the practical utility of some or all of these materials. For example, as of December 1999, the wholesale prices of Blankophor BBH and Blankophor HRS were $10.65 and $7.25 per pound ($23.43 and $15.95/kg), respectively. At 1% of 20 gal/acre (187 liters/ha), this would equate to $11.62/acre ($28.70/ha) for Blankophor HRS. As of January 2000, the suggested retail price of Coax was $20.00 per gallon ($5.28/liter). At 3% by volume of 20 gal/acre, the addition of Coax would cost $12.00/acre ($29.64/ha). Lignosite AN is by far the least expensive material that we tested; as of January 2000, the wholesale price of Lignosite AN was $345.00 per ton ($0.17/lb, $0.38/kg). The addition of 2% Lignosite AN to 20 gal/acre would thus cost $0.54/acre ($1.36/ha).

Fluorescent brighteners have been shown to render insects susceptible to viruses to which they are not normally susceptible (Shapiro and Dougherty 1994). Therefore, these materials have the potential to allow viruses applied against a pest to also infect other species. However, fluorescent brighteners have not been shown to allow NPVs of lepidopterans to infect insects in other orders. Usually, no caterpillar found on a crop plant would be considered a beneficial insect, though it may not be numerous enough to be considered a pest. In addition, fluorescent brighteners do not alter the NPV produced in an infected host (Shapiro and Dougherty 1993), so if this virus persists in the environment following the death of infected hosts, it should be no more infectious to nontarget insects than is NPV applied without fluorescent brighteners. Thus, infection of nontarget lepidopterans on a crop plant by NPVs applied with fluorescent brighteners against a pest species should not cause significant problems. How more desirable species feeding on weeds in the crop might be affected is unknown, however. Fluorescent brighteners should also pose little or no risk to humans and the environment. These materials have long been used in laundry detergents and are generally regarded as safe (Carter 1973).

Based on results of these and previous tests, Blankophor BBH and Coax may have the greatest potential of the materials included to be of practical use, at least with AfMNPV against the beet armyworm on collard. However, at least as UV protectants, these materials should have similar effects with other NPVs and possibly other microbial control agents. Testing on whole plants and in the field will be needed to fully evaluate these materials as adjuvants for NPVs.

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References Cited

Adams, J. R. and J. T. McClintock. 1991. Baculoviridae. Nuclear polyhedrosis viruses. Part 1. Nuclear polyhedrosis viruses of insects, Pp. 87-204. *In* J. R. Adams and J. R. Bonami [eds.], Atlas of Invertebrate Viruses. CRC Press, Boca Raton, FL.

Andrews, G. L., F. A. Harris, P. P. Sikorowski and R. E. McLaughlin. 1975. Evaluation of *Heliothis* nuclear polyhedrosis virus in a cottonseed oil bait for control of *Heliothis virescens* and *H. zea* on cotton. J. Econ. Entomol. 68: 87-90.

1977. Field tests of a nuclear polyhedrosis virus in a bait formulation for control of pink bollworms and *Heliothis* spp. on cotton in Arizona. J. Econ. Entomol. 70: 625-629.

Bell, M. R. and R. F. Knavel. 1978. Tobacco budworm: development of a spray adjuvant to increase effectiveness of a nuclear polyhedrosis virus. J. Econ. Entomol. 71: 350-352.

Bell, M. R. and C. L. Romine. 1980. Tobacco budworm field evaluation of microbial control in cotton using *Bacillus thuringiensis* and a nuclear polyhedrosis virus with a feeding adjuvant. J. Econ. Entomol. 73: 427-430.

Bonning, B. C. and B. D. Hammock. 1996. Development of recombinant baculoviruses for insect control. Annu. Rev. Entomol. 41: 191-210.

Burges, H. D. and K. A. Jones. 1998. Formulation of bacteria, viruses, and protozoa to control insects, Pp. 33-127. *In* H. D. Burges [ed.], Formulation of microbial biopesticides: beneficial microorganisms, nematodes, and seed treatments. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Carter, R. O. 1973. Safety of fluorescent whitening agents in detergents, Pp. 113-122. *In* Fluorescent Whitening Agents. Proc. Symp. Royal Inst. Technol., Stockholm, Sweden, April 11, 1973. MVC-Rep. 2. Cent. Environ. Sci., Royal Inst. Technol., Stockholm.

Chen, C.-J., D. J. Elise, and S. M. Them. 1996. Physical map of *Anagrapha falcifera* multinucleocapsid nuclear polyhedrosis virus. J. Gen. Virol. 77: 167-171.

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

Farrar, R. R., Jr. and R. L. Ridgway. 1994. Comparative studies of the effects of nutrient-based phagostimulants on six lepidopterous insect pests. J. Econ. Entomol. 87: 44-52.

195a. Enhancement of activity of *Bacillus thuringiensis* Berliner against four lepidopterous insect pasts by nutrient-based phagostimulants. J. Entomol. Sci. 30: 29-42.

1995b. Feeding behavior of gypsy moth (Lepidoptera: Lymantriidae) larvae on artificial diet containing *Bacillus thuringiensis*. Environ. Entomol. 24: 755-761.

1997. The celery looper (Lepidoptera: Noctuidae) baculovirus: potency and enhancement by Blankophor BBH against 3 lepidopteran species. Environ. Entomol. 26: 1461-1469.

