

Effects of Eight Brighteners as Solar Radiation Protectants for *Steinernema carpocapsae*, All Strain¹

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Abstract: Seven commercially available Blankophor fluorescent brighteners were compared with the standard Tinopal LPW as solar radiation protectants for *Steinernema carpocapsae* (All strain). Blankophor BBH and Tinopal LPW were the most successful UV screens, with 95% of the original nematode infectivity to larvae of the greater wax moth, *Galleria mellonella*, retained after 4 hours of exposure to direct sunlight. The Blankophor HRS and DML preserved 80 and 85% infectivity, and the P167 preserved 70% infectivity after the sunlight exposure. The other Blankophors (RKH, LPG, and BSU) were not as effective.

Key words: biological control, Blankophor, brightener, entomopathogenic nematode, *Steinernema carpocapsae*, solar radiation, stilbene.

Solar radiation is one factor that has hindered successful and consistent performance of nematodes and other entomopathogens in the field after foliar and irrigation water application (4-6,9). Infectivity of *Steinernema carpocapsae* Agriotos strain to the larvae of the greater wax moth, *Galleria mellonella*, was reduced by 94.9% after exposure to direct sunlight for 60 minutes (4). In a subsequent study, infectivity of wax moth larvae by *S. carpocapsae* All strain was reduced to 12% after direct sunlight exposure for 60 minutes (6). However, when a 1% solution of Tinopal LPW (Calcofluor white M2R) was added to the nematode suspension, the original infectivity retained (OIR) was 100% for periods of 60 to 240 minutes. Another entomopathogenic nematode, *Heterorhabditis bacteriophora*, is even more sensitive to ultraviolet light than *Steinernema* (6).

Recent research on UV protection for insect viruses has centered on optical brighteners (10-12,14). Considerable progress has been made especially for a nuclear polyhedrosis virus-brightener combination applied to gypsy moth. A se-

lected brightener also enhanced virus activity against this pest insect (12). The objective of our study was to determine whether any of the commercially available stilbene brighteners related to Tinopal LPW could also provide UV protection for *S. carpocapsae* All strain exposed to direct sunlight.

MATERIALS AND METHODS

Organisms and chemicals: *Steinernema carpocapsae* (All strain) was provided by biosys (Palo Alto, CA). Wax moth larvae were obtained from the Sunfish Bait (Webster, WI). Tinopal LPW (Calcofluor White M2R), a stilbene brightener, was obtained in chemically pure form from Sigma Chemical (St. Louis, MO). Chemically related stilbene brighteners were obtained from Burlington Chemical (Burlington, NC). The seven stilbene brighteners were as follows: Blankophor BBH, BSU, DML, HRS, LPG, P167, and RKH. All brighteners were dissolved in tap water and tested at a 1% (w/v) concentration.

Standardized nematode bioassay: A standardized nematode bioassay that consistently produces 90-100% wax moth larval mortality in 72 hours was used (I. Popiel and P. Pruitt, biosys, pers. comm.). A nematode suspension of 100-120 third-stage infective juveniles in 2 ml water was applied to two filter papers in a petri dish (100 × 15 mm), which contained 10 seventh-instar wax moth larvae. Mortality was recorded at 72 hours and was defined as

Received for publication 24 May 1994.

¹ This paper reports the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation for its use by the United States Department of Agriculture.

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We thank Bob Reise of the Nematology Laboratory for technical support. We also thank Ramon Georgis, biosys, for providing the nematodes used in this study.

"Original Infectivity" (OI). This OI was compared with mortality produced by the nematodes following sunlight exposure. Wax moth larval mortality produced in these treatments at room temperature was expressed as a percentage of the standard and designated as the original infectivity remaining (OIR) (7).

Testing of the stilbene brighteners: A suspension of 100–120 nematodes was placed in Syracuse watch glasses (5.5-cm-d) in 2–3 ml tap water or 1% brightener solution. The uncovered watch glasses were placed in a water-containing plastic pan to eliminate heating as a factor in this experiment. Outside temperature ranged from 31 to 33 C. The nematodes were exposed to direct sunlight from 11 a.m. to 3 p.m. in Beltsville, MD (August 1993) for 0, 15, 45, 120, and 240 minutes. After exposure, the nematode suspensions in water (2 ml) were added to the petri dishes for the standard infectivity bioassay. Each test was repeated four separate times.

Evaluation: In all tests, the percentage of nematode-caused wax moth larval mortality was the sole criterion for evaluation of UV protection. LC₅₀ values for percentage OIR were determined by probit analysis (2,3), and treatment means were compared by Duncan's multiple-range test (2).

RESULTS AND DISCUSSION

In all treatments, nematode infectivity remained high after a 45-minute exposure to sunlight. After 60 minutes of exposure, however, infectivity was reduced to 42% in the water suspension (data not shown). After a 120-minute exposure, the OIR was less than 10% in the water suspension, indicating the sensitivity of the nematodes to sunlight. In a 1% solution of Tinopal LPW and Blankophor BBH, the nematodes were completely protected (i.e., 100% OIR), even after a 240-minute exposure. Excellent protection was also obtained with Blankophor DML, HRS, and P167 (Table 1). Blankophor RKH, Blankophor LPG, and Blankophor BSU were ineffective as UV protectants for a 240-

TABLE 1. Effects of stilbene optical brighteners on infectivity of *Steinernema carpocapsae* to wax moth larvae after nematode exposure to sunlight.

Treatment†	Percentage mean larval kill after sunlight exposure (in minutes)				
	0	15	45	120	240
H ₂ O	90 a	89 a	92 ab	1 c	0 d
LPW	98 a	92 a	98 ab	93 ab	95 a
BBH	82 a	96 a	96 ab	98 a	95 a
BSU	88 a	88 a	90 ab	83 b	0 d
DML	96 a	84 a	100 a	93 ab	85 ab
HRS	76 a	76 a	84 ab	90 ab	80 ab
LPG	80 a	84 a	84 ab	85 ab	23 cd
P167	78 a	84 a	96 ab	93 ab	70 b
RKH	84 a	78 a	80 b	85 ab	25 c

Means in the same column followed by the same letter are not different ($P < 0.01$) by Duncan's multiple-range test.

Nematodes were used at final concentration of 100–120 per 2-ml suspension.

† Tinopal LPW was obtained from Sigma Chemical (St. Louis, MO) and the seven other stilbene brighteners were obtained from the Burlington Chemical (Burlington, NC).

minute sunlight exposure but were effective for a 120-minute exposure. For these brighteners, the times required to reduce nematode infectivity to 50% were approximately 150 minutes (BSU), 160 minutes (RKH), and 175 minutes (LPG).

The Blankophors used in the study are all available commercially and are much less expensive than the Tinopal LPW standard. Several (Blankophor BBH, Blankophor DML, and Blankophor HRS) are as effective as Tinopal LPW (9). These fluorescent brighteners are commonly used in the detergent, paper, plastics, and organic coatings industries (8), and as fluorochromes for microorganisms (1,13). Moreover, they appear to be promising candidates as sunlight protectants for insect viruses (12) and nematodes, and will undoubtedly be a key ingredient in future formulations.

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