SALINITY REDUCES RESISTANCE TO *TYLENCHULUS SEMIPENETRANS* IN CITRUS ROOTSTOCKS[†]

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ABSTRACT

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The effect of salinity on host resistance to the citrus nematode, *Tylenchulus semipenetrans*, was tested in a greenhouse experiment on six citrus rootstocks: the highly nematode-resistant Swingle citrumelo and trifoliate orange, the moderately nematode-resistant Carrizo citrange and Troyer citrange, and the highly nematode-susceptible Cleopatra mandarin and sour orange. Sodium chloride and $CaCl_2$ were added to irrigation water every other day during a 3-week period 6 months after seedling emergence. A total of 167 moles NaCl and 29.4 moles $CaCl_2$ was added per cubic meter soil. Soluble salts in soil were leached prior to inoculation with 73 000 nematodes per plant. Eight weeks after inoculation, resistance was evaluated in terms of female development and number of offspring produced. Salinity increased nematode egg production by two to 10-fold in all rootstocks but did not change the relative levels of nematode resistance among rootstocks.

Key words: Carrizo citrange, citrus nematode, Cleopatra mandarin, fecundity, resistance, salinity, sour orange, Swingle citrumelo, trifoliate orange, Troyer citrange, *Tylenchulus semipenetrans*.

RESUMEN

Mashela, P., L. Duncan y R. McSorley. 1992. La salinidad reduce la resistencia a *Tylenchulus semipenetrans* en patrones del cítrico. Nematrópica 22:7–12.

El efecto de la salinidad del suelo sobre la resistencia del hospedador al nematodo de los cítricos, *Tylenchulus semipenetrans*, fue evaluado en un experimento de invernadero para seis patrones del cítrico: los altamente resistentes citrumelo Swingle y naranjo trifoliado, los moderadamente resistentes citranjo Carizo y citranjo Troyer, y los altamente susceptibles mandarino Cleopatra y naranjo agrio. Cloruro de sodio y $CaCl_2$ fueron añadidos a la agua de riego cada día durante un período de 3 semanas, 6 meses después de la emergención de las plantas. Un total de 167 moles de NaCl y 29.4 moles de $CaCl_2$ fueron añadidos por metro cúbico de suelo. Sales solubles en el suelo fueron lixiviados antes la inoculación de macetas con 73 000 nematodos por planta. Ocho semanas despúes de la inoculación, se evaluó la resistencia midiendo el desarrollo de las hembras y el número de progenie producidos. La salinidad incrementó la producción de huevos de nematodos entre dos y 10 veces en todos los patrones, pero no cambió los niveles relativos de resistencia entre los patrones.

Palabras clave: citranjo Carrizo, citranjo Troyer, citrumelo Swingle, fecundidad, mandarino Cleopatra, naranjo agrio, naranjo trifoliado, nematodo de los cítricos, resistencia, salinidad, *Tylenchulus semipenetrans*.

INTRODUCTION

Nematode-resistant rootstocks play a major role in integrated management of the citrus nematode, *Tylenchulus semipenetrans* Cobb (7,13); however, no commercially available citrus rootstocks are both *T. semipenetrans* resistant and salt tolerant (3). Salt concentrations of irrigation water in several major citrus producing regions in the world are increasing

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(1,19,20) and higher population densities of the citrus nematode usually occur in areas with high salinity (4,15). Recent studies (16) showed that leaching soluble salts after a short period of plant stress increases infection of *T. semipenetrans* on nematode-susceptible citrus rootstocks. Salinity (1,19) and *T. semipenetrans* (5) can each reduce citrus growth and yield. It was recently (17) demonstrated that *T. semipenetrans* infection reduces salt tolerance in citrus rootstocks. However, the effect of salinity on the expression of host resistance to *T. semipenetrans* has not been studied. The objective of this study was to test the effect of salinity on host resistance to *T. semipenetrans* under greenhouse conditions.

MATERIALS AND METHODS

Six citrus rootstocks were selected to represent a wide range of resistance to *T. semipenetrans*. Highly resistant rootstocks were trifoliate orange (*Poncirus trifoliata*) and Swingle citrumelo (*Citrus paradisi* × *P. trifoliata*). Moderately resistant rootstocks were Carrizo citrange (*C. sinensis* × *P. trifoliata*) and Troyer citrange (*C. sinensis* × *P. trifoliata*). Highly susceptible rootstocks were Cleopatra mandarin (*C. reticulata*) and sour orange (*C. aurantium*). Seedlings of each rootstock were raised in plywood boxes containing a potting mix consisting of three volumetric parts of sandy soil (97% sand, 2% silt, 1% clay) and one part organic supplement PRO-MIX BX (Premier Brands, Inc., Stamford, Canada). Two months after emergence, plants were inoculated with a suspension of *Glomus intraradices* Schenck and Smith prepared by macerating 2 g of colonized Sudangrass, *Sorghum sudanense*, roots in a blender, passing the macerate through a 150-μm-pore sieve, and diluting the filtrate to 500 ml with water. Plants were irrigated twice weekly, and fertilized once weekly with 25% Hoagland's solution (10).

