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Leaching Soluble Salts Increases Population Densities of *Tylenchulus semipenetrans*[1]

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Abstract: The effect of salinity on population densities of *Tylenchulus semipenetrans* was measured on 3-month-old salt-tolerant Rangpur lime growing on either loamy sand, sand, or organic mix and on 4-month-old salt-sensitive Sweet lime in organic mix. Salinity treatments were initiated by watering daily with 25 mol/m^3 NaCl + 3.3 mol/m^3 $CaCl_2$ for 3 days and every other day with 50 mol/m^3 NaCl + 6.6 mol/m^3 $CaCl_2$ for one week, with no salt (NS) treatments as controls. Salinity was discontinued in one treatment (DS) by leaching with tap water prior to inoculation with nematodes, whereas the continuous salinity (CS) treatment remained unchanged. Overall, in Rangpur lime organic soil supported the highest population densities of *T. semipenetrans*, followed by loamy sand and sand. The DS treatment resulted in the highest ($P \leq 0.05$) mean population densities of *T. semipenetrans* in the three soil types. Similarly, the DS treatment in Sweet lime resulted in the highest ($P \leq 0.05$) nematode populations. The DS treatment predisposed citrus to nematode infection through accumulated salt stress, whereas leaching soluble salt in soil solution offered nematodes a suitable nonosmotic habitat. Nematode females under the DS treatment also had the highest ($P \leq 0.05$) fecundity.

Key words: *Citrus limettioides*, citrus nematode, *citrus reticulata*, electrical conductivity, nematode, Rangpur lime, salinity, soil type, Sweet lime, pH, *Tylenchulus semipenetrans*.

Worldwide salinity levels in irrigation water are increasing, whereas the cost of managing accumulated salt in soils is also increasing (17). Salinity generally decreased population densities of nematodes on some annual crops (8,10). Machmer (16), however, found that salinity increased population densities of the citrus nematode, *Tylenchulus semipenetrans* Cobb, on citrus and the root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, on papaya. No differences in population densities of juveniles or infectivity of *T. semipenetrans* were detected in fallow soil at osmotic potential (OP) levels from −0.18 to −9.36 MPa after 184 days (12). *In vitro,* juvenile motility of *T. semipenetrans* was inhibited by OP levels from −4.64 to −22.57 MPa (12). *In vitro* and in fallow, similar salt levels inhibited egg hatch of *T. semipenetrans* (unpubl.). Similar effects were observed for other plant-parasitic nematodes (5). Field observations in Israel (3) and South Africa (2) indicated that the highest population densities of *T. semipenetrans,* however, occur in citrus-producing areas with known salinity problems. It is unknown how salinity increases population densities of *T. semipenetrans* while inhibiting egg hatch and (or) motility. The objective of this study was to determine whether salinity increases population densities of the citrus nematode through direct salt effects on nematodes, indirect salt stress in plants, or both. Because cation exchange capacity depends on soil type and influences salinity of the soil solution (1), the effects of three soil types on salinity–nematode interactions were also investigated.

Materials and Methods

Salt-tolerant Rangpur lime (*Citrus reticulata* var. *austera* Swingle) seeds were germinated in sand and uniform 3-month-old plants were transplanted into 10-cm-d clay pots containing steamed loamy sand (82% sand, 5% silt, 13% clay; pH 6.9, 0.2% OM), sand (97% sand, 2% silt, 1% clay; pH 7.1, 0.1% OM), or organic mix (1:1, v/v) sand: PRO-MIX BX (Premier Brands, Inc.). Salinity treatments were discontinuous salt (DS), continuous salt (CS), and no salt (NS) for each soil type. Pots were arranged in the greenhouse in a complete 3 × 3 factorial block design with nine replications.

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Day/night air temperatures averaged 30/25 C. Plants were irrigated with 100 ml tap water every other day and fertilized weekly with 100 ml solution of 20:20:20 (N:P:K) mixture of 3 g/liter water.

