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Solarization for Nematode Disinfestation of Small Volumes of Soil[1]

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Abstract: Several polyethylene plastics were evaluated as potential materials for disinfesting small volumes of soil containing nematodes. *Bursaphelenchus seani,* cultured on the fungus *Monilinia fructicola* in petri dishes, was used to bioassay the survival and reproductive capability of nematodes buried 7.5 cm deep in Margate fine sand (soil moisture = 4.9%). The soil was exposed to sunlight for 6 days in May 1987. The highest mean temperatures recorded at 7.5 cm deep were 38 ± 1 C, 43 ± 1 C, 43 ± 1 C, and 50 ± 1 C for the no plastic, clear plastic, black plastic, and clear + black plastic treatments, respectively. The temperature in the clear + black plastic treatment exceeded 47 C for more than 2 hours on clear days. Nematode survival averaged 98 ± 3%, 78 ± 22%, 38 ± 38%, and 0 ± 0%, whereas the reproductive success of *B. seani* following treatment was 100, 100, 75, and 0% for the no plastic, clear plastic, black plastic, and clear + black plastic treatments, respectively. *Bursaphelenchus seani* in petri dishes and *Belonolaimus longicaudatus* and *Hoplolaimus galeatus* in soil died when exposed to 48 ± 2 C for 2 hours.

Key words: Belonolaimus longicaudatus, Bursaphelenchus seani, Hoplolaimus galeatus, lance nematode, physical control, potted plant, preplant control, solarization, sting nematode.

Exclusion of phytoparasitic nematodes from potted plants requires use of nematode-free planting stock and nematode-free soil, as well as isolating the pots from nematode-infested soil, water, or plants (1,5). An important step in this form of cultural management is disinfestation of the potting medium before planting. Commercial nurseries and universities often use steam or methyl bromide fumigation, but these are generally unavailable to the homeowner (1,5).

Soil solarization is being used with variable success for the control of certain soil-inhabiting nematodes and pathogens in field conditions (7–10,12,13). Little has been done to apply solarization to small volumes of soil for control of nematodes (5), even though conditions for solarizing small volumes of soil can be easily controlled. South Florida, like much of the southwestern United States, receives an annual mean daily solar radiation exposure of 450 g cal/cm², with the months of April through August receiving mean daily radiation levels of at least 500 g cal/cm² (5). These spring–summer solar radiation levels are high enough that solarization of small volumes of soil should be feasible (5).

The stylet bearing mycophagous nematode, *Bursaphelenchus seani* Giblin and Kaya (Aphelenchoididae) (3), was chosen to evaluate solarization efficacy because of its fast generation time (4.2 days at 25 C). Also, it destroys mycelia within 7–14 days after inoculation causing a reduction in sporulation and a characteristic sheen to appear on cultures of susceptible fungi, whereas uninoculated fungal plates appear feathery (3,4). These differences in fungal host response make *B. seani* a quick and reliable agent to bioassay whether a physical treatment, such as heat or radiation, alters the ability of the test nematode to reproduce. Aphelenchoidids are commonly encountered in Florida soils, and several species are known to cause severe problems to ornamental plants (11). Our objective was to evaluate the efficacy of three different plastic cover combinations for a simple solarization unit to pasteurize soil with *B. seani* as a bioassay organism.

MATERIALS AND METHODS

Bursaphelenchus seani was maintained monoxenically on *Monilinia fructicola* (Wint.) Honey on 5% glycerol-supple-

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mented potato dextrose agar (v/v) (GPDA) at 25 C (3,4). Cultures for bioassay purposes were established by inoculating sterile plates (100 × 15 mm) of GPDA with *M. fructicola* 2 weeks before aseptic inoculation with *B. seani*. *Bursaphelenchus seani–M. fructicola* plates were double wrapped with Parafilm "M" (American Can, Greenwich, CT) and stored for 2 weeks at 25 C before solar testing.

Wooden boxes (52 × 60 × 14 cm inside dimension) without bottoms were lined with 4-mil clear polyethylene plastic. A hole in the side 7.5 cm from the top provided thermometer access for temperature measurements.

