Activity and Persistence of the Nuclear Polyhedrosis Virus of the Celery Looper (Lepidoptera: Noctuidae) with A Feeding Stimulant and a Stilbene-Derived Enhancer[1,2]

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Abstract A nutrient-based feeding stimulant and a diaminostilbene disulfonic acid-derived enhancer (fluorescent brightener, Blankophor BBH®; Burlington Chemical, Burlington, NC) were evaluated as adjuvants for the nuclear polyhedrosis virus of the celery looper, *Anagrapha falcifera* (Kirby) (AfMNPV), against the beet armyworm, *Spodoptera exigua* (Hübner), on collard, *Brassica oleracea* L. (*Acephala* group), cv. 'Vates'. Tests included holding larvae on sprayed potted plants in the laboratory and bioassays of foliage collected from sprayed plants in the field. The feeding stimulant increased virus-caused mortality in all tests. The enhancer increased virus-caused mortality in the bioassays of field-collected foliage but not in the test of potted plants. Treatments with both materials maintained the greatest levels of activity over time in the field. At the concentration tested on potted plants (up to 0.5% of the spray), the enhancer may have acted as a feeding deterrent. Therefore, on the whole plants, where the larvae were free to move around, effects on feeding behavior may have reduced the effectiveness of the enhancer. In the bioassay of field-collected foliage, larvae were confined on small pieces of foliage and, thus, did not have the option of moving away from the enhancer. Because the enhancer and the feeding stimulant have both been previously reported to also protect viruses from degradation by ultraviolet light, exposure to sunlight in the field could also have contributed to differences in larval mortality.

Key Words *Spodoptera exigua,* nuclear polyhedrosis virus, feeding stimulant, stilbene, enhancer.

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 two to three decades, much research has been conducted on NPVs with the goal of utilizing them as pest control agents (Entwistle and Evans 1985, Granados and Federici 1986, Adams and McClintock 1991). Recently, the development of NPVs that have been genetically engineered to kill insects faster (Bonning and Hammock 1996) has prompted renewed interest in NPVs as biological control agents. While unformulated NPVs and other microbial control agents applied to crops have provided control,

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they are usually formulated with various adjuvants to improve stability, handling, persistence, and activity (Jones et al. 1997, Burges and Jones 1998).

Because NPVs must be ingested by the target pest, much work has been done on adjuvants that act as feeding stimulants or phagostimulants, thereby increasing the amount of virus ingested. Nutrient-based phagostimulatory spray adjuvants have been shown to improve the efficacy of NPVs, *Bacillus thuringiensis* Berliner, and some chemical insecticides, though positive results were not obtained in all cases (e.g., Bell and Romine 1980, Farrar and Ridgway 1994). Several spray adjuvants of this type, based primarily on vegetable flours, oils, and sugars, have been marketed for use with microbial insecticides.

NPVs are susceptible to degradation by ultraviolet (UV) light, so materials that protect NPVs from UV have been studied extensively (Shapiro 1995). At least one nutrient-based feeding stimulatory spray adjuvant, Coax® (AgroSolutions, San Marcos, CA), also acts as a UV screen (Shapiro et al. 1983). Another class of materials that act as UV protectants for NPVs is diaminostilbene disulfonic acid-based fluorescent brighteners or optical brighteners (Shapiro 1992). 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, Farrar and Ridgway 1997, Hamm 1999). 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 with the addition of a fluorescent brightener (Shapiro and Robertson 1992, Shapiro et al. 1992). Washburn et al. (1998) reported that fluorescent brighteners may act by inhibiting the sloughing of virus-infected midgut epithelial cells.

Fluorescent brighteners have been tested in the field with LdMNPV, yielding positive results (Webb et al. 1994a, b). However, published data on these enhancers on whole plants against pests of field or horticultural crops are limited. Hamm et al. (1994) obtained higher levels of mortality of larvae of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), by the addition of an enhancer to sprays of the fall armyworm NPV applied to whorl-stage corn, *Zea mays* (L.), in the field. Vail et al. (1993a) tested several NPVs with and without an enhancer and a feeding stimulant against the tobacco budworm, *Heliothis virescens* (F.), and cotton bollworm (corn earworm), *Helicoverpa zea* (Boddie), on cotton, *Gossypium hirsutum* L., in three localities. They obtained increased activity with both the enhancer and feeding stimulant in some tests but found no differences in other tests.

