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Effect of Temperature on Infection and Survival of *Rotylenchulus reniformis*

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Abstract: From infestation of lettuce with preinfective females to egg deposition, populations of *Rotylenchulus reniformis* from Baton Rouge, Louisiana; Lubbock and Weslaco, Texas; and Mayaguez, Puerto Rico, required 41, 13, 7, and 7 days at 15, 20, 25, and 34 C, respectively. No nematode infection occurred at 10 C with any *R. reniformis* population, and the population from Puerto Rico did not reproduce at 15 C. Nematode survival was not influenced by temperature, since populations from Texas and Louisiana survived for 6 months without a host at −5, −1, 4, and 25 C. Survival of *R. reniformis* was substantially influenced by soil moisture. Soil moistures greater than 7% (< 1 bar) aided nematode survival at storage temperature of 25 C, whereas moisture adversely affected nematode survival below freezing. Soil moisture below 4% (> 15 bars) favored nematode survival below freezing but adversely affected nematodes in soils stored at 25 C. Soil moisture effects on nematode survival were less accentuated at 4 and 0 C.

Key words: Cucumis melo, Lactuca sativa, postinfection development, reniform nematode, *Rotylenchulus reniformis,* soil moisture content.

Rotylenchulus reniformis Linford and Oliveira causes economic losses to many crops, primarily those in tropical and subtropical areas of the world (3). Its geographical distribution would appear to be limited by low temperatures. In Texas, for example, *R. reniformis* is an important pest only in the subtropical Rio Grande Valley, where it infects many vegetable and field crops throughout the year (2,6). The nematode does occur, however, to a limited extent in marginally temperate areas and has even been found on one farm on the high plains of northwestern Texas where soil temperatures are frequently below freezing in the winter (C. C. Orr, pers. comm.). Little experimental data is available on the effects of temperature on the biology of *R. reniformis.* Rebois (4) examined the effects of *R. reniformis* on soybeans at several soil temperatures maintained by water baths and found optimum temperature for nematode development to be 29.5 C. Nematodes did not produce egg masses at 15 or 36 C. Survival of *R. reniformis* has been studied only as related to soil moisture (1,3); the effects of temperature are unknown. The objective of this study was to obtain a better understanding of the extent to which soil temperature may limit the geographical distribution of *R. reniformis.* We compared the effects of temperature on infection, motility, and survival of four populations of *R. reniformis* from four latitudes ranging from tropical to temperate.

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MATERIALS AND METHODS

Effect of temperature on root infection

Rotylenchulus reniformis populations from Louisiana (Baton Rouge), northern Texas (Lubbock), Puerto Rico (Mayaguez), and southern Texas (Weslaco) were used in our experiments. Nematodes were propagated on lettuce, *Lactuca sativa* cv. Valverde, in a glasshouse in Weslaco. Motile stages of *R. reniformis* for inoculum were extracted using the Baerman funnel technique (5). Ten-day-old lettuce seedlings (one per pot) were transplanted into individual 6-cm-d plastic pots containing 80 cm^3 steam pasteurized sandy loam soil (12.7% clay, 14.8% silt, 72.5% sand, pH 7.1) (9). To minimize thermal shock during the inoculation of seedlings, inoculum and pots containing soil were equilibrated for 12 hours at 10, 15, 20, and 25 C, and one group for 4 hours at 34 C. Two thousand pre-infective adult females in 10 ml water per pot (25 nematodes/cm^3 soil) were poured directly on the roots of each seedling at transplanting. Plants were placed in growth chambers at the respective temperatures. Five seedlings from 20, 25, and 34 C were harvested every 2 days and five seedlings from 10 and 15 C were harvested every 5 days. Roots were washed gently in tap water, and infective females and egg masses were extracted by macerating roots in water for 30 seconds (18,500 rpm) in a blender with a rubber blade (5). Infective females and eggs in the gelatinous matrices were counted using a stereomicroscope. Motile nematode stages were extracted from soil by the Baerman funnel method to monitor the effect of temperature on the vitality of the nematode stages in soil. The experiment at each temperature was terminated when the first eggs were detected in the gelatinous matrices.

In addition, twenty 4-day-old cantaloupe, *Cucumis melo* cv. Perlita, seedlings were inoculated with the *R. reniformis* population from Weslaco and maintained at 37 C. Five seedlings were harvested at 2-day intervals for 8 days, and nematode infection and reproduction were determined as described for lettuce. Data were analyzed as count data and as log of the count. The design was a randomized complete block with five or eight blocks. Treatments were in a factorial arrangement. Significant differences ($P = 0.05$) between means were identified using Fisher's Protected Least Significant Differences multiple comparison test. The conclusion of the statistical analysis did not differ for the count data and the log count. Our choice was to present the results as counts.