Field soil (Margate fine sand—96% sand, 3% silt, 1% clay, 3% organic matter; pH 7.1) was dug and mixed, and three random samples were taken for pretreatment soil moisture measurement. Soil moisture was measured gravimetrically by comparing soil weights before and after oven drying at 60 C for 48 hours. Boxes were arranged in four rows 0.6 m apart on unshaded asphalt and replicated four times for each treatment. Each box was filled to the 6.5-cm level with compacted soil, and three plates of *B. seani*, each in a sealed plastic bag (15 × 26 cm), were randomly positioned on the soil. An additional 7.5-cm layer of compacted soil was used to cover the plates. The top and sides of filled boxes were covered with one layer of 4-mil clear polyethylene plastic, one layer of 6-mil black polyethylene plastic, one layer of 6-mil black polyethylene covered by one layer of 4-mil clear polyethylene plastic, or no plastic covering. Three additional plates of *B. seani* (without bags) were randomly positioned at the 7.5-cm level in the four replicates of the clear + black plastic treatment. A control without a plastic covering was also set up as described and placed indoors.

Temperatures at 7.5 cm deep were recorded every 2 hours starting at 0800 through at least 1800 hours daily for 6 days beginning 25 May 1987. The experiment was concluded on 31 May. Daily solar radiation output was measured by Solar Testing Service with an Epply pyranometer and integrator above the threshold of 0.08 Langleys (g cal/cm^2). Air temperature at the site was measured with a recording thermograph and thermometer. Following solarization, coverings were removed and three random soil samples (500 cm^3) per unit were analyzed for water content. Nematode bioassay plates were removed and survival of *B. seani* was determined from four 2-cm-d cores taken from each plate. Two cores were placed in distilled water for 1 hour and ≥ 50 nematodes per plate were probed for a movement response. The remaining two cores were inoculated onto a 2-week-old monoxenic culture of *M. fructicola* on GPDA, held at 25 C, and observed for reduction of fungal mycelia, nematode growth, and reproduction at 7 and 14 days. The identity of the nematodes was confirmed to insure that saprophytic nematodes or mites had not entered from the soil and colonized the plate.

Bursaphelenchus seani, *Belonolaimus longicaudatus* Rau, and *Hoplolaimus galeatus* (Cobb) Thorne were placed in an incubator set at 48 ± 2 C to determine the length of time they survived. Cultures of *B. seani* were incubated for 2, 4, and 6 hours, and four plates for each time period were assayed for survival and reproduction and compared with three control cultures kept at 25 ± 2 C. Margate fine sand (300 cm^3; soil moisture ca. 5%) from turfgrass plots with high levels of *B. longicaudatus* (> 150/100 cm^3) and *H. galeatus* (> 600/100 cm^3) was placed into 500-cm^3 vented Nalgene containers and incubated for 2 hours at 48 C. Nematodes were extracted from the incubated soil (100-cm^3 subsample) using the centrifugal-flotation technique (6), and mortality was assessed by probing and compared with controls left at 25 C. The experiment was repeated three times.

In a separate experiment, soil temperatures at 2.5, 7.5, and 11.5 cm deep in soil boxes without covering were compared with those covered with clear + black plastic. Offset holes were drilled into the side of each unit for access to the soil for temperature readings. Each treatment consist-

TABLE 1. Effect of different polyethylene plastics on the maximum mean daily soil temperature at 7.5 cm deep during 6 days (25–30 May 1987) of solarization.

	Soil temperature† (± standard deviation)					
	25 May	26 May	27 May	28 May	29 May	30 May
	31 C	29 C	31 C	31 C	31 C	30 C
Treatment‡	610	606	659	473	501	483
NP	38 ± 1 c	37 ± 1 c	37 ± 1 c	34 ± 1 d	35 ± 1 c	35 ± 1 c
CP	43 ± 1 b	42 ± 1 b	42 ± 1 b	37 ± 1 c	41 ± 1 b	40 ± 1 b
BP	43 ± 1 b#	42 ± 1 b	42 ± 1 b	39 ± 1 b#	41 ± 1 b	39 ± 1 b
CB	48 ± 1 a	50 ± 2 a	50 ± 1 a	43 ± 1 a	47 ± 2 a	45 ± 1 a

Values below each date are maximum air temperatures and solar radiation levels.

Means in a column followed by the same letter are not significantly different according to the Student-Newman-Keuls multiple-range test ($P > 0.05$).

† Recorded at 1600 hours except # (1800 hours).