Nutrient-based feeding stimulants increase mortality of insects due to NPVs (Farrar and Ridgway 1994). The use of fluorescent brighteners, however, may increase the need for feeding stimulants because at least some brighteners can, at some concentrations, be feeding deterrents to some insects. Farrar et al. (1995) found that a brightener, Blankophor BBH® (Burlington Chemical, Burlington, NC), applied to lettuce, *Lactuca sativa* L., at a concentration of 1% (w/v) of a dip, acted as a moderate feeding deterrent to gypsy moth larvae, but that the addition of a feeding stimulant, molasses, at least partially overcame this deterrence. Vail et al. (1996) reported that a similar brightener, Tinopal LPW® (Sigma, St. Louis, MO), applied to the surface of artificial diet at a concentration of 1% (w/v) of a liquid virus application deterred feeding by larvae of the corn earworm, tobacco budworm, beet armyworm (*Spodoptera exigua* [Hübner]), and cabbage looper (*Trichoplusia ni* [Hübner]). Recently, we found rates of *H. zea* larval feeding to be reduced by Blankophor BBH at 0.5 and 1.0%

(w/v) of a dip for bean leaf disks, but feeding was not affected by several similar compounds (R. R. F., unpubl. data).

The studies reported herein were undertaken to examine further the potential utility of fluorescent brighteners and feeding stimulants as adjuvants for NPVs against pests of field and horticultural crops. Tests were conducted on potted plants to measure increases in virus-caused mortality, and in field plots to measure effects on persistence (here defined as maintenance of activity of the treatment over time). These studies also were intended to evaluate further the NPV of the celery looper, *Anagrapha falcifera* (Kirby) (AfMNPV). This NPV is of interest as a biological control agent because it has a wider host range than most NPVs, being infectious to some 31 species of Lepidoptera in 10 families (Hostetter and Puttler 1991, Vail et al. 1993b). While AfMNPV is infectious to a similar number and many of the same species as the NPV of the alfalfa looper, *Autographa californica* (Speyer), (AcMNPV) (Gröner 1986, Adams and McClintock 1991), these viruses differ in potency against some insects (Hostetter and Puttler 1991).

The beet armyworm (feeding on collard, *Brassica oleracea* L., *Acephala* group), was chosen as the test insect because it is moderately susceptible to AfMNPV, and the activity of this virus can be enhanced by Blankophor BBH (Farrar and Ridgway 1997); enhancement should, thus, be more readily detectable than in an insect against which AfMNPV is highly potent. While the homologous NPV of the beet armyworm (SeMNPV) may be more potent against this insect than is AfMNPV(R. R. F., unpubl. data), AfMNPV is also active against other caterpillars that may be found with the beet armyworm on cole crops (e.g., cabbage loopers), or on tomato (e.g., corn earworm) (Hostetter and Puttler 1991).

Blankophor BBH was chosen as the enhancer based on previous results with LdMNPV (Webb et al. 1994a, b, Farrar et al. 1995) and AfMNPV (Farrar and Ridgway 1997).

Materials and Methods

Potted plant tests. All insects used in these studies were obtained from cultures at the Insect Biology and Population Management Research Laboratory (USDA-ARS, Tifton, GA). Larvae were reared, before and after virus treatment, on the artificial diet described by King and Hartley (1985).

The host plant was collard (cv. 'Vates'). Plants were grown for 5 to 6 wk in 10-cm diameter pots, 2 to 4 plants per pot, in a greenhouse using a commercial soil mix (Pro MixBX®, 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).

A sample of freeze-dried AfMNPV (lot Af052595), labeled to contain 1.97×10^{10} occlusion bodies (OB)/g, was provided by biosys (Columbia, MD; the assets of biosys, including this virus, were later acquired by Thermo Trilogy, Columbia, MD).

Treatments were applied to plants in a cylindrical spray chamber (45.7 cm in diam, 86 cm in height) made of clear acrylic plastic (6 mm thick). The cylinder was angled at 45° from vertical at a point about 40 cm from the bottom. A single Air Atomizing® nozzle (model SU 1, Tee Jet, Dillsburg, PA) was mounted in the center of the top of the cylinder (also at a 45° angle from vertical). The nozzle body was fitted with a venturi tube (3 mm outside diam copper tube) for uptake of premeasured aliquots of liquid and delivery into the nozzle. Spray was driven by CO_2 at a pressure of 4.22

kg/cm^2. A turntable (15 rpm) located at the bottom of the cylinder held one pot of plants. Each pot of collards was placed on an inverted empty pot (18 cm tall) on the turntable to place it directly in front of the nozzle. The cylinder was mounted on a frame and counterweighted to allow it to be easily raised and lowered to change plants.