Effect of temperature on nematode survival

Experiment 1: Lubbock sandy loam soils (17.9% clay, 16.0% silt, 66.1% sand, pH 8.0) had a natural density of 20 motile stages of *R. reniformis*/cm^3. Weslaco sandy loam soils (13.9% clay, 11.0% silt, 75.1% sand, pH 7.8) had a natural nematode density of 17 motile stages/cm^3. The silt loam soil (17.9% clay, 75.0% silt, 7.1% sand, pH 5.0) from Baton Rouge had a count of 90 motile stages/cm^3. Each soil was divided into 480 samples of 100 g each. Half of them were enclosed in plastic bags to avoid desiccation and half were air-dried for 24 hours at room temperature. Moist and air-dried samples were then stored for 6 months at −5, −1, 4, and 25 C. Forty air-dried and forty moist samples of each soil at each temperature were analyzed at 1-month intervals; 20 samples were processed by the Baerman funnel method to assess motility, and 20 were bioassayed to assess infectivity. Nematode densities in the soil were expressed as percentages of the initial nematode density. For bioassay, each soil sample was placed in a 6-cm-d plastic pot into which a 2-day-old cantaloupe seedling was transplanted, and plants were then grown in a glasshouse at 28–35 C. After 2 months plants were harvested, total roots were stained in hot lactoglycerol (1:1:1 lactic acid, glycerol, distilled water, and acid fuchsin) (7), and adult females in the roots were counted using a stereomicroscope. Nematode root infection was rated on a scale from 1 to 5, where 1 = no infection, 2 = 1–24% root system infected, 3 = 25–49% root system infected, 4 = 50–74% root system infected, and 5 = 75–100% root system infected.

Experiment 2: Lubbock and Weslaco soils were used with initial *R. reniformis* densities of 12.7 and 7.8 motile stages/cm^3 and moisture of 11.2 (< 1 bar) and 7.6% (1 bar), respectively. Each soil was divided into 128 units of 100 g each. Half of the samples were air dried as described in experiment 1 to moisture levels of 4.4 and 2.5% (> 15 bars) for Lubbock and Weslaco soils respectively. Samples were stored at −18 and 0 C for 60 days in plastic bags. Nematodes were extracted from eight samples from each moisture and each temperature level after 7, 15, 30, and 60 days of storage. Surviving nematode numbers were expressed as percentages of initial densities. Means were separated using a *t*-test ($P = 0.05$).

Results and Discussion

Effect of temperature on root infection

No *R. reniformis* were observed in lettuce roots maintained at 10 C 22 days after inoculation with the four populations. This

TABLE 1. Number of *Rotylenchulus reniformis* life stages representing reproduction in soil and in roots of Valverde lettuce at different temperatures after inoculation with 25 preinfective females/cm³ soil.

Temperature	Nematode population	First eggs (days after infection)	Females		Eggs in roots
			In soil	In roots	
10 C	Baton Rouge	†	586 a	0	0
	Lubbock	†	227 c	0	0
	Mayaguez	†	54 d	0	0
	Weslaco	†	345 b	0	0
15 C	Baton Rouge	41	20 a	17 b	3 b
	Lubbock	41	4 a	211 a	68 a
	Mayaguez	No reproduction	0.5 a	4 b	0 b
	Weslaco	41	1.2 a	55 b	3 b
20 C	Baton Rouge	13	132 a	133 a	8 a
	Lubbock	13	25 b	100 b	5 a
	Mayaguez	13	42 b	66 bc	4 a
	Weslaco	13	18 b	41 c	5 a
25 C	Baton Rouge	7	68 ab	152 a	30 a
	Lubbock	7	35 b	64 b	3 b
	Mayaguez	7	148 a	44 b	10 b
	Weslaco	7	21 b	31 c	4 b
34 C	Baton Rouge	7	193 a	28 b	4 a
	Lubbock	7	215 a	27 b	2 a
	Mayaguez	7	159 a	12 b	1 a
	Weslaco	7	236 b	58 a	5 a

Numbers in columns within temperature groups followed by same letter are not significantly different according to Fisher's Protected LSD test ($P = 0.05$).
† No reproduction after 22 days.