Plants were selected for uniformity 4 months post-emergence and transplanted individually into 15-cm-diam clay pots containing the previously described potting mix. Seventeen replications of each rootstock with and without salt treatment were arranged in a greenhouse in a randomized complete-block split plot design. Transplants were fertilized weekly with 20:20:20 ($N:P_2O_5:K_2O$) at 5 g/L and monthly with 25% Hoagland solution to provide micronutrients. Irrigation consisted of 150 ml tap water every second day. To achieve temporary saline conditions in the potting mix, irrigation water for half of the plants per rootstock species was supplemented with $NaCl$ and $CaCl_2$ for a 3-week period beginning 2 months after transplanting. The concentrations of $NaCl$ and $CaCl_2$ in the water were, respectively, 17 mM and 3 mM the first week, 50 mM and 8.8 mM the second week, and 100 mM and 17.6 mM the third week. Thus, salt concentrations were increased gradually and the total amounts of salt applied were 167 moles $NaCl$ and 29.4 moles $CaCl_2$ per cubic meter potting mix. The other half of the plants per rootstock species served as salt-free controls. At the end of the 3-week salinization period, non-saline conditions were recreated by leaching salt-treated soil with 300 ml tap water at 2-day intervals for 1 week. Salt-free controls were also leached. Leachate was collected from each plant at final leaching and the mean electrical conductivity was 0.806 dS/m, which converts to an osmotic potential of –0.290 MPa (2). Salt leaching prior to nematode inoculation was designed to simulate field conditions, to predispose host roots to infection, and to avoid direct, adverse effects of osmotic potential on nematodes (16).

Nematode-infected citrus roots were collected from a citrus orchard and placed in a plastic bag that was half filled with water. The bag was vigorously shaken and the contents were passed through a 150-μm-pore sieve onto a 25-μm-pore sieve. The contents of the 25-μm-pore sieve were aerated in 4.5 L water for 30 min to keep juveniles in suspension while allowing eggs, heavy soil particles, and some debris to settle. The suspension then was passed through a 150-μm-pore sieve onto a 25-μm-pore sieve from which inoculum was collected. Beginning 3 days after the final leaching, each plant was inoculated four times at 3-day intervals to obtain a total of ca. 73 000 nematodes/plant. Greenhouse temperatures from inoculation to harvest averaged 31 C maximum and 26 C minimum.

At harvest, 8 weeks after the initial inoculation, nematodes were extracted by macerating 2 g fresh roots per plant in a blender using 10% NaOC1, and passing the macerate through a 150-μm-pore sieve onto a 25-μm-pore sieve. The contents of the 25-μm-pore sieve were washed into a 96-ml glass tube and, after allowing nematodes to settle for 12 hr, contents were standardized to 25 ml water. 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. Female fecundity (the number of eggs plus juveniles per female) was calculated for each treatment. Total fresh fibrous roots of each plant were weighed. Also, shoots, fibrous roots, and tap roots were weighed after drying at 70 C for 48 hr. Nematode counts were transformed to $\log_2(X+1)$ prior to analysis of variance, but untransformed data are reported. Data for all variables were subjected to a factorial analysis of variance appropriate to the experimental design. Because the block term was not significant ($P \leq 0.10$), it was removed prior to final analysis. Only significant ($P \leq 0.05$) data are discussed unless stated otherwise.

RESULTS

Salinity increased nematode female development and reproduction on all rootstocks (Table 1). Salinity increased reproduction by two and to 10-fold, but nematode densities in resistant rootstocks were consistently lower than those in sus-

Table 1. Number of three life stages of *Tylenchulus semipenetrans* per gram of fresh roots 8 weeks after inoculation on six citrus rootstocks previously grown under nonsaline (control) and saline (salt) conditions.[y]

	Females		Juveniles		Eggs		Fecundity[z]	
Rootstock	Control	Salt	Control	Salt	Control	Salt	Control	Salt
Sour orange	162 a	193 a	49 b	73 a	711 a	1 283 a	5	7
Cleopatra mandarin	245 a	270 a	112 a	88 a	704 a	1 406 a	4	6
Carrizo citrange	45 b	49 b	52 b	73 a	156 b	547 b	5	13
Troyer citrange	29 b	41 b	56 b	74 a	93 b	169 c	6	6
Swingle citrumelo	4 c	15 c	26 c	53 b	23 c	227 c	13	19
Trifoliate orange	9 c	17 c	30 c	52 b	46 c	92 d	6	9

Datas are the means of 17 replications. Means with a column with the same letter are not different ($P \leq 0.05$) according to Duncan's multiple-range test.

[y]Salt was leached from soil before inoculating with nematodes.

