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The Effects of Soil Type, Particle Size, Temperature, and Moisture on Reproduction of Belonolaimus longicaudatus[1]

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Abstract: Effects of soil type, particle size, temperature, and moisture on the reproduction of *Belonolaimus longicaudatus* were investigated under greenhouse conditions. Nematode increases occurred only in soils with a minimum of 80% sand and a maximum of 10% clay. Optimum soil particle size for reproduction of the Tarboro, N.C. and Tifton, Ga. populations of the nematode was near that of 120-370 μm (65-mesh) silica sand. Reproduction was greatest at 25-30 C. Some reproduction by the Tifton, Ga. population occurred at 35 C, whereas the Tarboro, N.C. population declined, as compared to the initial inoculum. Both populations reproduced slightly at 20 C. Nematode reproduction was greater at a moisture level of 7% than at a high of 30% or a low of 2%. Reproduction occurred at the high moisture level only when the nutrient solution was aerated. *Key Words:* nematode ecology, populations.

The geographic distribution of the sting nematode, *Belonolaimus longicaudatus* Rau, in the United States is limited primarily to the coastal plains of the southeastern states; however, it occurs as far north as Connecticut, in the Gulf States, and in the midland states of Arkansas, Kansas, and Oklahoma. This limited distribution suggests that ecological factors may be more specific for this pest than for most other nematodes.

Field observations and sampling (8, 9, 11) indicated that *B. longicaudatus* is limited to sandy soils. For example: in Virginia, Miller (9) found sting nematodes limited to the "A"-horizon of soils with a sand content of 84% - 94%, whereas in Georgia it was found only in the top 30 cm of soil containing 88% sand and 5% clay (3). Thames (11) postulated that fine-textured soils inhibit movement of *B. longicaudatus* and its ability to reproduce.

Most reports of temperature effects on sting nematode reproduction are limited to field observations. In weekly sampling over a 1-yr period at Tifton, Ga., sting nematodes were recovered in greatest numbers from June through September, when the soil temperature at 0-30 cm deep was above 20 C (3). Perry (10) observed that reproduction of *B. longicaudatus* was greater at 29.4 C than at 26.7 C, and was greatly reduced at 35 C. Boyd and Perry (2) concluded that this nematode either died or migrated downward when the soil temperature at 2.5 cm below the bare soil surface reached 39.5 C or higher. In greenhouse experiments, the sting nematode reproduced on, and damaged, cotton roots maintained at 30-32 C (6). Barker et al. (1) found that 13 C was the optimum storage temperature for survival of *B. longicaudatus* in soil, and that it declined rapidly at 36 C.

Soil moisture may also limit the distribution of *B. longicaudatus*. Miller (9) reported that sands in which this nematode is not normally found, apparently due to excessive drying, would support it when supplemented with water during dry periods. In Georgia, nematodes were most numerous when soil moisture averaged 15-20% by volume (3).

General ecological investigations of this pest were undertaken to evaluate the factors responsible for its limited distribution. Studies included the effects of soil particle size, temperature, and moisture on reproduction of sting nematode populations from North Carolina and Georgia.

MATERIALS AND METHODS

Soil type: Reproduction of the Tarboro, North Carolina, population of *B. longicaudatus*, was tested in the following "soils": clay, coarse

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TABLE 1. Soils used and their composition in relation to reproduction of the Tarboro, N.C. population of *Belonolaimus longicaudatus.*

Component[a] (%)	Muck[b]	Clay	Coarse sandy loam	Fine loamy sand	Beach sand	1:1 mix (FLS & 65-sand)	65-mesh silica sand
Clay	--	34	10	8	0	4	0
Silt	--	24	10	4	0	2	0
Sand	--	42	80	88	100	94	100

[a]Bouyoucos method (12) of soil analysis used.
[b]No soil analysis made, mainly organic matter.

sandy loam, fine loamy sand, beach sand, 120-370 μm (65-mesh) silica sand, a 1:1 mixture of fine loamy sand, and a mixture of 65-mesh silica sand and muck. The soils were fumigated 1 mo before use with 98% methyl bromide and 2% chloropicrin. The percentages of clay, silt, and sand were determined by the Bouyoucos method (12) for all soils except muck, for which no analysis was made because it consisted largely of organic matter and some sand (Table 1).

