Fluorescent Brighteners Affect Feeding Rates of the Corn Earworm (Lepidoptera: Noctuidae) and Act as Enhancers and Sunlight Protectants for its Nucleopolyhedrovirus[1,2]

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J. Entomol. Sci. 38(2): 286-299 (April 2003)

Abstract Seven diaminostilbene disulfonic acid-derived fluorescent brighteners, including Blankophor BBH®, Blankophor HRS®, Blankophor P167®, Blankophor RKH®, Blankophor LPG®, Blankophor DML®, and Blankophor BSU® (Bayer, Rock Hill, SC), were tested for effects on feeding rates of *Helicoverpa zea* (Boddie) and as enhancers and sunlight protectants for the *H. zea* nucleopolyhedrovirus (HzSNPV). All tests were done using virus applied to plant material. Blankophor BBH and Blankophor RKH were feeding deterrents; the other brighteners did not affect feeding. Blankophor HRS caused the greatest increases in activity of HzSNPV, both with and without exposure to simulated sunlight.

Key Words Corn earworm, nucleopolyhedrovirus, stilbene, enhancer, deterrent, ultraviolet

Nucleopolyhedroviruses (NPVs) form a large group of viruses in the family Baculoviridae that includes many important pathogens of insect pests. An extensive body of literature documents research on NPVs as pest control agents (Entwistle and Evans 1985, Granados and Federici 1986, Adams and McClintock 1991, Hunter-Fujita et al. 1998). NPVs have found only limited use in production agriculture, however, due to a number of factors, including short residual activity in the field, low potency, and cost of production. The development of NPVs that have been genetically engineered to kill insects faster (Bonning and Hammock 1996) has prompted renewed interest in NPVs, but problems of cost, persistence, and potency remain.

NPVs are highly susceptible to degradation by the ultraviolet (UV) spectrum of sunlight, so materials that protect NPVs from UV have been studied extensively (Shapiro 1995). One class of materials that has been studied as UV protectants for NPVs is diaminostilbene disulfonic acid-based fluorescent brighteners or optical brighteners (Shapiro 1992). Fluorescent brighteners absorb energy in the form of UV and re-emit it as visible light. In addition, certain of these materials are of particular interest because, independently of their UV protectant activity, they are strong enhancers of the activity of many NPVs (Shapiro 1995, Farrar and Ridgway 1997, Hamm 1999). For example, in laboratory bioassays, median lethal concentrations

[1]Received for publication 04 January 2002; accepted for publication 03 July 2002.

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

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(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. Wang and Granados (2000) report that enhancement may be related to disruption of the peritrophic membrane by fluorescent brighteners. Fluorescent brighteners thus have the potential to help solve several of the problems that have kept NPVs from being more widely used as pest control agents, including low potency (through enhancement), short residual activity (through UV protection), and cost (through the use of lower rates of NPV).

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, and results have been inconsistent. 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. (1993) 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. Farrar et al. (1999) tested the effect of a brightener, Blankophor BBH® (Burlington Chemical, Burlington, NC), on the persistence of the NPV of the celery looper, *Anagrapha falcifera* (Kirby) (AfMNPV), on collard, *Brassica oleracea* (L.), in the field, and its activity on sprayed whole plants in the laboratory. Activity in bioassays of field-collected foliage against the beet armyworm, *Spodoptera exigua* (Hübner), was maintained longer in treatments with the brightener, but no effect was seen in the laboratory tests. Vail et al. (1999) found no effect of Blankophor BBH in similar bioassays against *H. zea* of cotton foliage treated with AfMNPV.

Though fluorescent brighteners can improve the activity of NPVs, some reports have indicated that at least some fluorescent brighteners can deter feeding by at least some species of Lepidoptera. As NPVs must be ingested in order to infect, feeding deterrence could be a serious limitation for the use of brighteners as adjuvants for NPVs. Farrar et al. (1995) quantified consumption of lettuce, *Lactuca sativa* L., treated with Blankophor BBH (concentration of 1% (w/v) of a dip) by gypsy moth larvae. They found that Blankophor BBH reduced consumption, but that the addition of a feeding stimulant, molasses, at least partially overcame this effect. Vail et al. (1996) tested 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 against four species of noctuid larvae. They reported that fewer larvae were found on treated diet than on untreated diet in a choice test, but did not quantify consumption. In Farrar et al. (1999), feeding deterrence by Blankophor BBH in tests of potted plants, where larvae are free to move to untreated parts of the plant, may have prevented the brightener from significantly affecting mortality, but consumption rates were not quantified.