The area of the base of the cylinder was 1,641 cm^2, or 1.641×10^{-5} ha. This value was used to calculate rates of spray approximately equivalent to those applied in the field. A volume of 3.07 ml/1,641 cm^2 was, thus, equivalent to 187 liters/ha. This volume was used to treat each pot of collards in all tests. At a pressure of 4.22 kg/cm^2, 3.07 ml could be applied in about 4 sec, during which time the pot rotated once.

Plants were allowed to air dry, and each pot was then enclosed in an organdy sleeve cage (15 cm diam × 61 cm long). Twenty-five late first-instar (showing head capsule slippage) to very early second-instar beet armyworms (reared on artificial diet) were placed on the plants in each pot. Pots were held in the laboratory at 24 ± 3°C for 48 h. Larvae were then collected and placed individually in cells of plastic bioassay trays (Bio-BA-128©, C-D International, Pitman, NJ) filled with artificial diet. Cells were covered with ventilated clear plastic covers. Larvae were held at 27°C, and mortality was recorded 8 d later. Each pot (25 larvae) was treated as the experimental unit.

The initial test was designed to determine the quantity of virus needed to obtain moderate levels of mortality that could be used to test the enhancer and feeding stimulant. All treatments were applied in distilled water. Rates of AfMNPV of 100 (3.07×10^5), 500 (1.54×10^6), 1,000 (3.07×10^6), 3,000 (9.21×10^6), and 6,000 (1.84×10^7) OB/µl (OB per pot), plus a control (water) were included. Each treatment in this and subsequent tests also included a wetting agent, Triton X-155® (Union Carbide, Danbury, CT), at 0.01% (v/v), and a sticker, Bond® (Loveland Industries, Greeley, CO), at 0.156% (v/v; 292 ml/ha). Three pots were treated with each rate of virus, and the test was replicated three times (9 pots and 225 larvae per treatment). Virus rates were logarithmically transformed. Percentage mortality was calculated for each pot, normalized by arcsine transformation, and analyzed by probit analysis (PROC PROBIT; SAS Institute 1988).

To test the enhancer, AfMNPV was applied at the LC_{50} rate as determined in the previous test: 1.385×10^3 OB/µl (4.25×10^6 OB/pot, $\approx 2.59 \times 10^{11}$ OB/ha). Treatments included 0, 0.125, 0.25, 0.375, and 0.5% Blankophor BBH (w/v), with a control of 0.5% Blankophor BBH only. Four pots were treated with each treatment, and the test was replicated four times (16 pots and 400 larvae per treatment). Data were adjusted for control mortality using Abbott's (1925) formula and analyzed by analysis of variance (ANOVA) with enhancer rate as an independent variable (PROC GLM; SAS Institute 1988).

A nutrient-based feeding stimulant similar to the commercial adjuvants reviewed by Farrar and Ridgway (1994) was prepared. It consisted of 20% Pharmamedia® flour (a cottonseed flour; Traders Protein, Memphis, TN), 5% cottonseed oil (Cotton, Inc., Raleigh, NC), 9% sucrose (ICN, Costa Mesa, CA), 4% emulsifier (Sponto Organic Emulsifier®, Whitco, Houston, TX), and 62% water. It was prepared by blending the emulsifier and 80% of the total oil in a small blender, blending the remaining ingredients separately, then blending the oil/emulsifier mixture into the other mixture. Treatments included 0, 0.3125, 0.625, 0.9375, and 1.25% feeding stimulant (dry w/v, where "dry" ingredients included oil and emulsifier; 0, 0.822; 1.645, 2.467, and 3.29% v/v), plus a control of 1.25% feeding stimulant only. The rate of AfMNPV was 1.385

$\times 10^3$ OB/μl (4.25×10^6 OB/pot). This test was replicated and analyzed as described previously.