Two days after transplanting, each pot was infested with 10 ml supernatant of greenhouse cultured *Glomus intraradices* Schenck & Smith (6) prepared by blending 2 g roots of infected Sudangrass, *Sorghum sudanense* Stapf, sieving (150-μm-pore sieve), and diluting to 500 ml with water. Two weeks after transplanting, salts were added to the irrigation water, first daily at 25 mol/m^3 NaCl + 3.3 mol/m^3 $CaCl_2$ in 100 ml solution for 3 days and then every other day at 50 mol/m^3 NaCl + 6.6 mol/m^3 $CaCl_2$ for one week. The soil for DS and NS treatments was leached 10 days after initiating salt treatment by irrigation with 250 ml water daily for 3 days. The soil for CS treatments was leached with 250 ml 25 mol/m^3 NaCl + 3.3 mol/m^3 $CaCl_2$ solution. Leachates were collected a day before leaching, 9, and 33 days after leaching. Electrical conductivity (EC) of leachates was determined using the EC meter and converted to OP values (1).

One week after leaching, the nematode inoculum was prepared. Citrus roots infected with *T. semipenetrans* were collected from the field, placed in a 2-liter plastic bag half-filled with water, and vigorously shaken. The contents were then passed through a 150-μm-pore sieve nested on a 25-μm-pore sieve. The contents of the 25-μm-pore sieve were aerated in 4.5 liters of water to keep the nematode juveniles in suspension while allowing eggs, soil particles, and some debris to settle. The suspension was passed through a 150-μm-pore sieve nested on a 25-μm-pore sieve and the contents of the latter were collected for inoculum. Plants were inoculated three times using a 10-ml glass syringe by placing nematodes in four 5-cm-deep holes in the soil around each plant at 2-day intervals to give a total of ca. 84,000 juveniles/plant.

At harvest, 45 days after initiating salt treatments, the shoots were cut at the surface of the soil and weighed. The pot contents were emptied, and roots were collected and weighed. Nematodes were separated from 1 g roots/plant by maceration and blending for 30 seconds in 10% NaOCl, and this mixture was passed through a 150-μm-pore sieve onto a 25-μm-pore sieve. The contents of the latter were washed into 96-ml glass tubes. After 12 hours to allow nematodes to settle, the tubes were standardized to 25-ml volumes. Five drops of acid fuschin stain were added to each tube, and the contents were brought to a boil. Eggs, juveniles, and adults were counted from a 5-ml aliquot. All roots and fully developed leaves were dried at 60 C for 48 hours and powdered separately in a porcelain mortar. Chloride concentration of leaves and roots, used as an index of plant stress, were measured by a Buchler-Cotlove Chloridometer (Haake Buchler Instruments, Inc.). Nematode data were transformed to ln (x + 1) prior to analysis of variance to homogenize the variance (15). All data were analyzed using three-way analysis of variance. The degrees of freedom and their associated sum of squares were partitioned to determine the relative (percentage) contributions of each factor to mean total treatment variations observed (11,14).

The experiment was repeated using salt-sensitive Sweet lime (*C. limettioides* Tan.) on organic mix only and the three salinity treatments under the conditions and procedures described for Rangpur lime. Each treatment (DS, CS, NS) was replicated 15 times, and pots were arranged in a complete randomized-block design. The methods used were similar to those used for Rangpur lime, except that 4-month-old Sweet lime seedlings were inoculated twice at 2-day intervals with a total of ca. 73,000 juveniles/plant. Data were analyzed by two-way analyses of variance, and means were compared by Duncan's multiple-range test. Only significant ($P \leq 0.05$) data are discussed unless stated otherwise.

RESULTS

Mean nematode female densities per gram of root weight were the highest in the

DS treatments and in the organic mix as compared to the loamy sand and sand (Table 1). Nematode female densities on plants grown in loamy sand were also higher than those on plants grown in sand. Mean female densities were not different between NS and CS treatments. Using partitioning of the degrees of freedom and their associated sum of squares (14), salinity, soil type, and interaction contributed, respectively, 52%, 36%, and 12% ($P \leq 0.10$) of the total treatment variation (TTV) in mean female densities. Mean juvenile numbers were not correlated with numbers of eggs, suggesting that at least some juveniles were the remnants from inocula (data not shown). Nematodes in DS treatments produced the most eggs, whereas those in the CS and NS treatments were not different. Nematodes in the organic mix also produced the most eggs followed by those in loamy sand and sand. The major sources of variation in mean egg counts were 83% for salinity and 14% ($P \leq 0.10$) for soil type. Fecundity was expressed as number of eggs per female. Females in DS treatments had the highest fecundity, whereas those in the NS and CS treatments were not different. The only source of treatment variation in mean fecundity was salinity.