‡ NP = no plastic covering, CP = one layer of 4-mil clear polyethylene plastic, BP = one layer of 6-mil black polyethylene plastic, and CB = one layer of black + one layer of clear polyethylene plastic.

ed of four randomized units. The experiment was conducted on 25 June 1987. Temperatures were measured as described.

Temperature and nematode survival data were subjected to analysis of variance (ANOVA) and a Student-Newman-Keuls multiple-range test for separation of means.

RESULTS AND DISCUSSION

Pretreatment soil moisture (4.9 ± 0.5%) remained relatively constant throughout the experiment. Respective soil moisture levels were 6.0 ± 0.6%, 6.3 ± 1.5%, 5.3 ± 1.4%, and 4.0 ± 0.8% for the no plastic, clear plastic, black plastic, and clear + black plastic treatments at the end of the experiment. The clear + black plastic treatment had a lower soil moisture level ($P < 0.05$) than the other treatments, suggesting that the higher temperatures generated under the unit might have increased the rate of water loss. Soil moisture is an important factor in pasteurizing soil and sand because they are relatively poor conductors of heat (7).

The clear + black plastic treatment had a higher ($P < 0.05$) mean daily soil temperature at 7.5 cm deep than did the other treatments (Table 1). The clear plastic and black plastic treatments did not differ from each other on 5 of 6 days (Table 1). The highest temperatures recorded at 7.5 cm deep for the no plastic, clear plastic, black plastic, and clear + black plastic treatments were 8, 13, 13, and 21 C above ambient temperature.

On 26 May the solar radiation reading was 606 g cal/cm^2, and there were a few scattered clouds. The clear + black plastic treatment produced higher temperatures throughout the day than did any of the other treatments ($P < 0.05$) (Fig. 1). Maximum temperatures were achieved sometime between 1330 and 1600 hours for all treatments. Between 1400 and 1800 hours temperatures above 47 C were recorded under the clear + black plastic, whereas they were 40 C under the clear plastic and black plastic treatments, and 36 C under no plastic. Solarization in nursery beds where two, one, and no layers of clear plastic were used reached soil temperatures of 60, 47.5, and 32.2 C at 10 cm (10).

Survival and reproduction of *B. seani* occurred in all treatments except clear + black plastic which had none (Fig. 2). One hundred percent growth and reproduction occurred 7 and 14 days after treatment and subculture in the control, no plastic, and clear plastic treated units. Soil temperatures for the indoor control unit ranged between 20 and 25 C. Reproduction occurred in 58% and 75% of bioassay cultures in the black plastic treatment at 7 and 14 days after inoculation (Fig. 2).

Bursaphelenchus seani survived and reproduced better under the clear plastic than under the black plastic (Fig. 2). The black plastic was used to examine its ability to

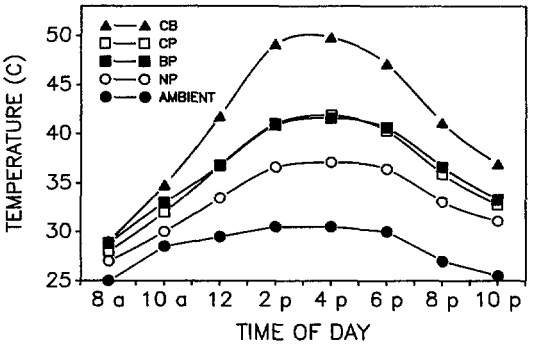

FIG. 1. Mean hourly soil temperatures measured 7.5 cm deep for 26 May 1987. AMBIENT = ambient temperature; NP = no plastic; CP = one layer of clear plastic; BP = one layer of black plastic; CB = one layer of black plastic + one layer of clear plastic. The mean solar radiation output for this day was 606 g/cal cm² (5° Langleys).

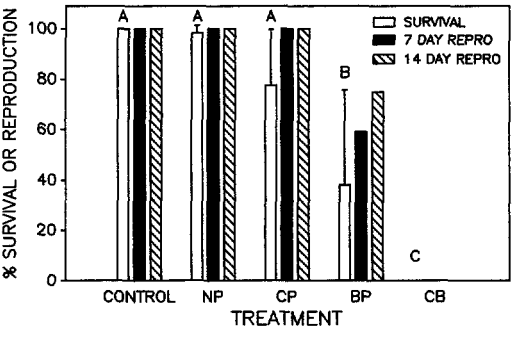

FIG. 2. Effect of solarization treatments on survival and reproduction of *Bursaphelenchus seani* in petri dishes buried in soil 7.5 cm deep. SURVIVAL = percentage survival; bars with the same letter are not significantly different based on a Student-Newman-Keuls multiple-range test comparison ($P > 0.05$); 7 day repro = percentage of reproduction 7 days after inoculation; 14 day repro = percentage of reproduction 14 days after inoculation. Control = no plastic, no solar exposure; NP = no plastic; CP = one layer of clear plastic; BP = one layer of black plastic; CB = one layer of black plastic + one layer of clear plastic.

