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Influence of Soil pH and Oxygen on Persistence of *Steinernema* spp.[1]

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Abstract: Survival of infective juveniles of *Steinernema carpocapsae* and *Steinernema glaseri* gradually declined during 16 weeks of observation as the tested soil pH decreased from pH 8 to pH 4. Survival of both species of *Steinernema* dropped sharply after 1 week at pH 10. Survival of *S. carpocapsae* and *S. glaseri* was similar at pH 4, 6, and 8 during the first 4 weeks, but *S. carpocapsae* survival was significantly greater than *S. glaseri* at pH 10 through 16 weeks. *Steinernema carpocapsae* and *S. glaseri* that had been stored at pH 4, 6, and 8 for 16 weeks, and at pH 10 for 1 or more weeks were not infective to *Galleria mellonella* larvae. *Steinernema carpocapsae* survival was significantly greater than that of *S. glaseri* at oxygen : nitrogen ratios of 1:99, 5:95, and 10:90 during the first 2 weeks, and survival of both nematode species declined sharply to less than 20% after 4 weeks. Survival of both nematode species significantly decreased after 8 weeks as the tested oxygen concentrations decreased from 20 to 1%, and no nematode survival was recorded after 16 weeks. *Steinernema carpocapsae* pathogenicity was significantly greater than that of *S. glaseri* during the first 2 weeks. No nematode pathogenicity was recorded at oxygen concentrations of 1, 5, and 10% after 2 weeks and at 20% after 16 weeks.

Key words: entomopathogenic nematode, pathogenicity, *Steinernema carpocapsae, Steinernema glaseri,* survival.

Steinernematid nematodes possess many key attributes for biological control, including a broad host range, high virulence, high power of host seeking, ease of mass production, and safety. Field tests, however, have frequently produced inconsistent and discouraging results, particularly against foliage-feeding pests, because of poor nematode persistence in exposed habitats (5,8). A more suitable target habitat, soil, is the natural reservoir for steinernematid nematodes, as it provides shelter from environmental extremes and offers the potential for establishment and recycling.

Steinernema glaseri (Steiner), the only steinernematid species considered to be tropical or subtropical in origin; and *Steinernema carpocapsae* (Weiser), widely distributed in temperate areas of the world (13), have been extensively tested against soil pests. Soil applications of these two nematodes have provided encouraging, although often unpredictable, results (6). Soil factors influencing their successful use are poorly understood. Scant information is available on the effects of soil pH and oxygen on nematode persistence and efficacy. Information is needed on the relationship between soil abiotic factors and nematode persistence before steinernematid nematodes can be used effectively against soil insects. The objectives of this research were 1) to determine the interraction between soil pH and oxygen and the persistence of *S. carpocapsae* and *S. glaseri* and 2) to provide information on optimal and threshold conditions for the persistence of these nematodes in soil.

MATERIALS AND METHODS

Infective juveniles of *S. carpocapsae* (All strain) and *S. glaseri* were obtained from BIOSYS (Palo Alto, CA). Infective juveniles of both nematodes were produced in last-instar larvae of *Galleria mellonella* (L.) using methods described by Dutky et al. (4). Stock suspensions of infective juveniles were stored in 0.1% formaldehyde solution at 7 C for 2 weeks before the tests were conducted.

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Soil pH: Sandy loam soil, pH 6, was collected on the Cook College Campus of Rutgers University, New Brunswick, New Jersey. Soil was air dried, sieved through a 2-mm-pore sieve, and heated to 121 C for 24 hours. Soil pH was adjusted to pH 4, 8, and 10 by adding 3.75 ml glacial acetic acid, 37.50 g pulverized lime, and 1.88 g sodium carbonate, respectively, to 75 g soil. Water was then added to the soil in a water : soil ratio of 1:1 (v/v). The soil-water mixture was stirred to make a slurry, the soil suspension was allowed to settle for 1 week, and the pH was measured with a pH meter at the supernatant–soil interface to give pH readings of 4, 8, and 10 as desired. Soil samples with pH readings of 4, 6, 8, and 10 were recovered by air drying and sifting to uniform texture after 5–6 weeks and heated to 121 C for 24 hours prior to the test. Soil moisture was adjusted to 12% (= 50% field capacity for this soil) in the pH tests.

