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# Use of a Stilbene Brightener, Tinopal LPW, as a Radiation Protectant for *Steinernema carpocapsae*

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*Abstract:* A stilbene fluorescent brightener, Tinopal LPW, was used as an ultraviolet (UV) protectant for the entomopathogenic nematode *Steinernema carpocapsae* (All strain). Irradiation of an aqueous suspension of nematodes produced a $LC_{50}$ in 15.7 minutes under a sunlamp and in 31.7 minutes in direct sunlight. Irradiation by both sunlamp and sunlight of a suspension of nematodes in Tinopal LPW did not reduce their biological activity as measured by their ability to parasitize wax moth larvae after exposure of 8 hours and 4 hours, respectively. Tinopal LPW appeared promising as a radiation protectant.

*Key words:* Biological control, entomopathogenic nematode, nematode, *Steinernema carpocapsae*, stilbene, Tinopal LPW, ultraviolet light.

Solar radiation is one of the factors that has precluded the foliar application of nematodes and other entomopathogens in the field (3,4,6,8). Infective juveniles of *Steinernema carpocapsae* Weiser (Agriotos strain) exposed for 7 minutes to short UV radiation (254 nm) were unable to infect larvae of the greater wax moth, *Galleria mellonella* (3). Moreover, exposure to direct sunlight reduced pathogenicity from 6.9% to 94.9% at 30 and 60 minutes of exposure. Para-aminobenzoic acid, an ingredient in many commercial sunscreen lotions, was found to be an effective radiation protectant for this nematode. This material produced a three- to fourfold increase in the period over which nematode pathogenicity and reproduction could occur (4). Currently, entomopathogenic nematodes are available commercially mainly to control soil-inhabiting insects. To expand the field use of nematodes as biocontrol agents (i.e., foliar application), this sensitivity to UV light needs to be solved.

In studies of optical brighteners as radiation protectants for gypsy moth nuclear polyhedrosis virus, 23 compounds were tested (10). One of these materials, Tinopal LPW (a stilbene), provided complete virus protection (i.e., 100% original activity remained) at a 1% (w/v) concentration. In this study, the use of Tinopal LPW was investigated as a protectant of infective juveniles of *Steinernema carpocapsae* (All) from UV irradiation (sunlamp and natural sunlight).

## Materials and Methods

*Nematode and wax moth larvae:* The nematode used in this study was *Steinernema carpocapsae* (All) provided by Biosys (Palo Alto, CA). The wax moth larvae, *Galleria mellonella L.*, were obtained from the Sunfish Bait Co., Webster, WI. The fluorescent brightener Tinopal LPW (Calcofluor white M2R) was obtained in chemically pure form from Sigma Chemical Co., St. Louis, MO. It was dissolved in tap water and tested at a 1% (w/v) concentration.

*Radiation sources:* Irradiation was provided in the laboratory by a 275-W high-intensity mercury sunlamp (Model RS-40, Sylvania Lighting, Danvers, MA), with built-in aluminum reflectors. This sunlamp has been used in other photoinactivation studies of insect pathogens (7–10). Direct sunlight provided irradiation on five different sunny days, from 11:00 to 3:00, in Beltsville, Maryland, in July and August. Data from one cloudy day were collected.

*Standardized nematode infectivity test:* A standardized nematode infectivity test that regularly provides 90–100% wax moth larval mortality in 72 hours was used (I. Pop-

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iel and P. Pruitt, Biosys, pers. comm.). This method used a nematode suspension of 100–120 third-stage infective juveniles in 2 ml of water applied to two filter papers in a petri dish (100 × 15 mm) into which 10 wax moth larvae were added. The resultant 90–100% wax moth larval mortality in 72 hours was calculated as "original activity" (OA). This OA was compared with mortality produced by the same number of UV-treated nematodes. Wax moth larval mortality produced in these treatments was then expressed as a percentage of the standard and called the original activity remaining (OAR).

