Interaction of *Rotylenchulus reniformis*, Soil Salinity, and Cotton[1]

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Abstract: *Rotylenchulus reniformis* occurred equally in relatively non-saline (4.0 mmhos/cm) and highly-saline (16.5 mmhos/cm) soils in sampling transects across zones of depressed plant growth in six Texas cotton fields.

Results from greenhouse pot experiments indicated progressive positive interaction of salinity and *R. reniformis* pathogenicity in the range 6–18 mmhos/cm. *Key Words:* Reniform nematode, *Gossypium hirsutum*.

The plant-parasitic nematode syndrome has been confused with symptoms of fertilizer deficiency, microorganism-caused root disease, soil salinity, "physiological disorders", and various other factors which cause stunting of plants.

In the irrigated Lower Rio Grande Valley of Texas, soil salinity is a major problem. During the past 10 years, the reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira) has also become a major pest of cotton (*Gossypium hirsutum* L.). Birchfield *et al.* (1) reported that 40% of samples from 80 cotton fields in the Valley contained reniform nematodes. Lambe and Horne (3) described *R. reniformis*-infected cotton to be dwarfed, chlorotic, with fewer secondary roots, and greater mortality among infected young plants. High soil salinity causes cotton plants to become stunted and their leaves to turn a dark green color (5). The symptoms of nematode injury and soil salinity in cotton are somewhat similar. Recognizing the general belief that one is independent of the other, this study was conducted to determine: (i) whether reniform nematode populations are high in saline cotton fields; (ii) whether the nematode causes additional damage to cotton under saline conditions; and (iii) if so, to measure the correlation between the two factors.

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MATERIALS AND METHODS

Six fields showing evidence of stunting were assayed for nematodes and soil salinity. Soil and roots were collected from five locations in each area with a nursery spade at depths of 15 cm and placed in plastic bags. Normal growth areas in each of the fields were also sampled in the same manner for comparison. Another field with a known history of salinity and nematodes was sampled in more detail. Two soil samples were taken with a nursery spade at depths of 15 to 20 cm at intervals of 7.6 m for a distance of 151 m. The paired samples were blended and half was used for nematode analysis and the other half was used to determine the electrical conductivity in mmhos/cm of the saturated extract (EC_e) (6). Nematode counts were obtained by 48-hr Baermann extraction of 100 g of soil for each composite sample placed on a screen-supported double layer of Scottie® (Scott Paper Company, Chester, Pennsylvania) paper tissue in a 15.2-cm glass funnel containing 0.01% $CaCl_2$

solution. The $CaCl_2$ solution was used to prevent deflocculation and passage of clay particles through the supporting filter. After the specified waiting period, 40 ml of water containing the nematodes was released from the bottom of the funnel for nematode analysis. The number of females per g of root was determined by counting attached females in 3 g of roots, stained with lactophenol and acid fuschin, with the aid of a dissecting microscope. Females per g of root is expressed as an average of the three one-gram counts per root system.

A 2 × 4 factorial greenhouse experiment was designed to determine the effect of salinity on pathogenicity of the reniform nematode to cotton. We used a methyl bromide-treated Hidalgo fine sandy loam with a natural EC_e value of 2, 6, 12, and 18 mmhos/cm, representing low to high salinity levels. Total salts, *p*H, cations and anions were determined for the soils by standard procedures (6). Predominant salts in the saline soil were $CaCl_2$ and NaCl. Sixteen 14 × 20-cm cans, lined with 4-mil polyethylene bags (to prevent leaching of salts) and filled with 3,000 g of soil, were used for each of the four salinity levels. Two seeds of *G. hirsutum* L., var. 'Stoneville 7A', were planted in each can and the moisture level for each treatment, as determined by pressure plate analysis (6), was maintained at one atmosphere throughout the experiment. Water was added daily on a weight basis to bring the water level to one atmosphere. After 31 days the plants were thinned to one per pot and 75,000 *R. reniformis* in 40 ml of water were added to each of eight pots per treatment into four 0.6-cm (diam.) holes per pot, equally spaced 2.5 cm from base of plant stem and 10 cm deep. Nematodes for inoculation were collected from a greenhouse culture of cowpea roots (*Vigna sinensis* [Torner] Savi, var. 'Blackeye') by the Baermann funnel technique and surface-sterilized in 0.001% 8-hydroxyquinoline sulfate for 30 minutes. Beginning 3 days after inoculation, 0.216 g KNO_3/pot was added four times at 2-week intervals. After 104 days, the experiment was terminated and plant heights, top weights (fresh and dry), and nematodes per g of root were recorded.

TABLE 1. Correlation of soil salinity (EC_e) and population densities of *Rotylenchus reniformis* and *Meloidogyne incognita*.

	EC_e	*R. reniformis*	*M. incognita*	Total nematodes
EC_e	1.000	0.141	0.119	0.225
R. reniformis	——	1.000	–0.360	0.446[a]
M. incognita	——	——	1.000	0.674[a]
Total nematodes	——	——	——	1.000

[a] Correlation coefficients >0.423 or <–0.423 necessary for significance at P = 0.05.

RESULTS AND DISCUSSION

Nematode and salinity data from all six fields showing symptoms characteristic of soil salinity, reniform nematode infection, or both, suggested that the two etiological causes could not be accurately identified on the basis of plant symptoms. Some areas with stunted plants had a high EC_e value and high nematode counts, while others had low EC_e values and high nematode counts. The average EC_e value from the fields without stunting symptoms was 4.7 mmhos/cm with an average nematode count of 538 per 100 g soil. Stunted areas in the same field had an average EC_e value of 4.9 mmhos/cm and 1,235 nematodes/100 g soil. In the field with a large saline area, *R. reniformis* and *Meloidogyne incognita* [Kofoid & White] Chitwood) were found in mixed populations (Table 1). The reniform nematode distribution was spotted in this field, but the nematodes were apparently not influenced by the degree of soil salinity.