supports previous reports that this predominantly tropical species is unable to infect plants at low temperatures (Table 1). All *R. reniformis* populations infected lettuce at 15 C, but fewer ($P = 0.05$) females of the Puerto Rico population were found (Table 1). The Puerto Rico populations did not reproduce at 15 C, indicating a greater sensivity to low temperatures. At temperatures above 15 C the duration of postinfection development was the same for all populations (Table 1). At 25 and 34 C the interval from root invasion to egg deposition was 7 days as opposed to 6 days reported for *R. reniformis* development in soybean roots at 29.5 C (4). *Rotylenchulus reniformis* did not reproduce on soybean at 36 C (2); however, reproduction did occur on lettuce at 34 C in our experiment. Optimum temperatures for postinfection development on lettuce appear to range from 25 to 34 C. The *R. reniformis* population from Weslaco penetrated but did not reproduce on cantaloupe seedlings maintained at 37 C for 8 days, agreeing with the findings of Rebois (4). There is also the possibility that the host may cause the nematode to respond differently to temperature.

Effect of temperature on nematode survival

Experiment 1: Motile stages of *R. reniformis* were recovered after 6 months storage at 25 C in moist soils, but densities of Baton Rouge, Lubbock, and Weslaco populations decreased 50, 82, and 75%, respectively (Fig. 1A). No nematodes were recovered from air-dried Lubbock soil after 2 months, Weslaco soil after 4 months, and Baton Rouge soil after 6 months, indicating that low soil moisture content adversely affected nematode survival at 25 C (Fig. 1B).

At 4 C, nematode densities of all *R. reniformis* populations decreased greatly after 6 months. Very low densities and no nematodes were recovered at this time interval in air-dried and moist soils, respectively (Fig. 1C, D).