Soil test units were made of plastic tubes (5 cm high × 4 cm i.d.), each holding 75 g soil (dry weight), with nylon cloth (39-μm-pores) glued to the bottom. Humidity chambers were rectangular plastic boxes (16.5 × 31 × 8.5 cm). Perforated aluminum support plates (15 × 29 cm) were placed inside the humidity chambers and suspended 2.5 cm above the box bottom with rubber stoppers. Water (300 ml) was added to a depth of 1 cm to provide 100% RH.

Approximately 3,500 infective juveniles suspended in 1.5 ml distilled water were placed on the soil surface of each test unit with a pipet. Twelve test units (three replicates of four soil pH) were placed on the support plate of each humidity chamber. The chambers were then sealed with polyethylene tape and incubated at 25 C. One chamber was opened to assess survival and one was opened to assess pathogenicity at 1, 2, 4, 8, and 16 weeks postinoculation.

Soil oxygen: To determine the effects of soil oxygen on nematode persistence, different soil oxygen levels were established and maintained in closed systems. Serum bottles (100 ml) containing 75 g soil and sealed with rubber flange stoppers and aluminum seals were used as the test units. Approximately 3,500 infective juveniles suspended in 1.5 ml distilled water were placed on the surface of the test soil (sandy loam with 50% field capacity = 12% moisture, pH 5.8) in each bottle. Different oxygen concentrations were provided by commercial oxygen : nitrogen mixture tanks at ratios of 20:80, 10:90, 5:95, and 1:99. To increase humidity, each gas mixture was passed through tubing into a water bath consisting of a 500-ml Erlenmeyer flask. The gas flowed to a Y connection leading to syringe needles inserted through the stoppers of two empty, sealed serum bottles. These two bottles were used as reservoirs to distribute gases to test bottles. Six additional syringe needles inserted through the stoppers of each reservoir bottle led through tubing to each of 12 sealed serum test bottles containing soil and nematodes. Gas mixtures were infused into the test bottles through 3.7-cm-long syringe needles which penetrated the rubber stoppers and reached to the bottom of the soil to insure complete flushing. An oxygen analyzer, connected to one of the test bottles with a syringe needle and tubing, was used to monitor the oxygen concentration until it stabilized at the required concentration for 5 minutes. All syringes were then removed from the test bottles and the bottles were incubated at 25 C.

Nematode survival: A centrifuge flotation technique was used to extract nematodes from soil (7,9). Soil from each pH test unit or each oxygen test bottle was placed in a 1,000-ml beaker and 700 ml water was added. The mixture was roiled and allowed to settle for 1.5 minutes. The soil suspension was rinsed through 420-μm-pore and 37-μm-pore sieves. The filtrate was rinsed into four 15-ml centrifuge tubes and spun at 4,800 rpm (500 *g*) for 10 minutes, and the supernatant was discarded. The best efficiency of extraction for *S. carpocapsae* was 52 ± 2% with a 45% sucrose solution; for *S. glaseri* it was 65 ± 3% with a 75% sucrose solution. The above sucrose concentrations were added to the soil, the contents

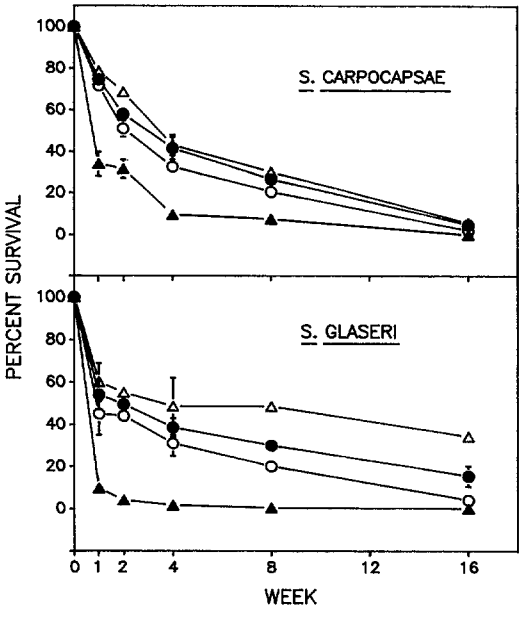

FIG. 1. Mean survival of infective juveniles of *Steinernema carpocapsae* and *S. glaseri* at soil pH 4 (○), pH 6 (●), pH 8 (△), and pH 10 (▲) over 16 weeks. Brackets indicate standard errors.