[z](Eggs + juveniles)/female.

ceptible rootstocks. Similar trends were observed in nematode female development. After partitioning degrees of freedom and associated sums of squares (14), rootstock treatment contributed 95%, 64%, and 86% to the total treatment variation (TTV) for females, juveniles, and eggs, respectively, while salinity contributed 3%, 22%, and 24%. There were no rootstock × salinity interactions except for a small effect (2%, $P \leq 0.10$) on mean female counts. Mean separations of nematode densities among the rootstocks generally followed the degree of nematode resistance on the rootstocks.

Sour orange had the highest mean fresh fibrous root weight and trifoliate orange had the lowest, while weights of Cleopatra mandarin, Swingle citrumelo, Carrizo citrange, and Troyer citrange were intermediate in both the salt-free control and the salt treatment (Table 2). Only the rootstock effect contributed (98%) to TTV in mean fresh fibrous root weights. Similar trends were observed in dry fibrous roots. Sour orange and Cleopatra mandarin had, respectively, the highest and lowest mean total dry root weights; the intermediate weights of other rootstocks were not different. Rootstock contributed 79% and salinity 13% to TTV in mean dry tap root weights, with no evidence of an interaction effect. Rootstock contributed 89% and salinity 5% to TTV in mean dry total root weights, with no evidence of an interaction effect. There was no evidence of a salinity effect on top weights.

DISCUSSION

Inherent differences (3) in rootstocks were the major source of variation in root weights. Salinity had no measurable effect on fresh or dry fibrous root weight but did cause small (*ca.* 20%) decreases in dry tap and total root weights. Thus, the primary effect of salinity on the root system was the reduction of tap root growth. The role of the citrus tap root in salt tolerance is not clear, since tap roots generally contain lower Cl concentrations than fibrous roots (9). *Tylenchulus semipenetrans* primarily feeds on fibrous roots.

The most notable effect of salinity was to increase nematode egg production by several fold in all rootstocks. Citrus resist-

Table 2. Root and shoot weights (in grams) of 9-month-old citrus rootstocks that were exposed to a 3-week salt treatment (salt) or not exposed (control) when 6 months old and then inoculated with *Tylenchulus semipenetrans* when 7 months old.[z]

	Root							
	Fresh fibrous		Dry fibrous		Dry tap		Dry shoot	
Rootstock	Control	Salt	Control	Salt	Control	Salt	Control	Salt
Sour orange	6.5 a	6.0 a	1.0 a	0.9 a	1.6 a	1.8 a	5.4 a	4.5 a
Cleopatra mandarin	3.1 b	3.7 b	0.3 b	0.4 b	0.5 b	0.5 b	2.4 c	2.7 c
Carrizo citrange	3.1 b	2.9 b	0.3 b	0.3 b	1.0 ab	0.7 b	2.4 c	2.7 c
Troyer citrange	2.7 bc	2.7 bc	0.3 b	0.3 b	0.8 b	0.7 b	2.4 c	2.7 c
Swingle citrumelo	3.5 b	3.1 b	0.6 ab	0.5 b	1.1 ab	0.8 b	4.2 b	3.0 b
Trifoliate orange	1.7 c	1.5 c	0.3 b	0.4 b	0.7 b	0.5 b	1.8 d	1.4 d

Data are the means of 17 replications. Means with a column with the same letter are not different ($P \leq 0.05$) according to Duncan's multiple-range test.

[z]Salt was leached from the soil before inoculating with nematodes.

ance to *T. semipenetrans* is expressed as suppression of female development to maturity (12,22). Development to maturity, even in resistant rootstocks, invariably leads to egg production (12). Females on Swingle citrumelo, the most widely used and the most salt-sensitive citrus rootstock (3), had the greatest relative increase in egg production (10-fold) due to salt treatment. Swingle citrumelo and trifoliate orange possess differential resistance (12), which often is readily overcome by pathogens, including plant-parasitic nematodes (6,21). The enhanced female development and increased fecundity due to salt stress on the host may eventually increase the selection pressure against resistant genes. Biotypes of *T. semipenetrans* capable of reproducing prolifically in resistant trifoliate orange rootstocks have, in fact, been reported from citrus producing regions with high salinity (8,11).

Generally, under field conditions, salinity in citrus production is a seasonal problem. Salts accumulate in the rhizosphere during extended irrigation seasons and leach from the rhizosphere during rainy seasons (1,20). Results of this and previous studies (16) suggest that salt accumulation and leaching cycles can augment *T. semipenetrans* populations even in resistant rootstocks, and may also explain higher population densities of this nematode in areas with high salinity (4,15).

Since salinity increased nematode development and fecundity in all citrus rootstocks tested, and since all nematode resistant rootstocks lack salt tolerance (3), increased salinity in irrigation water (1, 20) affects both citrus breeding and nematode management. As in cereal crops (18), salt tolerant genes should be incorporated into multiple resistance rootstocks such as Carrizo and Troyer citranges. Alternatively, nematode resistance genes could be introduced into the salt tolerant rootstocks Cleopatra mandarin and Rangpur.

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