Development of Crown and Root Rot Disease of Tomato Under Irrigation with Saline Water

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ABSTRACT

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We studied the effect of water salinity on the incidence and severity of crown and root rot disease of tomato, as well as on the pathogen and on the plant's response to the pathogen. Irrigation with saline water significantly increased disease severity in tomato transplants inoculated with *Fusarium oxysporum* f. sp. *radicis-lycopersici*, and mineral fertilization further increased it. In one field experiment, disease incidence in plots irrigated with saline water (electrical conductivity [EC] = 3.2 ± 0.1 dS m⁻¹) and in those irrigated with fresh water (EC = 0.4 ± 0.1 dS m⁻¹) was 75 and 38%, respectively. Disease onset was earlier and yield was

lower in plots irrigated with saline water. In a second field experiment, final disease incidence 250 days after planting, was 12% in plants which had been irrigated with saline water (EC = 4.6 ± 0.1 dS m⁻¹) and 4% in those irrigated with fresh water (EC = 1.2 ± 0.1 dS m⁻¹). Irrigation of tomato transplants with 20 mM NaCl did not inhibit plant development, but partial inhibition was observed at higher NaCl concentrations. Growth of the pathogen in culture or survival of conidia added to soil were not affected by saline water. Plants which were preirrigated with saline water were more severely diseased than those preirrigated with tap water. It was concluded that disease increases effected by saline water are associated with the latter's effect on plant response.

Additional keywords: soilborne pathogens, stress.

Crown and root rot disease of tomato, caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *radicis-lycopersici* W. R. Jarvis & Shoemaker, can result in total crop destruction. Since 1982, this disease has become widespread in Israel in both greenhouses and the open field (18,19). Disease symptoms include root and basal stem rot, discoloration of the vascular system in the basal stems, and wilting of the plant before fruit ripening. A pink layer of macroconidia usually appears on the stems of diseased plants (16,32). Infection of the plant by the pathogen is via chlamydospores which survive in the soil, root-to-root contact, or airborne propagules (32).

Agricultural crops frequently are irrigated with saline water at various salinity levels in arid and semiarid areas, where the availability of fresh water (nonsaline, usually the electrical conductivity [EC] at 1 dS m⁻¹ or less) is limited. In some areas such as the Arava valley (which is an important agricultural area), saline water from local wells is the only source of water for irrigating crops. Irrigation with saline water may have adverse effects on the physical and chemical properties of the soil, on crop production, and on plant metabolism which may, in turn, affect disease severity and plant resistance (5,15,20,27). Salinity generally affects plant growth through either excess ions or water deficit, with different plant organs responding differently. Plant responses to salinity depend upon various factors, such as the duration and degree of the stress and growth stage at stress exposure. However, most agronomical crops (vegetable and fruit) do not develop well

at a salinity level equivalent to 5 dS m⁻¹ or higher (23). Regarding tolerance to saline water with high EC, tomato (*Lycopersicon esculentum* Mill.) is considered to be moderately sensitive to salinity but adversely affected by irrigation with saline water at levels exceeding 2.5 dS m⁻¹. Plant development usually is reduced with increasing salt concentrations but sugar accumulation in the fruit increases (6,23).

Various pathogens are highly tolerant to salts in culture, including *Aspergillus*, *Penicillium*, and *Fusarium* spp. (37), *Pythium aphanidermatum* (30), *Phytophthora* spp. (2,21), and *F. oxysporum* f. sp. *vasinfectum* (28).

High salinity may increase the incidence and severity of diseases caused by various species of *Phytophthora* (3,21,22,33,36), *F. oxysporum* f. sp. *vasinfectum* (38), *F. oxysporum* f. sp. *radicis-lycopersici* (17,39), and *Verticillium dahliae* and *Alternaria solani* (25). On the other hand, suppression of diseases under saline irrigation has been reported with *Fusarium* wilt of date palm (4), *Rhizoctonia* crown and root rot of table beet (9), *Fusarium* crown and root rot of asparagus (11,12), and *Fusarium* wilt of cyclamen (10). The effect of salinity on plant disease may result from its effect on one or more of the biotic components involved in the disease: the pathogen, the host, microbial activity in the soil, or abiotic components of the soil. Soil disinfestation is done frequently in this region, which is cropped under saline water irrigation. Chemical fumigation alone or combined with other methods is effective in controlling the disease (35). However, the build up of the pathogen in soil is very fast, leading to the need for repeated fumigation prior to each crop. In the new era of phase out of fumigants, it is important to better understand the behavior and etiology of the pathogen in order to manage the disease with the limited measures which still are available.