Farrar, R. R., Jr., R. L. Ridgway, S. P. Cook, K. W. Thorpe and R. E. Webb. 1995. Spray adjuvants for the nuclear polyhedrosis virus of the gypsy moth (Lepidoptera: Lymantriidae): effects on viral potency and insect feeding behavior. J. Entomol. Sci. 30: 417-428.

Farrar, R. R., Jr., R. L. Ridgway and G. P. Dively. 1999. Activity and persistence of the nuclear polyhedrosis virus of the celery looper (Lepidoptera: Noctuidae) with a feeding stimulant and a stilbene-derived enhancer. J. Entomol. Sci. 34: 369-380.

Granados, R. R. and B. A. Federici [eds.]. 1986. The Biology of Baculoviruses. vol. 2. Practical Applications for Insect Control. CRC Press, Boca Raton, FL.

Gröner, A. 1986. Specificity and safety of baculoviruses, Pp. 177-202. *In* R. R. Granados and B. A. Federici [eds.], The Biology of Baculoviruses. vol. 1. Biological Properties and Molecular Biology. CRC Press, Boca Raton, FL.

Hostetter, D. L. and B. Puttler. 1991. A new broad spectrum nuclear polyhedrosis virus isolated from a celery looper, *Anagrapha falcifera* (Kirby), (Lepidoptera; Noctuidae). Environ. Entomol. 20: 1480-1488.

Jaques, R. P. 1977. Stability of entomopathogenic viruses, Pp. 99-116. *In* C. M. Ignoffo and D. L. Hostetter [eds.], Environmental Stability of Microbial Insecticides. Misc. Publ. Entomol. Soc. Am. 10.

1985. Stability of insect viruses in the environment, Pp. 285-360. *In* K. Maramorosch and K. E. Sherman [eds.], Viral Insecticides for Biological Control. Academic Press, Orlando, FL.

Jones, K. A., A. J. Cherry, D. Grzywacz and H. D. Burges. 1997. Formulation: is it an excuse for poor application? Pp. 173-180. *In* Proceedings, British Crop Protection Council, Microbial Insecticides: Novelty or Necessity? 16-18 April 1997, Univ. Warwick, Coventry, UK. No. 68.

King, E. G. and G. G. Hartley. 1985. *Heliothis virescens,* Pp. 323-328. *In* P. Singh and R. F. Moore [eds.], Handbook of insect rearing, vol. II. Elsevier, NY.

Luttrell, R. G., W. C. Yearian and S. Y. Young. 1983. Effect of spray adjuvants on *Heliothis zea* (Lepidoptera: Noctuidae) nuclear polyhedrosis virus efficacy. J. Econ. Entomol. 76: 162-167.

Maramorosch, K. and K. E. Sherman [eds.]. 1985. Viral Insecticides for Biological Control. Academic Press, Orlando, FL.

Martignoni, M. E. and P. J. Iwai. 1985. Laboratory evaluation of new ultraviolet absorbers for protection of Douglas-fir tussock moth (Lepidoptera: Lymantriidae) *Baculovirus.* J. Econ. Entomol. 78: 982-987.

Reardon, R. C. and J. D. Podgwaite. 1994. Summary of efficacy evaluations using aerially applied Gypchek® against gypsy moth in the U.S.A. J. Environ. Sci. Health B29: 739-756.

Ridgway, R. L. and R. R. Farrar, Jr. 1999. Mortality of two lepidopterans in response to selected commercial formulations of *Bacillus thuringiensis* Berliner. J. Entomol. Sci. 34: 273-285.

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

1995. Radiation protection and activity enhancement of viruses, Pp. 153-164. *In* F. R. Hall and J. W. Barry [eds.], Biorational Pest Control Agents: Formulation and Delivery. Am. Chem. Soc. Symp. Ser. 595, Am. Chem. Soc., Washington, DC.

Shapiro, M. and E. M. Dougherty. 1993. The use of fluorescent brighteners as activity enhancers for insect pathogenic viruses, Pp. 40-46. *In* R. D. Lumsden and J. L. Vaughn [eds.], Pest Management: Biologically Based Technologies. Proc. Beltsville Symp. XVIII, Agric. Res. Serv., U.S. Dept. Agric., Beltsville, MD, May 2-6, 1993. Am. Chem. Soc., Washington, DC.

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

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

Shapiro, M., P. P. Agin and R. A. Bell. 1983. Ultraviolet protectants of the gypsy moth (Lepidoptera: Lymantriidae) nucleopolyhedrosis virus. Environ. Entomol. 12: 982-985.

Shapiro, M., J. J. Hamm and E. Dougherty. 1992. Compositions and methods for biocontrol using fluorescent enhancers. U.S. Patent 5,124,149.

SAS Institute. 1992. Technical Report P-229, SAS/STAT Software Changes and Enhancements, Release 6.07. Cary, NC.

Vail, P. V., D. F. Hoffman, D. A. Streett, J. S. Manning and J. S. Tebbets. 1993. Infectivity of a nuclear polyhedrosis virus isolated from *Anagrapha falcifera* (Lepidoptera: Noctuidae) against production and postharvest pests and homologous cell lines. Environ. Entomol. 22: 1140-1145.

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

Washburn, J., B. A. Kirkpatrick, E. Haas-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.