*Testing of Tinopal LPW:* Between 100–120 nematodes were placed in 2–3 ml solution of either tap water or 1% Tinopal in Syracuse watch glasses. The watch glasses were positioned 47 cm below the sunlamp and exposed for periods of 5, 10, 15, 20, 25, 30, and 60 minutes and hourly to 8 hours. The depth of the solution in the Syracuse watch glasses never exceeded 2 mm. Similarly treated nematodes in watch glasses were exposed to direct sunlight in Beltsville, Maryland, for 15 and 30 minutes and half hourly to 4 hours. The Syracuse watch glasses were placed in a plastic pan with water that helped to reduce heating and loss of water due to the sunlamp and sunlight. The nematode suspension (in 2 ml) was then added to the petri dishes with the 10 *Galleria* larvae and held at room temperature in the dark. Mortality was determined at the end of 72 hours. There were five replications per treatment.

*Evaluation:* In all tests, the percentage of wax moth larval mortality caused by nematodes was the sole criterion for evaluation of UV protection. Mortality caused by nonirradiated *Steinernema* (in water) was compared with mortalities caused by irradiated *Steinernema* (in water and in Tinopal LPW) and nonirradiated *Steinernema* (in Tinopal LPW). In addition, UV protection was measured in terms of original activity remaining (OAR), after irradiation (5). $LC_{50}$ values were determined by probit analysis (1,2) at the 95% fiducial limits.

## Results and Discussion

The OAR was reduced from 100% to 0% when the nematodes in water were irradiated for 60 minutes under the sunlamp, and from 100% OAR to 2.0% OAR when irradiated by sunlight for up to 90 minutes. Tinopal LPW at 1% concentration was not toxic to the nematodes. Nematodes remained viable and infective for 1 week at room temperature. Nematodes in 2–3 ml of 1% Tinopal LPW were protected and maintained 100% of their OAR over periods of 8 hours under the sunlamp and 4 hours in direct sunlight (Table 1). Irradiation of an aqueous suspension of nematodes produced a $LC_{50}$ of 15.7 minutes (14.0–17.5) under the sunlamp and in about 31.7 minutes (27.6–36.3) in sunlight.

Table 1. Effect of UV light on the infectivity of *Steinernema carpocapsae* on wax moth larvae as influenced by Tinopal LPW.

| UV exposure (min) | Average % OAR† | |
|---|---|---|
| | Water | Tinopal LPW‡ |
| | Sunlamp | |
| 0 | 100 a | 100 a |
| 5 | 100 a | 100 a |
| 10 | 80 b | 100 a |
| 15 | 44 c | 100 a |
| 30 | 22 d | 100 a |
| 60 | 0 e | 100 a |
| 120 | 0 e | 100 a |
| 240 | 0 e | 100 a |
| 360 | 0 e | 100 a |
| 480 | 0 e | 100 a |
| | Sunlight | |
| 0 | 100 a | 100 a |
| 15 | 100 a | 100 a |
| 30 | 70 b | 100 a |
| 60 | 12 c | 100 a |
| 90 | 2 c | 100 a |
| 120 | 0 c | 100 a |
| 150 | 0 c | 96 a |
| 180 | 0 c | 100 a |
| 210 | 0 c | 100 a |
| 240 | 0 c | 100 a |

Means followed by the same letter are not significantly different ($P > 0.05$) by Duncan's multiple-range test.

† Original activity remaining was based on mortality of wax moth larvae before and after UV irradiation. Nematodes were used at a final concentration of 100–120 per ml, with 10 *Galleria* larvae used as hosts per treatment per each of five replicates.

‡ 1.0% aqueous.

On a cloudy day, the nematodes retained 100% of their OAR in water as well as in 1% Tinopal. This lends credence to the practice of spraying nematodes on cloudy days.

The fluorescent brightener Tinopal LPW is commonly used in soaps, detergents, bleaches, and fabric softeners. Tinopal LPW absorbs ultraviolet energy and converts it to visible light (11). In the present study, Tinopal LPW appeared to act as a very effective radiation protectant and provided complete protection for nematodes for up to 8 hours in the laboratory and 4 hours in full sunlight. Moreover, the material appeared to be harmless to both the nematode and its associated bacteria, as indicated in the wax moth larval mortality studies.

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