The corn earworm is a major pest of a variety of important crops, including cotton, tomato, *Lycopersicon esculentum* Mill.; corn, and beans, Fabaceae. It is also susceptible to a virus, the *H. zea* NPV (HzSNPV), that is commercially available as Gemstar® LC (Certis USA, Columbia, MD). Though HzSNPV is highly potent against

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the corn earworm (Farrar and Ridgway 1999), Shapiro and Vaughn (1995) showed that its potency can be further increased by the addition of fluorescent brighteners, but only tested them in surface treatments of artificial diet. To more rigorously evaluate fluorescent brighteners with HzSNPV, we conducted a series of tests of these materials applied to excised foliage and whole plants. These tests were designed to evaluate effects of brighteners on food consumption, UV protection, and enhancement of the virus.

Materials and Methods

Insects, plants, virus and brighteners. We obtained all insects from a stock culture (Crop Protection and Management Research Laboratory, USDA-ARS, Tifton, GA). Larvae were reared, before and after virus treatment, on artificial diet (King and Hartley 1985).

The host plant was lima bean, *Phaseolus lunatus* L., cv. 'Maffei 15.' Plants were grown in 10-cm diam pots, 2 to 4 plants per pot, in a greenhouse. A commercial potting medium (Pro Mix BX®, Premier Brands, Red Hill, PA) was used. Plants were grown under a regime of 24 ± 3°C, with the photoperiod supplemented to 16:8 (L:D) h by low-pressure sodium vapor lamps, and were fertilized weekly (Peters Professional 20-20-20®, Grace-Sierra, Milpitas, CA). Plants were 5- to 6-wk old when used.

We obtained samples of HzSNPV (Gemstar® LC), labeled to contain 2.00×10^9 occlusion bodies (OB)/ml, from Thermo Trilogy (now Certis USA, Columbia, MD).

We obtained samples of seven fluorescent brighteners from Bayer (Rock Hill, SC): Blankophor BBH®, Blankophor HRS®, Blankophor P167®, Blankophor LPG®, Blankophor RKH®, Blankophor DML®, and Blankophor BSU® (hereafter referred to by their three to four character suffixes; e.g., BBH). These materials were selected because they represent the range of various chemical structures (all stilbene derivatives) produced as fluorescent brighteners by Bayer.

Feeding rates. Feeding rates of corn earworm larvae on brightener-treated foliage were measured by the method of Farrar and Ridgway (1994), except that late first instar larvae were held in cells of plastic bioassay trays (Bio-BA 128©, CD International, Pitman, NJ) with moist filter paper and starved for 18 h. Only those larvae that molted to the second stadium during this time interval were used.

Treatments included the seven fluorescent brighteners listed above and controls (treated with water). Suspensions of 0.5 and 1.0% of each brightener were prepared in deionized water. A wetting agent, Triton X-155® (Union Carbide, Danbury, CT), and a spreader, Kinetic® (Setre Chemical Memphis, TN) at 0.01 and 0.125%, respectively, were included in all treatments. Leaf disks (9 mm diam) were dipped in the suspensions and allowed to dry. In a nonchoice test, ten larvae were fed control disks, while five larvae were fed disks of each concentration of each brightener. Larvae were held at 27°C for 24 h, then killed by freezing. The test was replicated five times over time (total of 50 larvae per brightener, 25 larvae at each concentration). Relative consumption rate (RCR, dry weight of food eaten divided by initial dry weight of larva) was calculated for each larva (Farrar et al. 1989). Data were analyzed by analysis of variance (ANOVA) with replication, treatment, and concentration of brightener as independent variables, and RCR as the dependent variable; means were separated by the least significant difference (LSD) test (PROC GLM, SAS Institute 1988).

A test in which each larva was offered a choice between one brightener-treated disk and one control disk was also conducted as described by Farrar and Ridgway

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(1994). The same brightener treatments as above were included. This test was replicated four times (40 larvae per brightener, 20 larvae per concentration). Dependent variables included RCR of brightener-treated foliage, and a Modified Lavene's Number (consumption of treated foliage as a percentage of total foliage consumed, after Schiff et al. [1988]). The Modified Lavene's Number was normalized by arcsine $\sqrt{\%}$ transformation. All variables were analyzed as above, except that consumption of treated disks was also compared with that of control disks by paired t-tests for each brightener.

Feeding deterrence on whole plants. To evaluate the influence of feeding deterrence by brighteners on their efficacy as enhancers, we compared BBH, which deterred feeding, to HRS, which did not deter feeding (see Results), as enhancers of HzSNPV applied to whole plants. BBH and HRS both appeared to enhance the virus in nonchoice tests of treated leaf disks, though the increase in mortality by BBH was not statistically significant (see Results). On whole plants, larvae have the option of feeding on less heavily treated foliage, so feeding deterrence could reduce mortality by causing larvae to feed on surfaces with less brightener and virus. All plants were in the vegetative stage (i.e., no flowers or pods were present), so all feeding by larvae was on leaves.