The objectives of the present work were to (i) study the effect of water salinity on the incidence and severity of crown and root

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rot disease of tomato under controlled and field conditions and (ii) study the effect of water salinity on the pathogen in culture and in the soil, and on the plant response to the pathogen.

MATERIALS AND METHODS

Soils. Three types of soil, referred to as soils 1, 2, and 3, were used. Soil 1 was an agricultural sandy soil from the central part of Israel, at Rehovot. Soil characteristics obtained by routine procedures were: contents of clay, silt, and sand, 4, 2, and 94%, respectively; organic matter, 1.0 g kg⁻¹; pH determined in 1:1 water extract (wt/wt), 7.9; water-holding capacity, 9% wt/wt. Soil 2 was collected from the southern desert, Arava region, at Ein Tamar. Soil characteristics obtained by routine procedures were: contents of clay, silt, and sand, 4, 7, and 89%, respectively; organic matter, 1.4 g kg⁻¹; pH determined in 1:1 water extract (wt/wt), 8.1; water-holding capacity, 8% wt/wt. Soil 3 was collected from the Ashalim Experimental Station at Ramat Negev, in the central Israeli desert. Soil characteristics obtained by routine procedures were: contents of clay, silt, and sand, 4, 3, and 93%, respectively; organic matter, 2.7 g kg⁻¹; pH determined in 1:1 water extract (wt/wt), 7.6; water-holding capacity, 8% wt/wt. Soils 2 and 3 both were from fields with diseased tomato plants infected with *F. oxysporum* f. sp. *radicis-lycopersici*. For laboratory studies, the infested soils were collected from the root zone of diseased plants at the end of the crop season and air dried.

Fusarium population counts by soil dilution. Soil samples (2.5 g) were added to 22.5 ml of sterile water agar (0.1% wt/vol) containing MgSO₄·7H₂O (0.1% wt/vol). The samples were shaken for 45 min in a reciprocal shaker (200 rpm). Aliquots of 0.2 ml were spread on four petri dishes which contained peptone-pentachloronitrobenzene selective agar medium (FSM) (14). Plates were incubated in the dark at 28°C for 4 to 6 days. Randomly selected colonies of *F. oxysporum* isolates originating from soil or stems of diseased plants were tested for pathogenicity using tomato transplants, in order to verify their identity as *F. oxysporum* f. sp. *radicis-lycopersici* (13). Results are expressed as CFU per gram of dry soil (105°C for 48 h).

***Fusarium oxysporum* f. sp. *radicis-lycopersici* inocula.** First, soil 2, naturally infested with the pathogen, was stored for over 9 months at room temperature (25°C). Before each test, a population of the pathogen was assayed as already described. Second, the basal parts of stems from diseased tomato plants (cv. 5656), which were covered with a typical pink-orange layer of conidia of *F. oxysporum* f. sp. *radicis-lycopersici* (60% macroconidia), were collected from the field at the end of the crop season and stored in paper bags at room temperature (25°C). Water suspensions of the conidia at predetermined concentrations were used. Their viability was determined by incubating on FSM for 17 h at 17°C and assessing germination. Conidia were considered viable when the length of the germ tube was twice that of the conidium. We used conidial stocks with >90% germination.

Effect of saline water irrigation and fertilizers in greenhouse tests on crown and root rot disease. Two weeks after emergence (with two true leaves), tomato transplants (cv. 870 contributed by Shorashim nurseries, Ein Habesor, Israel) were exposed for 2 weeks to irrigation with tap or saline water (20 mM NaCl). Then, tomato transplants were inoculated with *F. oxysporum* f. sp. *radicis-lycopersici* by first rinsing the root with water, to remove adhering growth medium, then dipping for 5 min in conidial suspension of *F. oxysporum* f. sp. *radicis-lycopersici* obtained from tomato stems (1×10^6 ml⁻¹ in tap or saline water). After inoculation, the tomato transplants were replanted in non-infested Rehovot soil and were irrigated with either tap or saline water, as specified. Three plants were planted in each pot (12-cm diameter, 15-cm depth) and were fertilized with liquid fertilizer Shefer 1 (Deshanim Ltd., Israel) containing 7% N (60% N-NO₃), 3% P₂O₅, 7% K₂O, and the necessary amounts of iron, man-