In the first of two experiments, four 6-day-old 'Lee' soybean [*Glycine max* (L.) Merr.] seedlings/pot were used. In the second experiment one 'Earlibelle' strawberry [*Fragaria virginiana* Duch.] plant/pot was used. Inoculum for both tests consisted of 1000 nematodes/20-cm pot. The nematode inoculum for all experiments was obtained from greenhouse cultures by decanting-sieving with 12.5 ppm Separan® (Dow Chemical Co., Midland, Mich.) added to the water to aid in obtaining cleaner nematode samples. Randomized complete block designs with five replications were used.

The experiments were terminated after 90 days. Soil from each pot was thoroughly mixed by passing it through a soil sample divider four times. The nematodes in a 100-g soil sample were extracted by a sugar-flotation-sieving method (4), which was used in all other experiments unless otherwise indicated.

Particle size: The effects of three sands of varying texture (fine, medium, and coarse) on reproduction of the Tarboro, N.C. population of *B. longicaudatus* on Earlibelle strawberry were investigated. The fine sand was 65-mesh silica sand with maximum diam mean of 245 μm (range 120-370 μm, number = 50). The medium sand was 35-mesh silica sand with maximum diam mean of 578 μm (range 255-1420 μm, number = 50). The coarse sand was washed river sand with maximum diam mean of 741 μm (range 50-3190 μm, number = 50). All conditions and procedures were the same as for the previously described soil type experiment except treatments were replicated four times.

Glass beads of various sizes also were used as growing media to test the effect of particle size on reproduction of the Tarboro, N.C. and Tifton, Ga. populations of this nematode on soybean. The glass beads (average diam 100, 180, 300, 500, and 1000 μm) were soaked overnight in dilute HCl followed by a thorough rinsing prior to use. Eight hundred grams of dry beads were placed in 1-liter plastic containers with filter paper placed over the drain holes. One 6-day-old Lee soybean seedling was transplanted to each container and inoculated

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FIG. 1. Influence of soil type, temperature and moisture on reproduction of the sting nematode, *Belonolaimus longicaudatus.* **A.** Reproduction of the Tarboro, N.C. population of this nematode on 'Lee' soybean in different soils. [Mean population 90 days after inoculation with 1,000 nematodes. Soil types tested were: muck, clay, coarse sandy loam (CSL), fine loamy sand (FLS), beach sand (BS), a 1:1 mixture of FLS and 65-mesh silica sand (1:1), and 65-mesh silica sand (65-S)]. **B.** Numbers of this population recovered from 'Earlibelle' strawberry growing in the above soils. **C.** Numbers of the Tarboro, N.C. population recovered from 'Lee' soybean in different textured sands 90 days after inoculation with 1,000 nematodes (sand textures tested were: fine = 65-mesh silica sand; medium = 35-mesh silica sand; and coarse = washed river sand). **D.** Effects of soil temperature on the reproduction of Tarboro, N.C. and Tifton, Ga. sting nematode populations. (Mean population 60 days after inoculation of 'Pioneer 3369 A' corn with 1000 nematodes; the interaction LSD may be used for comparisons in either direction). **E.** Numbers of nematodes recovered from soybean at different moisture levels (from 500 g of 65-mesh silica sand 5 wk after inoculation with 50 adults of each sex) and **F.** Moisture characteristics for the 65-mesh silica sand without host.