A spray booth (Research Track Sprayer, Model SB8®, De Vries Manufacturing, Hollandale, MN) was used to treat potted lima bean plants. The spray booth was modified to treat plants with three nozzles simultaneously. The hoses and fittings from a Tee Jet Row Application Kit, Model 23770® (Spraying Systems, Wheaton, IL) were mounted in the spray booth on a wooden support clamped to the spray head. Three Tee Jet 73039®(Spraying Systems, Wheaton, IL) flat fan nozzles were used, one positioned 30.4 cm above the plant, and two positioned at the sides, pointed toward the plant, 45.7 cm apart. Assuming that the plants represent one row in a field with 76 cm rows, this system was calibrated to deliver the equivalent of 187 liters/ha at a pressure of 3.52 kg/cm^2 and a speed of 1.92 km/h.

Sprayed plants were allowed to dry. Each pot (two to four plants) was then enclosed in a sleeve cage made of slightly elastic knit fabric tubing sold for use as sleeves for wire screen insect cages (Bio Quip, Gardena, CA). The tubing measures 20 cm across when flat, and it was cut into 38 cm sections to make sleeve cages. Thirty-two late first to early second-instar larvae were placed in each cage. Wire twist ties at the tops and rubber bands around the pots were used to close the cages. The cages were held at 27°C for 48 h. Larvae were then collected, transferred to artificial diet, and held for seven additional days at 27°C. Mortality was then recorded. In this and all tests described below, larvae were considered to have been killed by the virus if they showed characteristic symptoms, including dark coloration, liquefied tissues, and easily ruptured cuticle.

Preliminary tests (R. R. F., unpubl.) indicated that a rate of 2.52×10^9 OB/ha (14 OB/μl) would kill about 25% of larvae in this type of test. This rate of virus was then applied to plants alone or with 0.5 or 1.0% BBH or HRS. Initially, BBH, but not HRS, clogged the nozzles being used in this test. (In previous tests [Farrar and Ridgway 1999], BBH did not clog Tee Jet 11002® nozzles [Spraying Systems, Wheaton, IL], which have larger apertures.) Therefore, BBH was passed through a 100 mesh screen prior to being weighed and mixed with water. Control plants were treated with distilled water. All treatments also included Triton X-155, and Kinetic at 0.01 and 0.125%, respectively. The test was replicated six times. Percentage mortality on brightener treatments was calculated, and divided by that on the treatment with virus

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only. This percentage was analyzed by ANOVA to test for effects of replication, brightener, concentration, and the interaction of concentration and brightener (PROC GLM, SAS Institute 1988).

Enhancement of HzSNPV. The effect of seven fluorescent brighteners on mortality of second and fourth instar corn earworms fed relatively low doses of HzSNPV was measured. Bioassays with newly molted second instars were conducted by the method of Farrar and Ridgway (1999), in which known quantities of virus were pipetted onto small (6 mm diam) leaf disks, which larvae were allowed to consume completely, thus providing a known dose.

Farrar and Ridgway (1999) found that a dose of 2 OB per larva killed 25 to 30% of second instars; this dosage was therefore used in these bioassays. Treatments included HzSNPV alone and with each fluorescent brightener at a concentration of 1% (wt/vol). Control disks were treated with water. All treatments also included 0.01% Triton X-155. Twenty-four leaf disks were placed individually on moist filter paper in a cell of a bioassay tray with one late first instar (showing head capsule slippage) to early second instar per disk. Larvae were held in an incubator at 27°C for 48 h. Each larva that consumed the entire disk by 48 h was transferred to a new bioassay tray filled with artificial diet; other larvae were discarded. Larvae were held on diet at 27°C for seven additional days. Mortality (again as assessed by symptomatology) was recorded daily. This test was replicated six times. Percentage mortality was calculated, normalized by arcsine $\sqrt{\%}$ transformation, and analyzed by ANOVA with replication and treatment as factors. Means were separated by LSD (PROC GLM, SAS Institute 1988). Survival times of larvae that died of viral infection were analyzed by ANOVA with means separated by LSD (Farrar and Ridgway 1998).

Enhancement of HzSNPV against fourth-instar larvae was also measured. Dosage-mortality data of the type on which tests of second instars were based were not available for fourth instars; therefore, such data were developed in a preliminary test. Bioassays were similar to those for second instars, except that larger leaf disks (9 mm) were used, larvae were held in 5.5 cm diam Petri dishes for 24 h while on leaf disks, and larvae were held in 30-ml plastic cups with artificial diet after feeding on foliage. Treatments included HzSNPV at 10, 20, 50 and 100 OB per larva, and controls. Twenty-four larvae were offered disks of each treatment. The test was replicated five times. Data were analyzed by probit analysis (PROC PROBIT, SAS Institute 1988).