ganese, zinc, copper, and molybdenum, at 0.1% in the irrigation solutions, for 28 days in the greenhouse at 20°C. After those 28 days, the plants were uprooted and assayed for vascular discoloration after longitudinally sectioning the stem and rated on a scale of 0 to 4, where 0 = healthy plants without discoloration or wilt, and 1 to 4 = slight discoloration symptoms to dead plants. Both the average disease index of all plants and the percentage of severely diseased plants (ranked 2 to 4) were calculated.

In an additional experiment, the effect of fertilizers also was examined. Tomato transplants were irrigated with tap water and fertilized with Shefer 1 (0.1% in the irrigation solutions) before inoculation. After inoculation, plants were transplanted to non-infested soil and irrigated with tap or saline water with or without the addition of fertilizers at 0.1% in the irrigation solutions.

Effect of irrigation with saline water on crown and root rot disease under field conditions. The first field experiment was conducted at the Zohar Experimental Station at Ein Tamar (soil 2), in the southeastern desert in Israel, in soil with a long history of crown and root rot disease. At the end of the previous crop, the incidence of crown rot on tomato plants was >80%. Tomato transplants (cv. 5656, highly susceptible to the disease) were planted in the open field on 25 September 2001. Each plot was 11 m long and contained one row of plants, 0.5 m between plants. Half of the plots were irrigated with fresh water (EC = 0.4 ± 0.1 dS m⁻¹) and half were irrigated with local saline water (EC = 3.2 ± 0.1 dS m⁻¹) to the end of experiment, in February 2002, both via dripping system. The experiment consisted of five replicates per treatment in a randomized complete block design. The plots were prepared, cultivated, and treated according to the recommendations of the extension service in that region. Disease symptoms appeared 75 days after planting. The diseased plants with crown rot symptoms, which collapsed, were recorded periodically.

Soil samples from the root zone were taken at certain intervals after planting in order to assess *Fusarium* populations in the root system zone, as described earlier. Samples of soil and irrigation water were taken for chemical analysis, 120 days after planting. Chemical properties of soil irrigated with fresh water as tested in saturated paste were EC = 0.45 dS m⁻¹, Na = 0.60 mM, Ca = 1.13 mM, and Mg = 0.27 mM. The properties of soil irrigated with saline water were EC = 1.61 dS m⁻¹, Na = 4.30 mM, Ca = 3.92 mM, and Mg = 1.75 mM. Chemical properties of fresh irrigation water were EC = 0.44 dS m⁻¹, Na = 1.95 mM, and Ca = 5.01 mM, pH = 6.76 and for saline irrigation water were EC = 3.24 dS m⁻¹, Na = 14.24 mM, and Ca = 4.78 mM, pH = 7.59.

The second experiment was conducted in a greenhouse at the Ashalim Experimental Station in Ramat Negev (soil 3), in the central Israeli desert, in a soil with a long history of irrigation with saline water. The incidence of crown root disease in tomato plant at the end of the previous season was 40 to 50%. Tomato transplants (cv. 144, moderately susceptible to the disease) were planted on 4 September 2002. Each plot was 8 m long and contained eight rows of plants, with 0.5 m between plants. All plots were irrigated with fresh water (EC = 1.2 ± 0.1 dS m⁻¹) by dripping system until the end of October 2002. After that, half of the plots were irrigated with local saline water (EC = 4.6 ± 0.1 dS m⁻¹) and the other half with fresh water (EC = 1.2 ± 0.1 dS m⁻¹) until the end of the experiment in May 2003. The experiment consisted of four replicates per treatment in a randomized complete block design. The plots were prepared, cultivated, and treated according to the recommendations of the extension service in that region. Disease symptoms appeared 80 days after planting, and plants showing typical disease symptoms were recorded periodically and removed from the greenhouse to prevent possible aerial infection from sporulating diseased plants with spores on the stems (31). At the end of the experiment, in May 2003, all remaining plants were uprooted and evaluated for late symptoms of crown and root rot disease, which were not visible until that time.