Salinity accounted for over 97% of the TTV in mean OP variations throughout the study, with small contributions from soil type and interactions. Leachates from the CS treatments had the highest mean OP for all sampling dates, whereas mean OP of the DS and NS treatments, or that of loamy sand and sand, were not different (Table 2). Organic mix had the highest mean pH, whereas those of loamy sand and sand were not different. The strongest source of variation in mean pH throughout the study was soil type.

There was no evidence of treatment effects on either fresh shoot or root weights (data not shown). Mean leaf Cl contents were highest in the CS (1.4% Cl), moderately high in the DS (0.5% Cl), and low in the NS (0.2% Cl) treatment. Salinity and interaction effects contributed 94% and 4% ($P \leq 0.10$), respectively, of the TTV in mean leaf Cl levels. Mean root Cl levels across all treatments or soil types were not different.

Numbers of *T. semipenetrans* females and eggs on Sweet lime in the DS treatments were greater than those in the CS and NS treatments, whereas in the latter they were not different (Table 3). Mean juvenile numbers were neither different among treatments nor correlated with numbers of eggs (data not shown). Fecundity was higher in the DS treatment than in other treatments and was not different between the CS and NS treatments.

The effects of soil salinity on OP or pH for Sweet lime were similar to those for Rangpur lime on organic mix (Table 4). Salinized plants had higher leaf Cl levels than controls on all sampling dates. Root Cl levels in all treatments were not different (data not shown).

TABLE 1. Numbers of females and eggs of *Tylenchulus semipenetrans* per gram fresh root on salt-tolerant Rangpur lime as affected by soil type, discontinuous salt (DS), continuous salt (CS), or no salt (NS) treatments.

	Females				Eggs				Fecundity†			
Soil	NS	DS	CS	Mean	NS	DS	CS	Mean	NS	DS	CS	Mean
Loamy sand	75	217	57	117b	13	363	13	130ab	0.2	1.7	0.2	0.7a
Organic mix‡	121	588	123	278a	19	533	69	207a	0.2	0.9	0.6	0.6a
Sand	32	94	45	57c	31	159	24	72b	1.0	1.7	0.5	1.1a
Mean	76b	300a	75b		21b	352a	35b		0.5b	1.4a	0.4b	

Means followed by the same letter within a column or row for each variable are not different ($P \leq 0.05$) according to Duncan's multiple-range test. Each value is an average of nine replicates.

† Fecundity = number of eggs per female.

‡ Sand: PRO-MIX BX (1:1, v/v), Premier Brands, Inc.

TABLE 2. Osmotic potential and pH of leachate as affected by three soil types, discontinuous salt (DS), continuous salt (CS), or no salt (NS) treatments.

Sampling time	Soil	Osmotic potential (×10⁻² MPa)				Soil pH			
		NS	DS	CS	Mean	NS	DS	CS	Mean
1†	Loamy sand	−7.9	−24.1	−23.8	−18.7a	6.6	7.1	6.6	7.0b
	Organic mix‡	−7.2	−27.4	−23.0	−19.1a	7.2	7.9	7.4	7.5a
	Sand	−7.6	−23.0	−23.8	−18.0a	6.9	7.7	6.9	7.2b
	Mean	−7.6b	−24.8a	−23.5a		6.9b	7.6a	7.0b	
2	Loamy sand	−6.5	−6.5	−21.2	−11.5a	6.9	7.0	6.8	6.9b
	Organic mix	−6.8	−7.2	−23.4	−12.6a	7.3	7.4	7.3	7.3a
	Sand	−5.8	−7.2	−19.8	−10.8a	7.3	6.8	6.9	7.0b
	Mean	−6.5b	−6.8b	−21.6a		7.2a	7.1a	7.0a	
3	Loamy sand	−7.2	−8.3	−40.3	−18.7a	5.9	6.0	5.6	5.8b
	Organic mix	−7.9	−8.6	−37.1	−18.0a	6.8	6.7	6.8	6.8a
	Sand	−7.2	−6.5	−37.4	−16.9a	5.6	5.3	5.9	5.6b
	Mean	−7.6b	−7.9b	−38.2a		6.1a	6.0a	6.1a	

Means followed by the same letter within a column or row for each variable are not different ($P \leq 0.05$) according to Duncan's multiple-range test. Each value is an average of nine replicates.
† 1 = One day before leaching; 2 = 9 days after leaching; 3 = 33 days after leaching.
‡ Sand:PRO-MIX BX (1:1, v/v), Premier Brands, Inc.