The above test indicated that a dose of HzSNPV of 12 OB per larva should kill about 25% of fourth instars (see Results). Brighteners were thus tested with this dosage of virus. Treatments were the same as with second instars, except that three brighteners were omitted: BBH (feeding deterrent), LPG (no enhancement with second instars), and RKH (feeding deterrent, difficult to suspend) (see Results). Twenty-four larvae were offered disks of each treatment. The test was replicated and analyzed as for the test of second instars.

We tested the effects of concentration of HRS and P167 (two of the most active brighteners; see Results) on the enhancement of the activity of HzSNPV against second instars. Each brightener was included at concentrations of 0.00, 0.10, 0.25, and 0.50%. Control treatments of 0.50% HRS and P167 without virus were also included. The bioassays were similar to those described above. This test was replicated six times. Data were adjusted for mortality on control treatments with the same brightener with Abbott's (1925) formula, normalized by arcsine $\sqrt{\%}$ transformation,

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and analyzed by ANOVA with brightener, concentration, and the interaction thereof as factors (PROC GLM, SAS Institute 1988).

Blankophor HRS, the most active material in the above test, was tested again at lower concentrations, including 0.00, 0.01, 0.05, 0.10, 0.15 and 0.20%, and a control of 0.20% HRS with no virus. This test was replicated seven times. Data were analyzed as before for effects of concentration of HRS, except that quadratic (nonlinear) as well as linear effects of concentration were evaluated. This test was otherwise similar to those described above.

Activity under simulated sunlight. The combined effects of the seven brighteners as enhancers and as sunlight protectants for HzSNPV were evaluated with second instars. We used a sunlight simulator, Suntester CPS+®, (Atlas Electric Devices, Chicago, IL) with a xenon lamp as source of light, including ultraviolet (UV) A and B, and visible light. McGuire et al. (2000) tested an identical device and found that the rate of degradation of an NPV under simulated sunlight was similar to that under natural sunlight when exposure was expressed as cumulative total energy (joules/m^2). The simulator was operated at 500 W/m^2, which was expected to provide UV intensities similar to those of sunlight at noon in June at Beltsville, MD (R. R. F., unpubl.). The chamber containing the xenon lamp is cooled by a current of chilled air, as well as by chilled water circulating under a pan at the bottom of the chamber. To prevent virus-treated leaf disks (see below) from being blown away and/or desiccated by the air current, a box with a top made of UV-transparent acrylic plastic, 2.5 mm thick (UVT®, Polycast Technology, Stamford, CT) was used to hold the disks during exposure to UV. This plastic transmits > 95% of UV energy (Ridgway and Farrar 1999). The box is 2.5 cm deep with a thin aluminum flashing bottom, and was made to fit inside the pan at the bottom of the UV-exposure chamber. Moist paper under, as well as inside, the box allowed excess heat to be conducted away from the chamber by the circulating chilled water.

A preliminary test was conducted to determine the rate of degradation of activity of the virus in the sunlight simulator at 500 W/m^2. Virus was pipetted onto 6 mm diam leaf disks as described above with 28 OB per disk. Treated disks were exposed to light in the simulator for 0, 5, 10, 15, 20, 25 or 30 min. Bioassays with second instars were then set up as described above, except that mortality was recorded only at the end of the test. Control disks, treated with water and not exposed, were also included. Twenty-four larvae per exposure interval, and control, were included. The test was replicated five times. Percentage mortality on the virus treatment with 0 min exposure was calculated and treated as original activity (OA). Percentage mortality on the exposed treatments was calculated and divided by OA to give original activity remaining (OAR). OAR was normalized by arcsine $\sqrt{\%}$ transformation and analyzed by ANOVA for linear and quadratic (nonlinear) effects of the logarithm of exposure time. OAR was then regressed against the logarithm of exposure time to calculate the length of exposure needed to degrade 90% of viral activity.

The above test indicated that exposure of 28 OB per disk for 26.5 min would degrade activity by approximately 90% (see Results). This dosage and exposure were therefore used in all subsequent tests of UV. Leaf disks were treated with virus and each of the seven brighteners as described above. All treatments with brightener, and a treatment of virus with no brightener, were exposed to UV. Control treatments (water only) and a second treatment with virus only were kept unexposed. (Time constraints precluded our simultaneously including unexposed treatments with all brighteners; this comparison was made later with selected brighteners; see below.)

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Bioassays were conducted as described above. OAR was calculated for all exposed treatments relative to the unexposed virus treatment, normalized by arcsine √% transformation, and analyzed by ANOVA with replication and brightener as factors. Means were separated by LSD (PROC GLM, SAS Institute 1988).