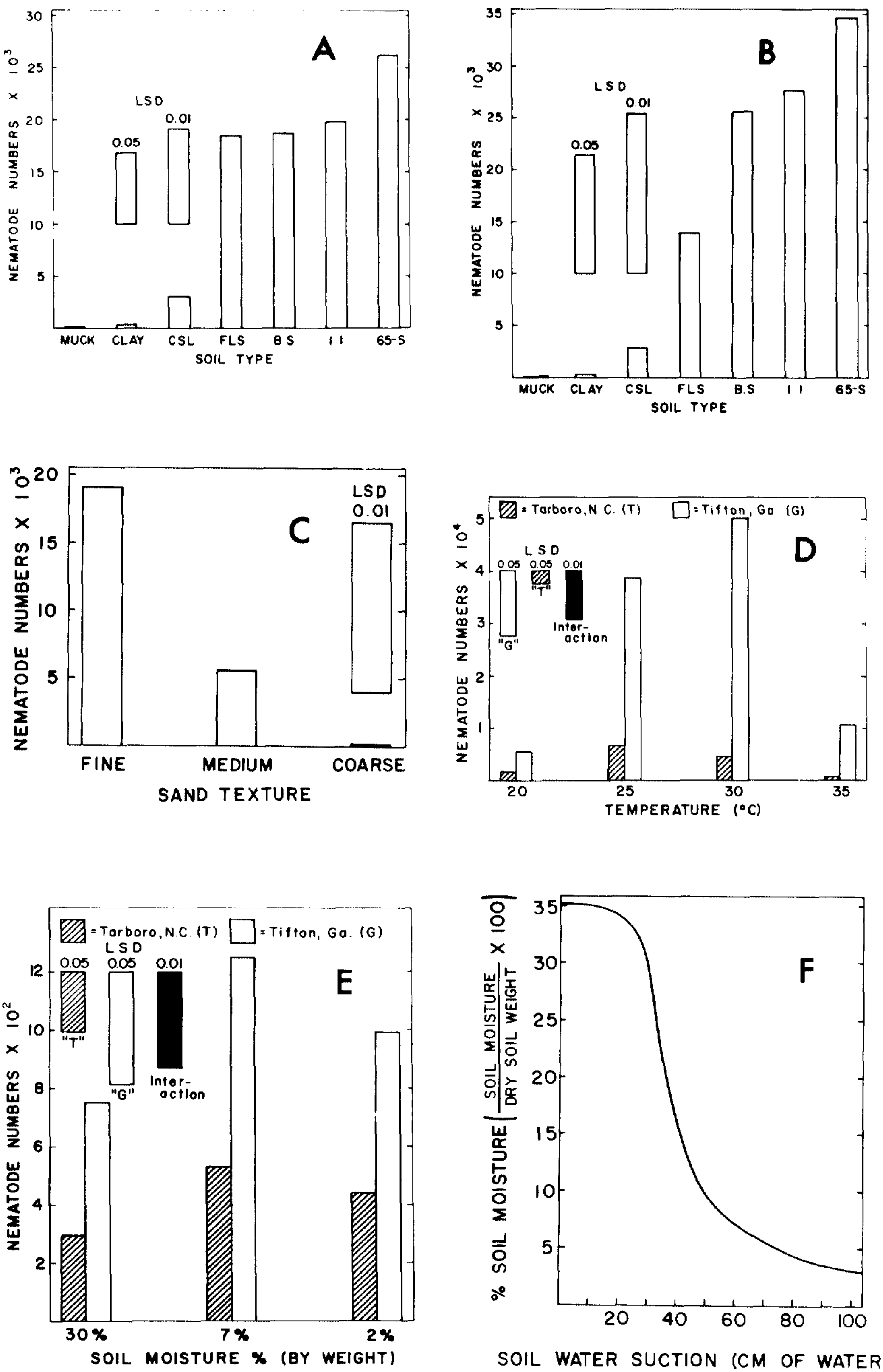
A
B
C
D
E
F
LSD
0.05
0.01
NEMATODE NUMBERS X 10^3
MUCK
CLAY
CSL
FLS
B S
I I
65-S
SOIL TYPE
FINE
MEDIUM
COARSE
SAND TEXTURE
= Tarboro, N.C. (T)
= Tifton, Ga (G)
"G"
"T"
Inter-action
NEMATODE NUMBERS X 10^4
TEMPERATURE (°C)
NEMATODE NUMBERS X 10^2
30%
7%
2%
SOIL MOISTURE % (BY WEIGHT)
% SOIL MOISTURE (SOIL MOISTURE / DRY SOIL WEIGHT X 100)
SOIL WATER SUCTION (CM OF WATER)

TABLE 2. Influence of glass bead size on the reproduction of *Belonolaimus longicaudatus.*

Nematode population	Mean nematode numbers at the indicated glass bead diam (μm)[a]				
	1000	500	300	180	100
Tarboro, N.C.	8	17	64	141	136
Tifton, Ga.	18	28	318	364	197
LSD: ($P = 0.05$) = 41; ($P = 0.01$) = 55					

[a]Mean of four replicates, 28 days after inoculation, initial inoculum 25 adults of each sex on 'Lee' soybean.

with 50 handpicked adult nematodes, 25 of each sex. Each container with plants was weighed daily and maintained at 70% "field-capacity" with 0.2 N Hoagland's solution (13). The field-capacity for each bead size was determined by saturating the beads with water, allowing them to drain 12 h and calculating the weight of water retained. Final numbers of nematodes were determined by the decanting-sieving procedure after 4 wk.