DISCUSSION

Discontinuous salt is similar to irrigation with poor quality water under field conditions where rainfall can leach salt from the soil profile. Although the CS treatment did not affect populations of *T. semipenetrans*, DS treatment had a tremendous influence. *Tylenchulus semipenetrans* in the DS treatments was not exposed to continuous salt stress in the soil environment, which inhibits nematode movement (12,13). In other studies, the CS levels decreased population densities of *Meloidogyne incognita* on tomato (8) and had no effect on populations of *Rotylenchulus reniformis* on cotton (10). Results in this study suggest that temporary salt stress on the plant predisposes the host to *T. semipenetrans* infection only in the absence of osmotic stress in soil solution.

In the DS or NS treatments the mean OP was less than −1.44 MPa and the pH was less than 8.5, which are considered to be the upper limits of nonsaline soils (1,4). In the CS treatment, the mean OP was greater than −1.44 MPa and the pH was less than 8.5, meeting the criteria for salinity affected soils. This confirms the ease with which leaching can convert saline to normal soil under suitable conditions (1).

TABLE 3. Numbers of females and eggs of *Tylenchulus semipenetrans* per gram of fresh root on salt-sensitive Sweet lime as affected by discontinuous salt (DS), continuous salt (CS), or no salt (NS) treatments.

Treatment	Females	Eggs	Fecundity†
DS	101a	386a	3.8a
CS	28b	15b	0.5b
NS	21b	8b	0.4b

Means followed by the same letter within a column are not different ($P \leq 0.05$) according to Duncan's multiple-range test. Each figure is an average of 15 replicates.
† Fecundity = number eggs per female.

TABLE 4. Osmotic potential and pH of leachate and salt-sensitive Sweet lime leaf chloride (Cl) levels as affected by discontinuous salt (DS), continuous salt (CS), or no salt (NS) treatments.

Sampling time†	Treatment	Osmotic potential (×10⁻² MPa)	pH	Leaf Cl (%)
1	DS	−23.8a	8.0a	0.76a
	CS	−21.2a	7.3a	0.61a
	NS	−2.9b	7.7a	0.07b
2	DS	−7.6b	7.7a	0.31b
	CS	−29.5a	6.9ab	1.58a
	NS	−6.8b	6.3b	0.10c
3	DS	−10.1b	6.0b	0.27b
	CS	−40.3a	7.1a	1.69a
	NS	−8.3b	5.8b	0.09c

Means followed by the same letter within a column for a given sampling time are not different ($P \leq 0.05$) according to Duncan's multiple-range test. Each value is an average of 15 replicates.
† 1 = One day before leaching; 2 = 9 days after leaching; 3 = 33 days after leaching.

The high pH in organic mix was probably due to the high cation exchange capacity of the soil (1). The mean pH range of 6.0–8.0 was within the optimum range for *T. semipenetrans* population development (7). The reason for higher pH in DS prior to leaching is unknown. The lowest nematode population densities in the sandy soil and the highest densities in organic mix in Rangpur lime confirmed earlier findings (18).

Plants in CS treatments had leaf Cl levels above the mean toxic level of 1% (21), but there was no noticeable defoliation over the short duration of this study. In both experiments, plants in DS treatments had higher leaf Cl content than the controls. These results suggest the inability of either rootstock to reduce Cl accumulation in shoots even after leaching salts from the root zone. Mean leaf Cl levels in the DS treatments were higher than the physiological damage threshold of 0.20% (21), suggesting that the plants were salt-stressed for the duration of the study. Roots or leaves in NS treatments had higher Cl levels than usually reported in NS control plants (22). It was previously shown that *G. intraradices* increases Cl levels in citrus (9). That may partly account for the higher Cl levels in NS plants in this study. However, because the magnitudes of Cl levels in our NS plants were higher than those in *G. intraradices* infected plants (9), and because all the NS plants were also infected with the citrus nematode, it seems that the citrus nematode may also be increasing the Cl levels. Because Machmer's (16) studies were conducted under field conditions over 3 years, it is conceivable that rainfall occasionally leached salts, creating conditions similar to those in our study. Periodic salinity and rainfall leaching similarly account for the higher population densities of *T. semipenetrans* observed in citrus-producing areas with known salinity problems (2,3). This study projects increasing *T. semipenetrans* problems in citrus-producing areas because 1) salt concentrations of irrigation water in citrus producing areas are increasing (20), 2) salt leaching, which increases population densities of *T. semipenetrans,* is the major strategy of controlling salinity in the root zone (1), and 3) salinity accentuates the severity of the citrus nematode damage (19).

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