To evaluate the relative roles of enhancement and UV protection of the NPV by brighteners, we tested two of the most active brighteners, HRS and P167, in treatments exposed to UV and, at the same time, in treatments not exposed to UV. UV-exposed and unexposed treatments included virus at doses of 28 and 2 OB per larva, respectively. Different doses were used because, in the absence of brighteners, after exposure to UV, 28 OB per larva should kill a low percentage of larvae, as would 2 OB per larva not exposed to UV. The statistical analysis was then designed to partition any remaining differences between these treatments from the effects of interest, i.e., those of the brighteners. UV exposures and bioassays were conducted as described above. Percentage mortality was calculated, normalized by arcsine √% transformation, and analyzed by factorial ANOVA with replication, brightener, exposure, and the interaction of brightener and exposure as factors (PROC GLM, SAS Institute 1988). Data also were analyzed separately for effects of brighteners within UV treatments and for effects of UV exposure within brightener treatments.

Results

Feeding rates. In the absence of virus, consumption of foliage was significantly affected by fluorescent brighteners ($F = 2.55$; df = 7, 289; $P = 0.0145$), but not by concentration of the brighteners ($F = 0.16$; df = 1, 289; $P = 0.6918$) (Table 1). Relative to untreated foliage, RCR was significantly ($P \leq 0.05$) reduced by BBH and LPG, but unaffected by the other brighteners.

When given a choice between foliage treated with brighteners and untreated fo-

Table 1. Feeding rates of *H. zea* larvae on lima bean foliage treated with fluorescent brighteners in a non-choice test

Brightener	Mean ± SE RCR* at a concentration of		Grouping**
	0.5%	1.0%	
Control	6.35 ± 0.568†		A
Blankophor BBH	4.55 ± 0.588	4.17 ± 0.494	C
Blankophor HRS	6.48 ± 0.787	6.28 ± 0.660	A
Blankophor RKH	6.02 ± 0.682	5.70 ± 0.634	AB
Blankophor P167	5.54 ± 0.620	7.04 ± 0.519	AB
Blankophor DML	5.70 ± 0.856	6.67 ± 0.578	AB
Blankophor LPG	5.15 ± 0.737	4.96 ± 0.680	BC
Blankophor BSU	6.40 ± 0.835	6.15 ± 1.044	AB

* RCR = Relative Consumption Rate = dry weight of food eaten/initial dry weight of insect.

** Means with the same letter are not significantly different by LSD ($P > 0.05$).

† Concentration does not apply to the control, which was treated with water only.

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liage, RCR of treated foliage was again affected by brighteners ($F = 3.36$; df = 6, 239; $P = 0.0034$) but not by concentration of brighteners ($F = 0.04$; df = 1, 239; $P = 0.8432$) (Table 2). In this analysis, consumption of foliage treated with RKH was significantly ($P \leq 0.05$) lower than that of the other treatments. When consumption of treated foliage was expressed as a percentage of total food consumption (modified Lavene's number), similar trends were evident, but the effect of treatments was not significant ($F = 1.86$; df = 6, 259; $P = 0.0888$ for brighteners; $F = 0.03$; df = 1, 259; $P = 0.8712$ for concentration of brighteners) (Table 2). When consumption of treated foliage was compared with that of untreated foliage for individual brighteners in paired t tests, consumption of foliage treated with BBH or RKH was significantly ($P \leq 0.05$) reduced. No differences were seen for the other brighteners (Table 2).

Feeding deterrence on whole plants. Mortality of larvae on treatments with virus and brightener, expressed as a percentage of mortality of larvae on virus only, was affected by brightener ($F = 7.73$; df = 1, 15; $P = 0.0140$), but not by concentration of brightener ($F = 1.58$, df = 1, 15; $P = 0.2283$) (Fig. 1). However, the effect of the interaction of concentration and brightener was significant ($F = 6.26$; df = 1, 15; $P = 0.0244$). Mortality increased with increasing concentration of HRS, but declined with increasing concentration of BBH. No mortality of control larvae occurred.

Enhancement of HzSNPV. The addition of brighteners to virus treatments significantly affected both mortality ($F = 3.80$; df = 7, 35; $P = 0.0036$) and survival time of larvae that were killed by virus ($F = 4.90$; df = 7, 277; $P = 0.0001$) (Table 3). Mortality was increased by HRS, RKH, and P167, but was unaffected by the other brighteners. Survival times were reduced by HRS and P167. Mortality of control larvae was 0.0%.

In the dose-mortality test of HzSNPV against fourth instars, the LD_{25} was 12 OB

Table 2. Feeding rates of *H. zea* larvae on lima bean foliage treated with fluorescent brighteners in a choice (treated versus control foliage) test. Because effects of concentrations of brighteners were nonsignificant ($P > 0.05$) in all cases, means across concentrations are presented.