were agitated by Vortex, and the suspension was centrifuged at 3,840 rpm (400 *g*) for 10 minutes. The supernatant was washed through 5-μm membrane filters, and the extracted nematodes were washed into a counting dish (60 × 15 mm) and counted with a dissecting microscope. The following formula provided an estimate of mean percentage nematode survival:

$$\frac{\text{Live nematodes extracted}}{\text{Nematodes inoculated} \times \text{extraction efficiency}}$$

Nematode pathogenicity: Nematode pathogenicity in the pH and oxygen tests was determined by the ability of surviving infective juveniles to kill *G. mellonella* larvae (9). Bioassays were conducted by placing soil from each pH test unit and each oxygen test bottle into petri dishes (150 × 25 mm). Ten last-instar larvae of *G. mellonella* were placed into each petri dish, and dishes were held at 25 C for 1 week. Insect cadavers were dissected; those containing nematodes were recorded as nematode-induced mortality, and those turning black and not containing nematodes were recorded as control mortality.

Experimental design: In soil pH tests, nematode survival and pathogenicity were determined by extraction and bioassay at exposure times of 1, 2, 4, 8, and 16 weeks, so that five humidity chambers were prepared for survival tests and five for pathogenicity tests for each species. In soil oxygen tests, nematode survival and pathogenicity were determined at exposure times of 1, 2, 4, 8, and 16 weeks, so that a total of 120 bottles (4 oxygen concentrations × 5 exposure times × 2 species × 3 replicates) were prepared for survival tests and 120 bottles for pathogenicity tests. The design was a 4 × 5 × 2 factorial with four treatments, five exposure times, and two species as the main factors. Data were analyzed with SAS (ANOVA and GLM) (14).

RESULTS

Soil pH: Survival of *S. carpocapsae* and *S. glaseri* infective juveniles was significantly different ($F = 13, P < 0.01$) at the four soil pH ($F = 147, P < 0.01$) over the 16-week test period (Fig. 1). Survival of *S. carpocapsae* was lower than that of *S. glaseri* at pH 8 after 8 weeks ($P < 0.05$) and 16 weeks ($P < 0.01$).*Steinernema carpocapsae* survival was significantly greater than *S. glaseri* at pH 10 throughout the 16 weeks (Fig. 1). Survival of both nematode species decreased gradually as the tested soil pH decreased from pH 8 to pH 4 throughout the 16 weeks; however, it declined drastically at pH 10 after 1 week. Survival was less at pH 10 than at any other pH over the 16-week test period (Fig. 1). *Steinernema carpocapsae* and *S. glaseri* survived best at pH 8, followed by pH 6 and pH 4, whereas survival dropped sharply when soil became highly alkaline (pH 10) (Fig. 1).

Pathogenicity of *S. carpocapsae* differed from that of *S. glaseri* ($F = 268, P < 0.01$) in the four soil pH ($F = 527, P < 0.01$) (Fig. 2). *Steinernema carpocapsae* pathogenicity was lower than *S. glaseri* at pH 4 and pH 6 after 2 weeks, but it sharply declined after 4 and 8 weeks. Moreover, *S. carpocapsae* pathogenicity was lower than *S. glaseri* at pH 8 throughout the 16 weeks.

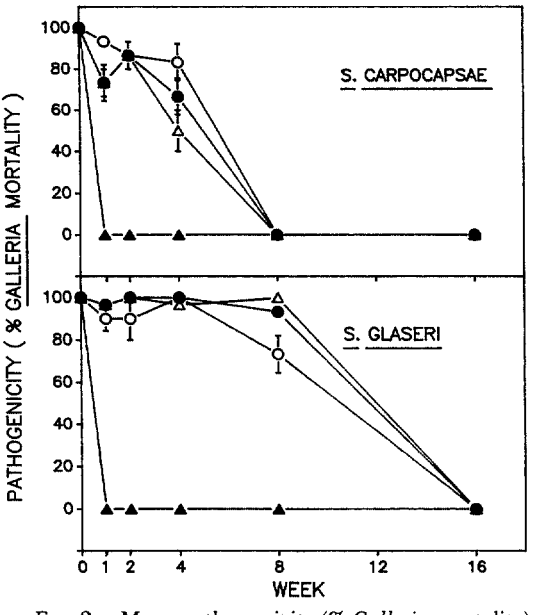

FIG. 2. Mean pathogenicity (% *Galleria* mortality) of infective juveniles of *Steinernema carpocapsae* and *S. glaseri* at pH 4 (○), pH 6 (●), pH 8 (△), and pH 10 (▲) over 16 weeks. Pathogenicity is measured by the ability of infective-stage juveniles to cause lethal host infection. Brackets indicate standard errors.