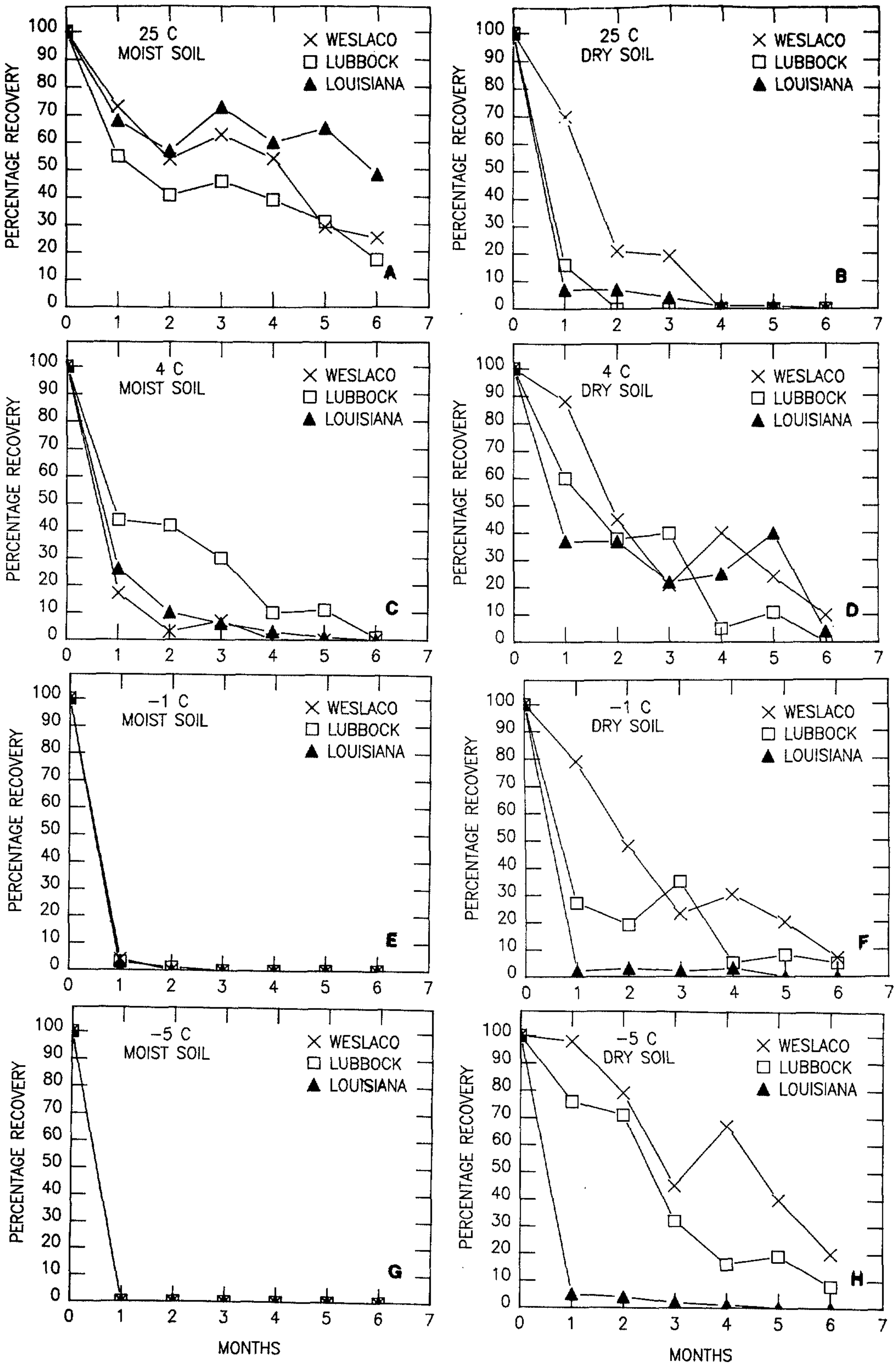

FIG. 1. Recovery percentages of three populations of *Rotylenchulus reniformis* from moist or air-dry soil stored at 25 to −5 C.

TABLE 2. *Rotylenchulus reniformis* index of *Cucumis melo* cv. Perlita 60 days after inoculation. Soil stored 1–6 months at 25 to −5 C.

Population	Storage period (months)	Root infection indices: Moist soil 25 C	4 C	−1 C	−5 C	Air dry soil 25 C	4 C	−1 C	−5 C
Baton Rouge	1	3.0	2.6	1.0	1.0	2.0	2.8	2.0	2.0
	2	5.0	4.6	1.0	1.0	2.0	4.0	2.0	2.0
	3	5.0	2.2	1.0	1.0	2.4	5.0	2.2	2.0
SE = 0.26	4	4.8	2.0	1.4	1.0	2.0	3.0	2.0	1.8
LSD = 0.7	5	4.8	2.0	1.0	1.0	2.0	2.8	1.8	1.8
	6	3.0	1.0	1.0	1.0	1.0	1.8	1.0	1.0
$\bar{x}$		4.3	2.4	1.1	1.0	1.9	3.2	1.8	1.7
Lubbock	1	2.4	2.2	1.0	1.0	2.0	2.0	2.0	2.0
	2	2.4	3.2	1.4	1.0	1.4	1.4	1.2	1.8
	3	2.6	2.2	1.0	1.0	1.4	1.4	1.4	1.6
	4	3.2	2.4	1.0	1.0	1.0	1.0	2.0	1.6
SE = 0.15	5	2.2	2.0	1.0	1.0	1.0	1.0	1.0	2.0
LSD = 0.4	6	2.4	1.4	1.0	1.0	1.0	1.0	1.0	1.0
$\bar{x}$		2.5	1.9	1.1	1.0	1.3	1.3	1.4	1.7
Weslaco	1	2.0	2.0	1.0	1.2	1.8	2.0	2.0	1.8
	2	2.4	2.8	1.0	1.0	1.0	2.0	1.8	1.8
	3	2.4	1.2	1.0	1.2	1.2	2.0	2.0	2.0
	4	2.8	1.0	1.0	1.4	1.0	1.2	1.2	2.0
SE = 0.16	5	2.0	1.0	1.0	1.0	1.0	1.8	2.0	2.0
LSD = 0.5	6	1.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0
$\bar{x}$		2.2	1.1	1.0	1.1	1.1	1.7	1.7	1.8

Index across moisture and storage within populations are statistically different ($P = 0.05$) if differences between the index values exceed Fisher's Protected LSD.

Infection indices are averages of five replicates: 1 = no root infection, 2 = 1–24%, 3 = 25–49%, 4 = 50–74%, 5 = 75–100%.

At −1 and −5 C, populations of *R. reniformis* survived longer in air-dried than in moist soils (Fig. 1E–H). At high moisture levels nematodes survived no more than 1 or 2 months (Fig. 1E, G). In air-dried soils, nematodes survived longer in sandy loam (Lubbock and Weslaco) than in silt loam (Baton Rouge) soils at both subfreezing temperatures (Fig. 1F, H).

These results were supported by the bioassay test. More severe cantaloupe root infections were detected in moist soils than in air-dried soils stored at 25 C; however, more severe nematode root infections were observed in air-dried soils than in moist soils stored below freezing (Table 2) and at 4 C ($P = 0.05$). Difference in nematode infection severity between moist and air-dried soil at 4 C were the same as those at temperatures below freezing. In one case, plants were more severely infected by the Lubbock population in moist soil than in air-dried soil stored for 5 months at 4 C.