Brightener	Mean ± SE RCR* of treated foliage†	Mean ± SE Modified Lavene's Number**†	Treated versus control	
			t	*P* > *t*
Blankophor BBH	4.24 ± 0.533 A	38.6 ± 3.78 B	2.6644	0.0117
Blankophor HRS	5.36 ± 0.780 A	45.8 ± 4.44 AB	0.0491	0.9611
Blankophor RKH	3.50 ± 0.629 B	39.2 ± 4.32 B	2.6432	0.0122
Blankophor P167	6.13 ± 0.712 A	53.2 ± 4.58 A	−0.6984	0.4898
Blankophor DML	5.28 ± 0.553 A	49.3 ± 3.55 AB	−0.0368	0.9708
Blankophor LPG	4.22 ± 0.569 A	41.2 ± 4.58 B	0.7633	0.4510
Blankophor BSU	5.47 ± 0.610 A	50.8 ± 4.44 AB	−0.2513	0.8030

* RCR = Relative Consumption Rate = dry weight of food eaten/initial dry weight of insect.

** Consumption of treated foliage as a percentage of total foliage consumed.

† Means with the same letter are not significantly different by LSD ($P > 0.05$).

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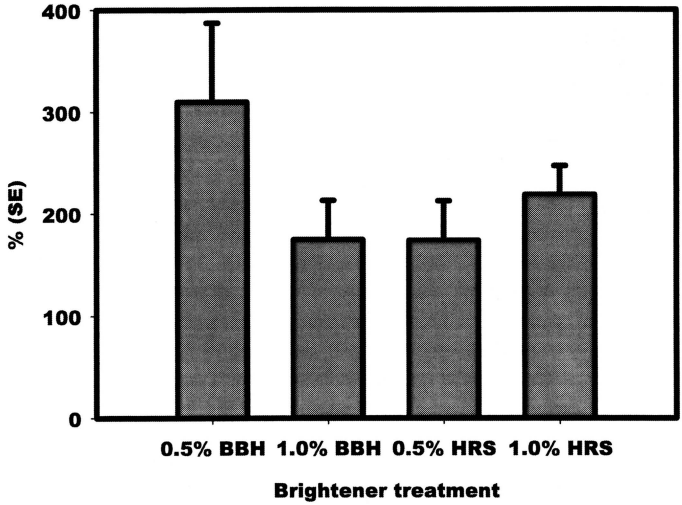

Fig. 1. Mortality (±SE) of *H. zea* larvae fed on whole lima bean plants treated with HzSNPV and Blankophor BBH or HRS expressed as a percentage of mortality on similar plants treated with virus only. Mortality on the latter treatment was 25.5% (±1.22%).

per larva (95% fiducial limits: 9-15 OB per larva) and the LD_{50} was 29 OB per larva (95% fiducial limits: 25-33 OB per larva), with a slope of 1.732 (SE ± 0.1602) and a χ^2 of 116.87. No mortality occurred in control larvae. (Similar data for second instars were published previously [Farrar and Ridgway 1999].)

Mortality of fourth instars treated with virus was affected by the addition of brighteners ($F = 35.76$; df = 4, 20; $P = 0.0001$), as was survival time ($F = 5.74$; df = 4, 286; $P = 0.0002$) (Table 3). HRS and P167 increased mortality and reduced survival times. No mortality was seen on control treatments.

In the test comparing HRS and P167 at three concentrations, mortality of second instars treated with virus only was 24.8% (±SE 6.53%). Mortality was greater on treatments with brightener, and overall greater for HRS than for P167 ($F = 18.72$; df = 1, 27; $P = 0.0002$) (means over all concentrations =61.3% ± SE 4.19% and 39.9% ± SE 4.10%, for HRS and P167, respectively). Mortality was unaffected by concentration of brighteners ($F = 0.91$; df = 1, 27; $P = 0.3481$) or by the interaction of brightener and concentration ($F = 2.27$; df = 1, 27; $P = 0.1438$). Mortality on control treatments with 0.50% HRS or P167 but no virus was 2.4% and 0.0%, respectively.

When HRS was tested at lower concentrations, mortality increased from 24.4% (±SE 3.31%) in larvae fed virus only to 49.6% (±SE 4.17%) in larvae fed virus with 0.20% HRS. Linear effects of concentration of brightener on mortality were significant ($F = 7.77$; df = 1, 33; $P = 0.0088$) but quadratic (nonlinear) effects were nonsignificant ($F = 2.11$; df = 1, 33; $P = 0.1562$). No mortality in control larvae was observed.