Soil temperature: The influence of soil temperature on reproduction of the Tarboro, N.C. and Tifton, Ga. populations of *B. longicaudatus* were determined on corn, *Zea mays* L. 'Pioneer 3369A'. Single seedlings were transplanted to 20-cm diam clay pots filled with methyl-bromide-treated Norfolk fine loamy sand. Three replicates/nematode population/temperature were used, with an inoculum level of 1,000 nematodes/pot. These pots were placed in larger waterproof pots with the intervening space filled with fine gravel. The larger pots were placed in water baths in a completely randomized design and maintained at 20, 25, 30, and 35 C for 60 days. The soil moisture was kept at approximately 8-10% by weight by providing water when needed as determined with an Agtronic® soil moisture meter (Agtronics Co., Barstow, California).

Soil moisture: In studying the influence of soil moisture on *B. longicaudatus*, single 6-day-old Lee soybean plants were placed in 500 g of 65-mesh silica sand in 350-ml fritted glass Büchner funnels of fine porosity and inoculated with 500 nematodes of the Tarboro, N.C. population. Air bubbles were expelled from the fritted glass and the bottom portion of the funnel. The funnels were connected to reservoirs of continuously aerated 0.1 N Hoagland's solution by rubber hoses for continuous irrigation (7). Solution surfaces of the reservoirs were placed 10, 40, or 90 cm below the level of the fritted glass disks to provide moistures of approximately 30, 20, and 2% oven dry wt, respectively (Fig. 1-F). A similar experiment was conducted with the Tifton, Ga. population. In a final experiment, four replicates of each population, each having 50 handpicked adults of each sex of a given population, were included at moistures of approximately 30, 7, and 2%. These experiments were terminated after 35 days.

RESULTS

Soil type: The rate of nematode reproduction varied greatly with soil type. In muck and clay soils, *B. longicaudatus* failed to maintain the inoculum level on soybean (Fig. 1-A). Some nematode reproduction occurred in the coarse sandy loam soil, but it was not statistically different from that in muck or clay. Greatest reproduction occurred in the 65-mesh silica sand, but this was not significantly different from that in the fine loamy sand, 1:1 mixture or beach sand. Nematodes had no detectable effect on plant growth, but soil type did.

Reproduction of *B. longicaudatus* on strawberry in the different soils, was similar to that on soybean in these soils, except for fine loamy sand. In this soil, reproduction was intermediate and did not differ statistically from either the lower or higher rates of reproduction in the other "soils" (Fig. 1-B).

Particle size: Sand texture affected sting nematode reproduction on strawberry. Reproduction in the fine sand was statistically greater than that in either the medium or coarse sand (Fig. 1-C). Reproduction occurred in the medium sand, but was not significantly greater than that in the coarse sand, where the nematode population declined.

The relationship of particle size to nematode reproduction was also evident when various-sized glass beads were used as a growing medium (Table 2). Reproduction of the Tarboro, N.C. population in glass beads with mean diam of 100 and 180 μm was statistically greater than in beads with mean diam of 300, 500, and 1000 μm. However, reproduction of the Tifton, Ga. population in glass beads with mean diam of 180 and 300 μm was statistically greater than in those of 100, 500, or 1,000 μm. Some difficulties were encountered when glass beads were used as test media. Seven to 10 days after inoculation with nematodes, the soybean plants became chlorotic, and their growth was reduced. Plant growth improved after daily foliar spray with Greenol®, a source of minor elements (Chevron Chemical Co., Ortho Division, San Francisco, Calif.) at 4,000 ppm. Generally, the Tifton, Ga. population increased more than the Tarboro, N.C. population (Table 2).