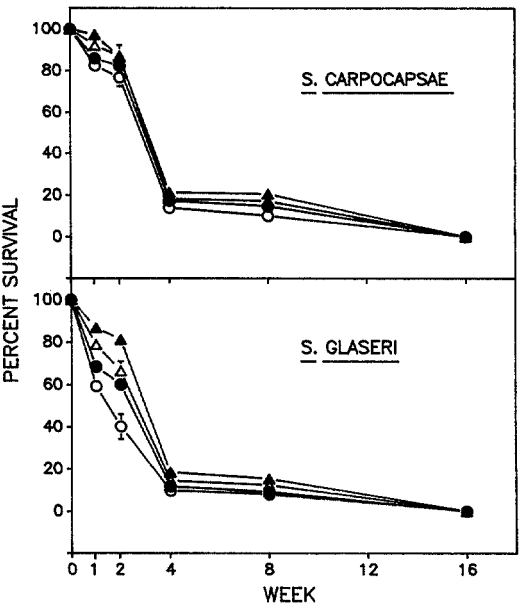

FIG. 3. Mean survival of infective juveniles of *Steinernema carpocapsae* and *S. glaseri* at soil oxygen concentrations of 1% (○), 5% (●), 10% (△), and 20% (▲) over 16 weeks. Brackets indicate standard errors.

Pathogenicity of *S. carpocapsae* and *S. glaseri* decreased as the tested soil pH decreased from pH 8 to pH 4 after 4 and 8 weeks, respectively, and no pathogenicity was observed after 16 weeks (Fig. 2). Pathogenicity assays showed that *S. carpocapsae* was significantly less pathogenic than *S. glaseri* at pH 4, 6, and 8, over the test period. Throughout the 16 weeks, neither nematode species killed *G. mellonella* larvae at pH 10 (Fig. 2).

Soil oxygen: Survival of *S. carpocapsae* and *S. glaseri* was significantly different ($F = 167, P < 0.01$) at the four oxygen concentrations ($F = 57, P < 0.01$) over the 16-week test period (Fig. 3). *Steinernema carpocapsae* survival was greater than *S. glaseri* survival at oxygen concentrations of 1, 5, and 10% during the first 2 weeks. Nematode survival declined sharply to less than 20% with no significant differences among oxygen concentrations after 8 weeks. No nematode survival was observed at any soil oxygen level tested after 16 weeks. Survival of the two nematode species at 20% oxygen was not significantly different throughout the 16 weeks, but survival decreased as the tested soil oxygen levels decreased from 20 to 1% after 8 weeks, and no nematodes survived after 16 weeks (Fig. 3).

Significant differences in pathogenicity between *S. carpocapsae* and *S. glaseri* ($F = 189, P < 0.01$) at the four oxygen concentrations ($F = 14, P < 0.01$) were observed (Fig. 4). *Steinernema carpocapsae* pathogenicity was significantly greater than that of *S. glaseri* during the firsts 2 weeks ($P < 0.05$). No nematode pathogenicity was recorded at oxygen concentrations of 1, 5, and 10% after 2 weeks or at 20% after 16 weeks (Fig. 4). Nematode pathogenicity (Fig. 4) was correlated closely with nematode survival (Fig. 3) in different oxygen concentrations. The ability of the two nematodes to cause lethal infection decreased as the tested soil oxygen decreased from 20 to 1% throughout the 16 weeks.

DISCUSSION

The effects of soil pH on survival and pathogenicity of plant-parasitic nematodes and insect-parasitic nematodes have been