Activity under simulated sunlight. Degradation of the activity of HzSNPV in the Suntester CPS+ tended to follow a negative logarithmic pattern. Without exposure to simulated sunlight, the virus killed 90.5% of larvae (OA). When exposed, OAR de-

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Table 3. Enhancement of activity of HzSNPV by fluorescent brighteners (1% wt: vol) against second (2 OB per larva) and fourth (12 OB per larva) instar *H. zea*

	Mean ± SE of instar			
	Second		Fourth	
Brightener*	Mortality, %**	Survival time, d**	Mortality, %**	Survival time, d**
None	22.4 ± 5.16 CD	5.86 ± 0.168 CD	25.3 ± 2.32 C	6.30 ± 0.102 C
BBH	36.6 ± 3.64 ABC	5.70 ± 0.130 BC	—†	—†
HRS	44.2 ± 4.66 A	5.41 ± 0.108 AB	76.2 ± 4.30 A	5.80 ± 0.065 A
RKH	44.9 ± 8.83 A	5.71 ± 0.087 BC	—†	—†
P167	40.6 ± 6.23 AB	5.29 ± 0.074 A	54.3 ± 3.60 B	5.93 ± 0.073 AB
DML	23.5 ± 4.07 BCD	5.89 ± 0.134 CD	30.7 ± 2.41 C	6.17 ± 0.107 BC
LPG	18.3 ± 3.91 D	6.18 ± 0.182 D	—†	—†
BSU	24.9 ± 6.68 CD	5.59 ± 0.117 ABC	24.5 ± 3.91 C	6.11 ± 0.080 BC

* All brighteners are sold under the trade name "Blankophor;" only suffixes shown; see text.
** Means with the same letter are not signficantly different ($P > 0.05$) by LSD.
† BBH, RKH, and LPG were not tested against fourth instars.

clined to 1.5% after 30 min. Significant effects of both linear ($F = 57.63$; df = 1, 20; $P = 0.0001$) and quadratic ($F = 16.94$; df = 1, 20; $P = 0.0005$) effects of exposure time were found. When exposure time was transformed logarithmically prior to the analysis, however, only the linear effect was significant ($F = 71.00$; df = 1, 16; $P = 0.0001$ for linear, $F = 0.19$; df = 1, 16; $P = 0.6655$ for quadratic). Regression analysis showed the relationship of OAR to exposure as:

$$\% \text{ Reduction in OAR} = 63.19 \times \log_{10} \text{Exposure Time}$$

($T = 25.51$; df = 1; $P = 0.0001$; $R^2 = .9673$). A reduction in OAR of 90%, thus, would require an exposure of 26.5 min. No control mortality occurred.

All brighteners significantly increased OAR when they were added to virus treatments exposed to simulated sunlight ($F = 32.56$; df = 1, 21; $P = 0.0001$) (Table 4). The greatest increases occurred with BBH, HRS, and P167; the least, with LPG. No mortality was seen in control larvae.

When virus treatments with no brightener, or with HRS or P167, were compared with and without exposure to simulated sunlight, mortality was affected by exposure ($F = 50.59$; df = 1, 15; $P = 0.0001$), brightener ($F = 3.69$; df = 2, 15; $P = 0.0497$), and the interaction thereof ($F = 26.89$; df = 2, 15; $P = 0.0001$) (Fig. 2). When exposure treatments were analyzed separately, effects of brightener treatments were significant for unexposed treatments ($F = 5.51$; df = 2, 6; $P = 0.0438$) as well as exposed treatments ($F = 62.61$; df = 2, 6; $P = 0.0001$). Exposed treatments included virus at 28 OB per larva, which, when not exposed, caused 87.9% mortality (Fig. 2). This value

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Table 4. Activity of HzSNPV against second instar *H. zea* following exposure to simulated sunlight with or without one of seven fluorescent brighteners. Mortality on virus with no brightener, not exposed to UV (original activity), was 97.9%

Brightener	Exposure, min	Mean ± SE OAR, %* **
None	26.5	9.3 ± 2.69 E
Blankophor BBH	26.5	97.6 ± 2.38 A
Blankophor HRS	26.5	100.0 ± 0.00 A
Blankophor RKH	26.5	86.0 ± 8.64 BC
Blankophor P167	26.5	95.3 ± 2.69 AB
Blankophor DML	26.5	89.9 ± 2.24 BC
Blankophor LPG	26.5	49.8 ± 4.35 D
Blankophor BSU	26.5	77.0 ± 6.78 C

* OAR = original activity remaining = (% mortality on exposed treatment/% mortality on unexposed treatment)* 100.

** Means with the same letter are not significantly different ($P > 0.05$).

is presented for comparative purposes, but was not included in the statistical analyses. No mortality of control larvae occurred.