The feeding stimulant and enhancer were tested in combination with rates based on the previously described tests. Treatments included AfMNPV alone (1.385×10^3 OB/μl; 4.25×10^6 OB/pot), AfMNPV with enhancer (0.5% w/v), AfMNPV with feeding stimulant (1.25% dry w/v), and AfMNPV with both enhancer and feeding stimulant at these rates, and a control with both enhancer and feeding stimulant but no virus. Five pots were treated with each treatment, and the test was replicated three times (15 pots and 375 larvae per treatment). Data were analyzed by factorial ANOVA (PROC GLM; SAS Institute 1988) for effects of enhancer, feeding stimulant, and the interaction.

Field persistence tests. A field of collards (cv. Vates) was planted by direct seeding in 76.2 cm (30 in) rows at the Wye Research and Education Center (Queenstown, MD) on 24 Aug 1995. It was divided into 24 plots (four blocks of six plots each) each 3.66 m long by two rows wide with two border rows between plots. The field was irrigated with overhead sprinklers as needed prior to the test. It was not irrigated during the test.

On 15 Apr 1996, a field of collards was planted as before, except that transplants were used instead of direct seeding. This field was divided into 20 plots (four blocks of five plots) each 3.05 m long by four rows wide with one border row between plots.

Plots were treated with a CO_2-backpack sprayer (model KQ-25, Weed Systems, Inc., Keystone Heights, FL) equipped with a boom with three flat fan nozzles (Tee Jet 1102®, Dillsburg, PA), one 45.7 cm above the plants, and two at the sides of the plants, 30.5 cm from the plants. The sprayer was calibrated to deliver 187 liters/ha at a walking speed of 6.44 km/h and a pressure of 2.11 kg/cm^2.

Because measurable natural infestations of susceptible insects were not present, treatments were evaluated by collecting foliage, returning it to the laboratory, infesting it with beet armyworms, and recording mortality. Each sample consisted of about 4 to 6 cm^2 of leaf tissue. Four samples were taken in each plot. Each sample was placed individually in a 5.5-cm diam Petri dish with moist filter paper. Six late-first to very early-second instars were placed in each dish. Dishes were sealed with Parafilm® (American National Can, Greenwich, CT) and held at 27°C for 48 h. All larvae were then transferred to bioassay trays and held at 27°C. Mortality was recorded 8 d later. Data from the four samples from each plot were pooled for calculation of percentage mortality (24 larvae per plot). Feeding rates were not measured.

In 1995, spray applications were made on 2 Oct, between 1000 and 1100 hours. All treatments were applied in distilled water. AfMNPV (lot Af052595) was applied to all plots, except the control, at a rate of 4.94×10^{11} OB/ha. Adjuvant treatments included Blankophor BBH at 0.25% and 0.50% (w/v; 0.45 and 0.9 kg/ha), feeding stimulant at 0.875% (dry w/v; 1.57 kg/ha; 2.58% v/v), and Blankophor BBH at 0.50% plus feeding stimulant at 0.875%. Feeding stimulant was prepared as described above. The control was distilled water. A spreader, Kinetic® (Setre Chemical Co., Memphis, TN), was included in all treatments at a rate of 0.125% (vol./vol.; 234 ml/ha).

Foliage was sampled 1, 24 and 48 h after application of treatments. Percentage mortality was calculated, normalized by arcsine transformation, and analyzed by factorial ANOVA for effects of the enhancer, feeding stimulant, and interaction thereof (PROC GLM; SAS Institute 1988). Data from each sample date were analyzed separately.

For the test in 1996, a sample of a liquid suspension of AfMNPV (lot Af091295, labeled to contain 2×10^9 OB/ml) was obtained from biosys. Two applications were made; 6 May and 13 May, between 1000 and 1100 hours. Treatments were the same as in 1995, except that Blankophor BBH at 0.25% was not included. Foliage was sampled 1, 24, and 48 h after each application. Data were analyzed as in 1995, except that some control mortality occurred after the second application, so these data were adjusted for control mortality using Abbott's (1925) formula.

Results

Potted plant tests. In the test of AfMNPV alone, the LC_{50} was 1.385×10^3 OB/µl ($\approx 4.25 \times 10^6$ OB/pot, 2.59×10^{11} OB/ha; slope ± SE = 1.116 ± 0.073; 95% fiducial limits = $1.156 \times 10^3 - 1.663 \times 10^3$ OB/µl). The LC_{95} was 4.123×10^4 OB/µl ($\approx 1.27 \times 10^8$ OB/pot, 7.72×10^{12} OB/ha; 95% fiducial limits = $2.697 \times 10^4 - 7.063 \times 10^4$ OB/µl). No mortality occurred in control treatments.