Soil temperature: Both *B. longicaudatus* populations reproduced more on corn at 25 and 30 C, than at 20 or 35 C. Increase of the Tifton, Ga. isolate was much greater than that of the Tarboro, N.C. isolate at 25, 30, and 35 C (Fig. 1-D). Neither nematode population reduced plant wt, but brace roots failed to penetrate soil infested with the Tifton, Ga. population at 25 and 30 C. Only slight damage was noted to brace roots in pots with the Tarboro, N.C. population at the same temperatures.

Soil moisture: Where 50 nematodes of each sex of the Tarboro, N.C. or Tifton, Ga., populations were used as inoculum, the latter increased more than the former at all moisture levels (Fig. 1-E). Reproduction of both populations was greatest at the 7% moisture level. The Tifton, Ga. population induced more severe root damage than did the Tarboro, N.C. population. Similar results were obtained in other experiments using 500 nematodes/funnel at moisture levels of 2, 20, and 30%. However, this inoculum level caused severe root damage, especially at 20% moisture, and fewer nematodes of the Tifton, Ga. population were recovered at this moisture level than at the 2 and 30% levels (tabular data not given).

DISCUSSION

The unusual sensitivity of *B. longicaudatus* to soil type is apparently the primary limiting factor in its geographic distribution. Greenhouse tests show that reproduction of this nematode is restricted to soils with a minimum of 80% sand and a maximum of 10% clay. This agrees with field observations of Thames (11) and Miller (9). Our studies also suggest that optimal particle size for maximum nematode activity is near that of 120-370 μm (65-mesh) silica sand. This was supported by evidence that reproduction and survival was greatest with glass beads of 100-180 μm diam for the Tarboro, N.C. nematodes and 180-300 μm diam for the larger Tifton, Ga. nematode.

Since nematode diam and soil pore size are directly related to nematode movement (15), the movement of *B. longicaudatus* undoubtedly is restricted in clay soil as suggested by Thames (11). However, *Xiphinema americanum* (Cobb), which is similar in size to *B. longicaudatus*, reproduces well in a wide range of soils, including clay (5). This suggests that movement through soil is more essential for reproduction of amphimictic species such as *B. longicaudatus*, than for reproduction of parthenogenetic species such as *X. americanum*.

Our investigations into the effects of soil temperature on reproductive capacity of two *B. longicaudatus* populations showed that reproduction of the Tifton, Ga. population was greater than the Tarboro, N.C. population at 25, 30, and 35 C. Furthermore, at 35 C the Tifton, Ga. population reproduced, but the Tarboro,N.C. population declined. Apparently, the Tifton, Ga. population has adapted to the higher soil temperatures of Tifton, Ga. (mean summer soil temperature at 15 cm = 29 C), and the Tarboro, N.C. population is adapted to the slightly cooler temperatures occurring in that location (mean summer soil temperature at 15 cm = 25 C).

Soil type, moisture and aeration are interrelated as discussed by Wallace (15, 16). He stated that replenishment of oxygen (O_2) in saturated soils is much slower than in well-drained soils. Van Gundy et al. (14) found that low O_2 concns reduced the activity of ectoparasitic nematodes (*X. americanum* and *Trichodorus christiei* (Allen) more than that of endoparasitic nematodes [*Meloidogyne incognita* (Kofoid & White) Chitwood, and *Tylenchulus semipenetrans* Cobb]. Thus, any factor reducing O_2 in the environment of nematodes probably would be more detrimental to ectoparasites than to endoparasites.

The ectoparasite, *B. longicaudatus*, reproduced best in moderately dry soil with 7% moisture. Inhibition of nematode movement under high moisture conditions may be partly responsible for reduced reproduction under such conditions (15). The nutrient solution in our tests was aerated to provide sufficient O_2 at all moisture levels, and this design apparently was successful; the nematodes failed to survive high moisture conditions in preliminary tests in which the nutrient solution was not aerated, but reproduced when the nutrient solution was aerated. These experiments suggest that the effect of soil moisture alone may not be as important to nematode activity and reproduction as are interrelated effects on O_2 concn and rate of O_2 replenishment.

Our greenhouse studies on soil type, temperature, and moisture, agree with the previous field observations and limited greenhouse experiments reported by various investigators. Apparently, the major limiting ecological factors responsible for the infrequent geographical occurrence of *B. longicaudatus* are soil type and temperature.

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