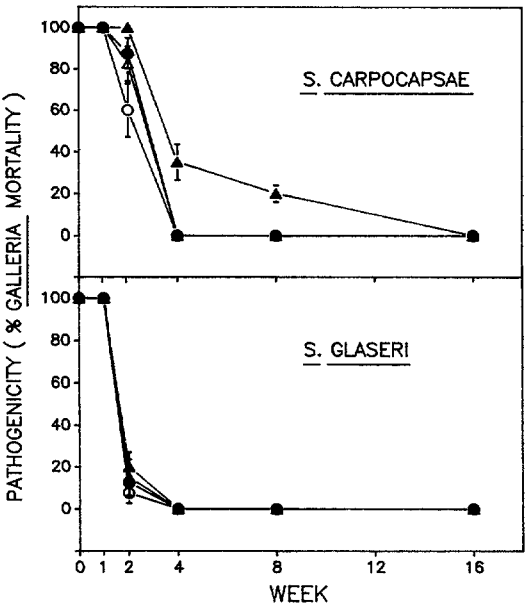

FIG. 4. Mean pathogenicity (% *Galleria* mortality) of infective juveniles of *Steinernema carpocapsae* and *S. glaseri* at soil oxygen concentrations of 1% (○), 5% (●), 10% (△), and 20% (▲) over 16 weeks. Pathogenicity is measured by the ability of infective-stage juveniles to cause lethal host infection. Brackets indicate standard errors.

studied very little. Banage and Visser (1) observed that *Dorylaimus* sp. survived a few minutes at pH 1.0–2.5, a few hours at pH 3.0–6.0, and several days at pH 6.5–10. Morgan and MacLean (12) reported that *Pratylenchus penetrans* survived over the pH spectrum of most agricultural soils (pH 5.1–6.5), and survived best at pH 5.5–5.8, but soil pH above 6.6 showed a nematicidal effect. No information is available on the effect of soil pH on survival and pathogenicity of entomopathogenic nematodes. In our study, steinernematid nematode survival and pathogenicity decreased only slightly as the tested soil pH decreased from pH 8 to pH 4, but their survival drastically declined and they showed no ability to kill *G. mellonella* larvae at pH 10. Moreover, no marked nematicidal effect on the persistence of these two nematode species were observed when they were exposed to soils with a pH range of 4–8. These findings are in agreement with previous studies on plant-parasitic nematodes.

Our study suggests that steinernematid nematode persistence and efficacy is unlikely to be adversely affected at the pH spectrum of most agricultural soils. In highly alkaline soils (> pH 10), however, a nematicidal effect can be expected.

Oxygen content of soil air in topsoils is lower than that of atmosphere. Normal oxygen concentrations in topsoils were reported at 15.10% in New York, 20.40% in Iowa, and 20.65% in England (11). Oxygen percentage in the soil air decreased with depth, and the rate of decrease was much more rapid in silty clay loam than in sandy loam. The average oxygen content decreased from 20% to ca. 18% in sandy loam as soil depth increased from 1 foot to 5 feet. By comparison, the average oxygen content decreased from 20% to ca. 5% in silty clay loam as soil depth increased from 1 foot to 5 feet (2). Most previous studies on the influence of soil oxygen have been conducted on the survival of plant-parasitic nematodes. Stolzy et al. (15) and Van Gundy and Stolzy (17) reported that nematode survival decreased as soil oxygen decreased.

Reports on the effect of soil oxygen on entomopathogenic nematodes are more limited, although our soil study is in agreement with previously published information. Infective juveniles of *S. carpocapsae* survived at a very low oxygen concentration (0.5% of saturation) after 43 days (3). *Steinernema carpocapsae* infective juveniles died at a low oxygen concentration (0.5% ml/5 × 10^5 nematodes) (10). Our study showed that survival and pathogenicity of both nematode species decreased as the tested oxygen concentrations decreased from 20 to 1%.

Infective juveniles of many nematodes rely on their stored food reserves for the energy required to survive, locate hosts, and initiate infections. Soil oxygen is one factor regulating the amount of energy produced for nematode survival and pathogenicity. Under normal oxygen conditions, energy is generated effectively by oxidative processes using lipid consumption from food reserves. Some soil-inhabiting nematodes can live in low oxygen hab-

itats, but they survive these conditions by undergoing fermentation processes (16). However, energy generated by fermentation processes using carbohydrate consumption from stored food reserves is used up rapidly, resulting in low survival, motility, and pathogenicity.

Our results indicate that steinernematid nematodes would provide poor inoculative biocontrol in areas subject to frequent flooding or excessive irrigation (e.g., cranberry bogs); water-filled soil pores result in reduced oxygen availability, which may reduce nematode survival and pathogenicity and, therefore, establishment.

Our data further demonstrate that soil pH and available soil oxygen are important factors that must be considered when devising biological control strategies against soil insect pests. They also provide information on optimal and threshold conditions in the field where steinernematid nematodes can be used effectively in most agricultural soils with pH ranges not exceeding pH 10 and in well-aerated soils without excessive irrigation or frequent flooding.

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