Experiment 2: Survival of *R. reniformis* was adversely affected by high soil moisture content in soils stored below freezing. No nematodes survived in moist soils after 7 days storage at −18 C (Fig. 2A); however, nematodes of both populations survived in air-dried soils stored at −18 C (Fig. 2B). In soil stored at 0 C, nematodes of both populations survived in air-dried and moist soils for several weeks. (Fig. 2C, D).

The results of these experiments indicate that *R. reniformis* is not able to infect lettuce roots at 10 C, supporting the belief that this species requires warm temperatures to develop and infect crops. Nematode populations from Texas and Louisiana were able to reproduce on lettuce at 15 C. This temperature occurs during the winter in top soils of the Rio Grande Valley, and nematode infections probably have less impact on crop production because of slow nematode development (41 days). Nematode population densities may in-

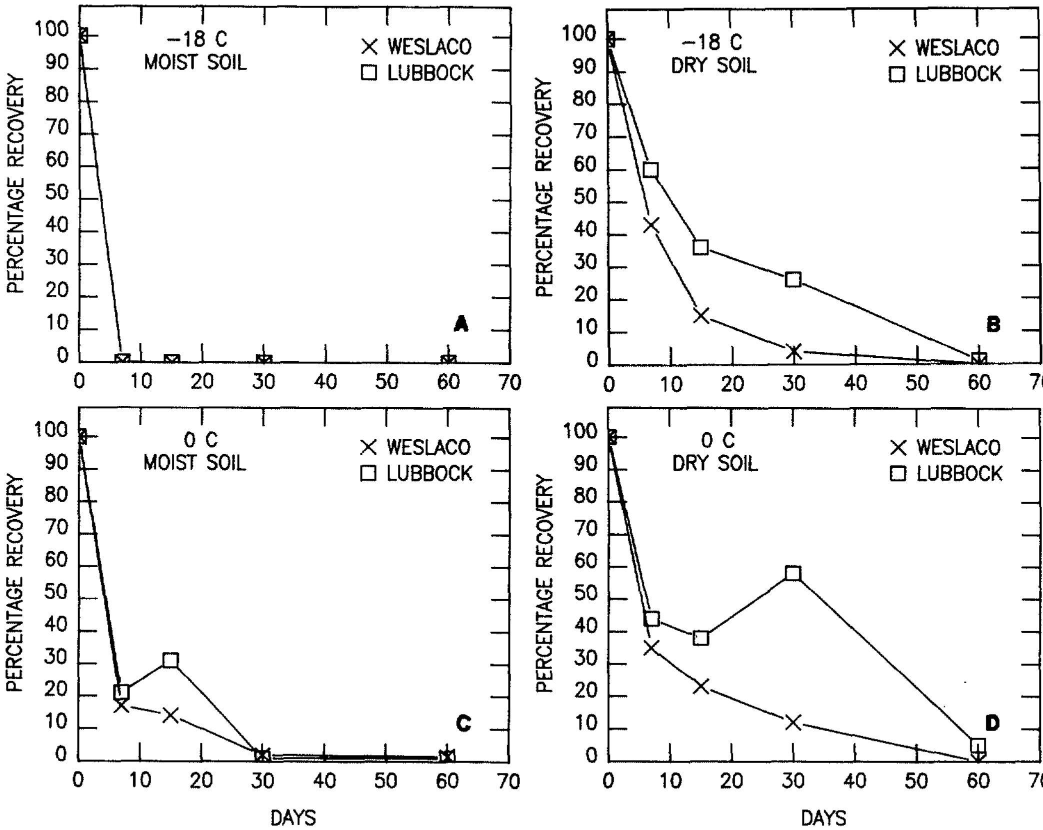

FIG. 2. Recovery percentages of two populations of *Rotylenchulus reniformis* from moist or air-dry soil stored at 0 or −18 C.

crease, however, during winter under these conditions provided a host is present. This suggests also that the populations from Texas and Louisiana are adapted to cooler temperatures than the tropical population from Puerto Rico, which did not reproduce at 15 C.

The effects of temperature on the survival of *R. reniformis* were greatly affected by soil moisture. At storage temperatures below freezing, soil moisture content above 7% (< 1 bar) decreased nematode survival, possibly through water crystal formation within nematodes. This adverse effect was reduced in soil with moisture contents below 4% (> 15 bars).

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