Mortality of beet armyworm larvae fed on potted plants was not significantly affected by the enhancer, Blankophor BBH. Although mortality increased from 50.8% in the absence of the enhancer to 61.2% with 0.5% enhancer, the effect was not significant ($F = 2.10$; df = 1, 73; $P = 0.1517$). Control mortality was 0.27%. In contrast, mortality of larvae fed on potted plants treated with the virus was significantly increased by the feeding stimulant. Mortality increased from 48.6% in the absence of the feeding stimulant to 75.7% with 1.25% feeding stimulant ($F = 13.68$; df = 1, 75; $P = 0.0004$). Control mortality was 0.83%.

In the test of both the enhancer and the feeding stimulant on potted plants (Table 1), mortality was significantly increased by the feeding stimulant ($F = 18.48$; df = 1, 52; $P = 0.0001$) but not by the enhancer ($F = 0.74$; df = 1, 52; $P = 0.3923$). The interaction of feeding stimulant and enhancer was not significant ($F = 0.87$; df = 1, 52; $P = 0.3544$).

Field persistence tests. The enhancer, Blankophor BBH, increased mortality of beet armyworm larvae on foliage collected from the field at 1 h after application in 1995 (Table 2) and both applications in 1996 (Tables 3 and 4). On foliage collected 24 h after application, mortality was not significantly affected by the enhancer in any test (Tables 2 to 4), though a trend to higher mortality on treatments with the enhancer

Table 1. Mean % mortality (±SEM) of beet armyworm larvae on potted collard plants sprayed with AfMNPV (4.25×10^6 OB/pot) and Blankophor BBH and/or feeding stimulant. See text for statistical results

Blankophor BBH, % (w/v)	Feeding stimulant, % (dry w/v)	% Mortality*
0.00	0.00	57.0 ± 4.47
0.50	0.00	60.9 ± 4.72
0.00	1.25	78.4 ± 5.39
0.50	1.25	76.6 ± 5.05

* Adjusted for control mortality (0.7%) by Abbott's (1925) formula.

Table 2. Activity and persistence of AfMNPV (4.94×10^{11} OB/ha) with Blankophor BBH and/or feeding stimulant on field-grown collards, Queenstown, MD, October 1995

Blankophor BBH, % (w/v)	Feeding Stimulant, % (dry w/v)	Mean mortality, %, ± SE*, on foliage collected at		
		1 h	24 h	48 h
0.000	0.000	18.4 ± 6.1	1.1 ± 1.09	1.3 ± 1.32
0.250	0.000	31.9 ± 10.13	3.3 ± 2.16	3.2 ± 2.02
0.500	0.000	48.1 ± 4.07	4.2 ± 2.95	0.0 ± 0.00
0.000	0.875	53.5 ± 7.72	13.3 ± 5.19	2.5 ± 1.49
0.500	0.875	50.2 ± 6.05	24.0 ± 4.62	4.4 ± 2.96
Statistical results for indicated effects**				
Blankophor BBH	*F*	10.38	0.98	0.32
	P	0.0067	0.3398	0.5407
Feeding Stimulant	*F*	15.47	10.94	0.05
	P	0.0017	0.0057	0.8247
Blankophor BBH x	*F*	6.28	0.24	0.42
Feeding Stimulant	*P*	0.0263	0.6291	0.5290

* No mortality on control treatments.
** Degrees of freedom = 1, 13 for all tests.

than on similar treatments without the enhancer occurred. On foliage collected 48 h after application, mortality was increased by the enhancer in the both applications in 1996 (Tables 3, 4).

The feeding stimulant increased mortality on foliage collected 1 h after application in 1995 (Table 2) and in both applications in 1996 (Tables 3, 4). It increased mortality at 24 h in 1995 (Table 2) and in the first application in 1996 (Table 3), with a nearly significant ($P = 0.0574$) trend to an increase in the second application in 1996 (Table 4). The feeding stimulant had no significant effect at 48 h in any field test, though a nearly significant ($P = 0.0567$) trend to an increase was seen after the second application in 1996.

The interaction between enhancer and feeding stimulant was significant for 1 h samples in 1995 (Table 2) and the second application of 1996 (Table 4) and for the 48 h sample in the first application in 1996 (Table 3).