Discussion

Fluorescent brighteners affected both feeding behavior and susceptibility to NPV in *H. zea.* However, among the seven brighteners included in this series of tests, activity varied widely. BBH and RKH reduced feeding rates on treated leaf disks, but other brighteners had no effect on feeding. The decline in virus-caused mortality on whole plants with increasing rates of BBH, in contrast with the increase in mortality with HRS, is consistent with feeding deterrence. Previous observations indicated that BBH can deter feeding in some insects (Farrar et al. 1995, 1999), as can Tinopal LPW (Vail et al. 1996), but consumption rates have not been quantified previously, except for BBH with the gypsy moth (Farrar et al. 1995). However, not all brighteners deterred feeding of corn earworm larvae. HRS and P167, which were among the most potent enhancers of viral activity, had no effect on feeding.

Several brighteners, including BBH, HRS, RKH, and P167, enhanced activity of HzSNPV against second instars. HRS and P167 also enhanced activity against fourth instars. The reader should note that BBH and RKH were not tested against fourth instars because they deterred feeding; LPG, because it did not enhance HzSNPV against second instars. RKH was also more difficult to suspend or dissolve in water, though relative solubility and sedimentation rates were not measured. HRS and P167 also decreased survival times of second and fourth instars that were killed by the virus, but these differences were small, usually <1 d. This small reduction in survival time is consistent with Farrar and Ridgway (1998) and Washburn et al. (1998). Enhancement of activity of HzSNPV by HRS and P167 did not vary greatly over a range

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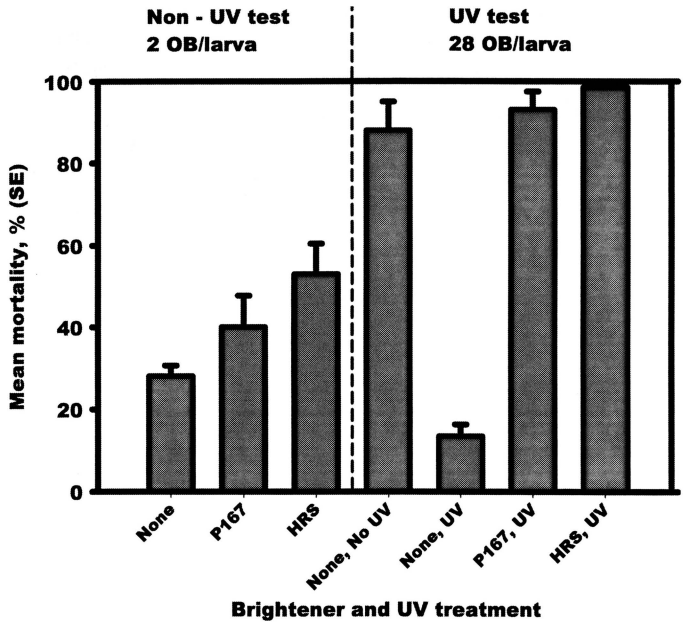

Fig. 2. Mortality (±SE) of second instar *H. zea* fed HzSNPV with or without Blankophor BBH or P167 and with or without exposure to simulated sunlight.

of concentrations of brightener from 0.10% to 0.50%. Only when HRS was tested at rates under 0.10% was a decline in activity seen.

Under simulated sunlight, the virus was protected to at least some degree by all brighteners tested, including LPG, which had no effect as an enhancer without exposure to simulated sunlight. HRS and P167, at least, appear to act both as sunlight protectants and as enhancers. This conclusion is evidenced by the greater effect of brighteners in the presence of UV than in its absence, which resulted in a significant interaction between these brightener treatments and exposure to simulated sunlight (Fig. 2). Dougherty et al. (1996) also concluded that brighteners could act as both UV protectants and as enhancers. Farrar and Ridgway (2000) found that for the NPV of the celery looper against the beet armyworm, BBH acted as both an enhancer and as a UV protectant, but that HRS acted only as an enhancer. The reasons for difference in activity of HRS between that study and the present one are not immediately evident, as the insect, virus, host plant, and UV source all differed.

Of the seven brighteners tested, HRS thus appears to be the most appropriate choice for use with HzSNPV against *H. zea*. HRS was not deterrent to feeding, and caused the greatest increases in activity of the virus with and without exposure to simulated sunlight. It is priced similarly to P167, which would appear to be the second best choice. As of December 1999, the wholesale price of HRS was $15.95/kg ($7.25/lb), versus $17.49/kg ($7.95/lb) for P167. P167, however, does have the potential advantage of being completely soluble in water at the concentrations tested. HRS is not completely soluble, but does suspend readily in water.

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Acknowledgments

The authors wish to thank J. J. Hamm (USDA-ARS) for comments on earlier drafts of the manuscript, and Ron Myers (USDA-ARS) for supplying insects.

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