TABLE 2. Effect of soil salinity and *Rotylenchulus reniformis* on the growth and development of cotton.

EC_e	Treatment	Plant top wt. (g) Fresh	Plant top wt. (g) Dry	Height (cm)	*R. reniformis* Females/g root
2	Inoculated	35.38	7.35	30.12	704
	Check	59.98	12.98	36.19	
6	Inoculated	30.22	6.21	31.59	700
	Check	52.13	11.13	37.79	
12	Inoculated	15.67	2.95	20.69	358
	Check	26.03	5.60	22.55	
18	Inoculated	5.98	1.37	17.59	292
	Check	13.79	3.11	19.53	
LSD Salinity ($P = 0.01$)		5.89	1.18	3.14	
LSD Nematodes ($P = 0.01$)		7.12	1.43	3.80	197.59

Data from the greenhouse experiment are shown in Table 2. As soil salinity increased, growth of cotton decreased. These differences were expected and have been shown by other workers (7, 9). Nematodes significantly reduced the fresh and dry top weight of inoculated plants at each salinity level. Stunting and lack of vigor was apparent in all inoculated plants; chlorosis of the top leaves (Fig. 1) appeared during the last 30 days.

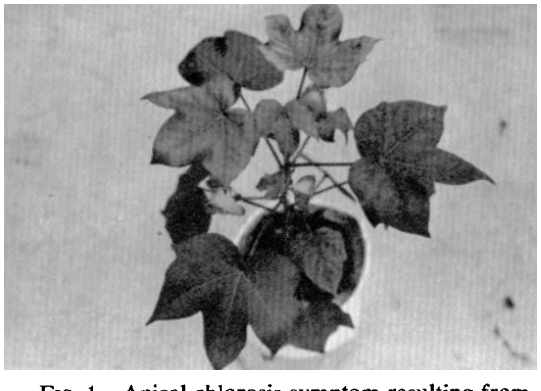

FIG. 1. Apical chlorosis symptom resulting from *Rotylenchulus reniformis* attack on cotton growing in soil with an EC_e value of 6 mmhos/cm.

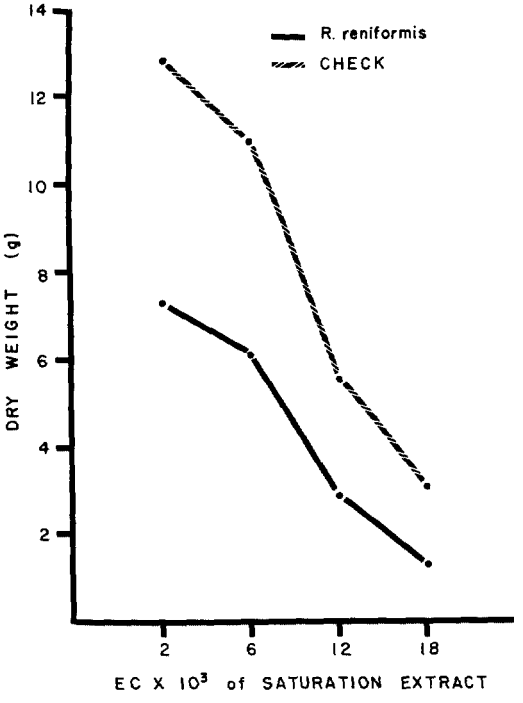

FIG. 2. Dry top weights of cotton plants affected by salinity and *Rotylenchulus reniformis*.

A significant ($P = 0.01$) interaction between soil salinity and inoculated plants is shown in Fig. 2. As soil salinity increased, the percentage reduction in plant weight attributable to nematode injury increased. Dry weight of inoculated plants was 43 and 44% less than that of check plants in soils with a salinity value of EC_e 2 and 6 mmhos/cm, respectively. Reductions of 47 and 56% plant weight occurred for inoculated plants in soil with an EC_e value of 12 and 18 mmhos/cm, respectively. Nematodes significantly reduced plant height at soil salinity levels of 2 and 6, but not at 12 and 18 mmhos/cm. Van Gundy and Martin (8) found that the effect of the citrus nematode was usually most severe under soil conditions that were unfavorable for the growth of citrus. We noted that nematode-inoculated plants at high salt

levels dropped several bottom leaves and plants were very weak, with thin stems. This accounts for the large difference in weight. Number of females per g of root was significantly higher in soils with an EC_e of 2 and 6 mmhos/cm than in those with an EC_e of 12 and 18 mmhos/cm (Fig. 1). These differences were apparently due to the limitation of the development of the root system by the salinity and not to the effect of the salinity on the nematode. Kirkpatrick and Van Gundy (2) found no significant change in citrus nematode larvae inoculated to fallow saline soil levels after 184 days. However, larvae were significantly reduced at the 26.0 mmhos/cm level after 68 and 184 days. Under field conditions, Machmer (4) concluded that more citrus nematodes were recovered from citrus roots subjected to high salinity conditions than from citrus roots grown at lower salinity levels. These results indicate that the various species of nematode are not affected by salinity levels which were used in the experiment.

Data from this research show that the reniform nematode attacks and significantly damages cotton at all soil salinity levels tested. They further show that nematode injury increases as soil salinity increases.

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