Discussion

Blankophor BBH increased virus-caused mortality of beet armyworm larvae in most cases in the field persistence test but had no effect in any of the potted plant tests. The inconsistency of these results may be related to effects of the enhancer on feeding behavior and to the design of the tests. Previous tests involving AfMNPV and Blankophor BBH fed to beet armyworm larvae on small leaf disks (at concentrations

Table 3. Activity and persistence of AfMNPV (4.94×10^{11} OB/ha) with Blankophor BBH and/or feeding stimulant on field-grown collards, Queenstown, MD, May 1996, first application

Blankophor BBH, % (w/v)	Feeding Stimulant, % (dry w/v)	Mean mortality, %, ± SE*, on foliage collected at		
		1 h	24 h	48 h
0.000	0.000	47.9 ± 7.12	8.5 ± 4.47	4.2 ± 1.70
0.500	0.000	73.5 ± 3.76	11.8 ± 3.53	10.3 ± 2.17
0.000	0.875	70.8 ± 2.74	38.6 ± 10.59	8.4 ± 0.09
0.500	0.875	79.8 ± 7.00	67.3 ± 7.57	36.8 ± 5.73
Statistical results for indicated effects**				
Blankophor BBH	*F*	6.68	0.46	5.22
	P	0.0296	0.5136	0.0482
Feeding Stimulant	*F*	5.20	9.08	3.51
	P	0.0485	0.0146	0.0937
Blankophor BBH x	*F*	0.95	1.20	5.63
Feeding Stimulant	*P*	0.3561	0.2908	0.0417

* No mortality on control treatments.
** Degrees of freedom = 1, 9 for all tests.

of up to 1% of a dip) showed enhancement of the activity of the virus (Farrar and Ridgway 1997). However, Blankophor BBH at 1% is known to be a feeding deterrent to gypsy moth larvae (Farrar et al. 1995) and corn earworm larvae (R. R. F., unpubl. data), and a similar enhancer, Tinopal LPW, at 1% was deterrent to feeding by beet armyworm larvae (Vail et al. 1996). Argauer and Shapiro (1997) found no effect of several optical brighteners applied without virus to artificial diet on mortality of gypsy moth larvae, but did not measure feeding rates. In the potted plant test, larvae were able to move to any part of the plant and could, thus, have chosen to feed on less heavily treated parts of the plant, such as the undersides of leaves. (The location of larvae was not recorded when larvae were collected because most larvae fell from the plants when the sleeve cages were removed.) In the field persistence test, larvae were confined to small pieces of foliage in Petri dishes and, thus, had limited opportunity to move to less heavily treated foliage. However, feeding rates were not measured in either test, so this conclusion remains somewhat speculative.

Another factor that may have contributed to the inconsistency of the results with Blankophor BBH is sunlight, especially UV. While the field persistence test was in full sun, the potted plant test was in a laboratory, where UV levels are minimal and the sleeve cages provided additional shading. If Blankophor BBH were acting as an enhancer in the field persistence test (at least as measured by our bioassay), differences between treatments with and without the enhancer should have been evident in samples taken immediately after application. Such differences were indeed found in the initial samples (Tables 2 to 4). However, because of labor constraints, we were

Table 4. Activity and persistence of AfMNPV (4.94×10^{11} OB/ha) with Blankophor BBH and/or feeding stimulant on field-grown collards, Queenstown, MD, May 1996, second application

Blankophor BBH, % (w/v)	Feeding Stimulant, % (dry w/v)	Mean mortality, %, ± SE*, on foliage collected at		
		1 h	24 h	48 h
0.000	0.000	30.8 ± 1.80	17.4 ± 5.25	0.0 ± 0.00
0.500	0.000	62.2 ± 6.70	35.0 ± 3.38	15.1 ± 3.78
0.000	0.875	67.4 ± 7.23	37.6 ± 9.25	8.3 ± 5.26
0.500	0.875	71.3 ± 5.10	53.7 ± 5.21	36.9 ± 2.59
Statistical results for indicated effects**				
Blankophor BBH	*F*	16.46	3.98	16.79
	P	0.0029	0.0773	0.0027
Feeding Stimulant	*F*	22.64	4.74	4.78
	P	0.0010	0.0574	0.0567
Blankophor BBH x	*F*	6.17	0.10	0.16
Feeding Stimulant	*P*	0.0348	0.7539	0.6989

* Adjusted for control mortality (3.1, 1.0, and 1.1% at 1, 24, and 48 h, respectively) by Abbott's (1925) formula.
** Degrees of freedom = 1, 9 for all tests.

unable to take the initial samples until about 1 h after application. It is, thus, not possible to rule out significant degradation of the virus by UV within this 1 h period. Because diaminostilbene disulfonic acid derivatives can also act as ultraviolet light screens (Shapiro 1992), photodegradation could have contributed to the differences.