absorb solar radiation (7). Although the temperature differences under the two were small, they may have been critical to the survival of *B. seani.* A single layer of clear plastic was more effective than black plastic at reducing the encysted juvenile stage of *Globodera rostochiensis* (Woll.) Behrens in field plots (8).

In other studies (4), *B. seani* could not survive incubation at 36 C for 3 weeks, but it cycled through one generation in 3.1 days at 33 C. *Bursaphelenchus seani* tolerated a maximum daily soil temperature between 34 and 38 C for 6 days in the no plastic treatment (Table 1). In the clear plastic and black plastic treatments, exposure of *B. seani* to temperatures between 37 and 43 C for 4 hours a day for 6 days decreased the ability of the nematodes to survive and reproduce; however, the temperatures were not eradicative.

The enclosure of the nematode cultures in sealed plastic bags under the clear + black plastic did not alter the ability of the nematodes to survive. Also, no mites or foreign nematode species were found in the sealed plates.

Bursaphelenchus seani did not survive or reproduce after incubation at 48 ± 2 C for 2, 4, or 6 hours. Survival and reproduction was 100% in plates maintained at 25 C for 6 hours. None of the *B. longicaudatus* or *H. galeatus* recovered from the soil held at 48 ± 1 C for 2 hours responded to probing at time of harvest or 24 hours later. Over 90% of *B. longicaudatus* and *H. galeatus* from the control soil were alive. This demonstrates that the *B. seani* culture plate bioassay results were applicable to two important phytoparasitic nematodes.

In the temperature × depth experiment (Fig. 3), the maximum temperature was recorded at 1400 hours and the day was partly cloudy with a radiation reading of 560 g cal/cm². The maximum mean temperature (60 C) was recorded in the top 2.5 cm under the clear + black plastic. The temperatures at 7.5 (47 C) and 11.5 cm (44 C) were not different under the clear + black plastic ($P < 0.05$); however, 44 C for 4 hours could be below the lethal temperature threshold for most nematodes. Temperature limits for different nematode species vary and can be influenced by the developmental stage and other factors such as artificial selection (2). *Aphelenchoides fragariae* (Ritzema Bos) Christie can be controlled with a hot water treatment of 47 C for 30 minutes or 48 C for 20 minutes (11). The minimum lethal exposures to hot water for *Heterodera glycines* Ichinohe were 30 minutes at 49 C or 4 hours at 46 C, 25

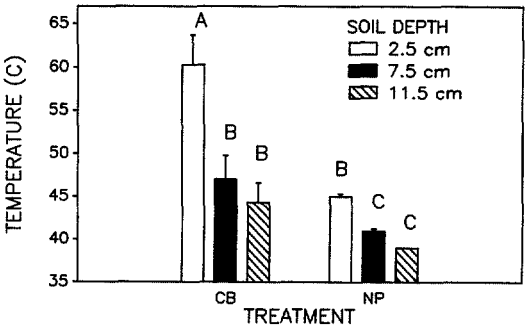

FIG. 3. Maximum mean soil temperatures for solarization treatments recorded at three depths. Bars with the same letter are not significantly different based on a Student-Newman-Keuls multiple-range test comparison ($P > 0.05$). CB = one layer of black + one layer of clear plastic; NP = no plastic.

minutes at 49 C or 4 hours at 46 C, and 15 minutes at 49 C or 30 minutes at 46 C for juveniles in cysts, free eggs, and free juveniles, respectively (2). These results and the results from our study suggest that exposure to a constant temperature above 46 C for 4 hours should be above the minimum lethal temperature exposure necessary for disinfestation of soil of many phytoparasitic nematodes. Since the clear + black plastic treatment achieved temperatures over 47 C for 4 hours on days receiving over 600 g cal/cm^2 (Fig. 1), a home solarization unit constructed to a depth of less than 7.5 cm with this treatment should be eradicative to most nematodes.

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