Increased persistence (i.e., reduced rate of decline in activity relative to initial levels of activity) of the virus in the field due to the enhancer occurred. Mortality on the 48 h sample was significantly increased, relative to treatments with virus only, by the enhancer in both the first and second applications in 1996. Nonsignificant trends to higher mortality at 24 h on treatments with the enhancer also were observed in all tests. This result, however, could be explained by protection of the virus from UV, enhancement of a small amount of virus remaining after degradation, or both. Tinopal LPW, a material similar to Blankophor BBH, acts both as an enhancer and as a UV protectant for LdMNPV and AcMNPV applied to artificial diet (Dougherty et al. 1996). Blankophor BBH acted similarly for AfMNPV applied to foliage in the laboratory (R. R. F., unpubl. data).

In contrast to results with the enhancer, results with the feeding stimulant were consistent across both the potted plant and field persistence tests. The feeding stimulant increased mortality, relative to treatments with virus only, of beet armyworm larvae in the potted plant tests, the initial (1 h) samples of all field tests, and some 24 and 48 h samples of the field tests. Significant results of the potted plant tests, in which UV levels were minimal, are consistent with feeding stimulation. Similar materials have previously been shown to increase food consumption by beet armyworm

larvae (Farrar and Ridgway 1994). One of these similar materials, Coax® (AgroSolutions, San Marcos, CA), has also been shown to be an effective UV screen (Shapiro et al. 1983), so, as with the enhancer, protection of the virus from UV could also be a factor in the field.

No evidence that the feeding stimulant altered any presumed feeding deterrent effects of the enhancer was seen. No significant interaction was seen in the potted plant test of both materials (Table 1). Some significant ($P < 0.05$) statistical interactions between feeding stimulant and Blankophor BBH were seen in the field persistence tests. For those interactions that occurred in the 1 h samples, data seem to indicate that these interactions were the result of mortality lower than would be expected from additive effects. If the feeding stimulant had overcome feeding deterrence by the enhancer, mortality higher than would be expected from additive effects should have occurred. It is possible that some component of the feeding stimulant could have formed an inactive complex with the enhancer. However, the interaction seen in the 48 h samples of the first application of 1996 would seem to indicate effects greater would be expected from additive effects. The interactions that were observed were not strong, though, and we believe that they are probably not biologically significant.

The virus itself, AfMNPV, caused levels of mortality of beet armyworm larvae in all tests that were near levels expected (Farrar and Ridgway 1997). No evidence was observed indicating that this virus would not be a useful management tool for the beet armyworm on cole crops. Though SeMNPV may be more potent against the beet armyworm (R. R. F., unpubl. data). AfMNPV also has activity against some other insects, such as the cabbage looper, that may also be present on cole crops (Hostetter and Puttler 1991).

All evidence found in this study indicates that nutrient-based feeding stimulants should be useful adjuvants for AfMNPV against the beet armyworm on cole crops. Evidence regarding the potential usefulness of the enhancer with AfMNPV against the beet armyworm of cole crops is inconsistent, however. While previous results clearly demonstrated that enhancement can occur (Farrar and Ridgway 1997), feeding deterrence may limit this effect on whole plants. Nevertheless, at least as measured by our bioassays, activity of treatments with Blankophor BBH was maintained in the field better than that of virus alone, and that of treatments with both feeding stimulant and Blankophor BBH was maintained better than that of any other treatments. Further tests on infested plants in the field, on which larvae can move around as they did in the potted plant tests, may be needed to fully evaluate the enhancer. More rates of the enhancer need to be evaluated; it may be possible to limit feeding deterrence by lowering rates without loss of enhancement activity. In addition, tests of other diaminostilbene disulfonic acid derivatives could reveal compounds that also enhance AfMNPV but are less deterrent to feeding, as has been found for the corn earworm (R. R. Farrar, unpubl. data).

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