Samples of soil and irrigation water were taken for chemical analysis, 120 days after planting. Chemical properties of soil irrigated with fresh water as tested in saturated paste were EC = 0.72 dS m⁻¹, Na = 4.00 mM, Ca = 0.62 mM, and Mg = 0.31 mM. The properties of soil irrigated with saline water were EC = 1.52 dS m⁻¹, Na = 10.00 mM, Ca = 0.98 mM, and Mg = 0.46 mM. Chemical properties of fresh irrigation water were EC = 1.20 dS m⁻¹, Na = 5.85 mM, and Ca = 1.59 mM, pH = 7.22 and for saline irrigation water were EC = 4.60 dS m⁻¹, Na = 27.93 mM, and Ca = 5.15 mM, pH = 7.15.

Chemical analysis of leaf contents was carried out 120 days after planting. Leaves of five plants (the fifth leaf from the top) were removed from each plot, dried at 65°C, and digested with H₂SO₄ and H₂O₂; N and P contents were determined by a colorimetric method with Lachat autoanalyzer and K content was determined by flame spectrometry.

Growth of tomato plants under saline water irrigation in the greenhouse. Tomato transplants (cv. 870, contributed by Shorashim nurseries) were planted in drained pots (15 cm in diameter) which contained a mixture of sandy Rehovot soil with perlite (15% vol/wt, no. 4; Agrikal, Haborim, Israel) to improve soil drainage. Plants were irrigated every 3 days to drainage with saline water at different rates of NaCl, as described. The control plants were irrigated with tap water. The EC of the drained water from each pot was assessed following every irrigation by conductivity meter (B-173, Horbita, Kyoto, Japan). The salts were found not to accumulate in the soil. The plants were fertilized once a week with Shefer 1 (0.1% in irrigation solutions) and were kept at controlled temperature (25°C) in the greenhouse. After 4 weeks, chlorophyll level was assessed by chlorophyll meter (SPAD 502, Minolta, Osaka, Japan). Then, plants and roots were removed to assess root volume (by the change of water height in a narrow water tube) and fresh and dry weight in grams (48 h of drying at 80°C).

Pathogen growth and development on salt-amended medium. Petri dishes containing Czapek agar (7) amended with 0 to 600 mM NaCl or KCl (five plates for each salt concentration) were inoculated with culture disks (8-mm diameter) of *F. oxysporum* f. sp. *radicis-lycopersici*. The plates were incubated in the dark at 28°C. The colony area was assessed at 24-h intervals and plotted against incubation time.

Pathogen survival in soil under saline water irrigation.

Conidial suspensions of *F. oxysporum* f. sp. *radicis-lycopersici* taken from diseased tomato stems were suspended in distilled water and agitated for 1 min at the highest speed (Vortex-Genie, Springfield, MA). The conidial suspension was mixed with soil 2 to a final concentration of 1×10^5 conidia/g. The infested soil was put into 1-liter drained pots (15 cm depth) which then were irrigated to drainage with saline water of 0, 2, 4, and 10 dS m⁻¹ NaCl every 3 days (1.17 g of NaCl liter⁻¹ of H₂O, ≈20 mM NaCl, ≈2 dS m⁻¹). The pots were kept for 28 days at 25°C. Soil samples were assayed every 7 days for population density of *F. oxysporum* f. sp. *radicis-lycopersici* by the soil dilution method. The indigenous population of *F. oxysporum* f. sp. *radicis-lycopersici* in this soil consisted of ≈1% of the conidia added to the soil, which alone cannot produce disease symptoms within 30 days of incubation.

Effect of saline water irrigation on plant predisposition to disease. Tomato transplants were grown in noninfested soil irrigated with either tap or saline (20 mM NaCl) water. After 14 days, the plants were removed and their roots were washed and inoculated with conidia of *F. oxysporum* f. sp. *radicis-lycopersici* obtained from stems of diseased plants. Half of the plants, from each of the two treatments, then were transplanted in noninfested soil and irrigated with tap water only to assess the effect on predisposition to disease. The other half of the plants were transplanted in noninfested soil and irrigated with saline water to assess the effect of irrigation with saline water, after inoculation, on disease. Plants from all treatment were fertilized every 3 days with Shefer 1 (0.1% in the irrigation solutions).

Statistical analyses. All laboratory and greenhouse experiments were conducted at least twice. Data which were taken as percentages were arcsin transformed before analysis. Nonparametric variables such as disease index were analyzed using the rank procedure followed by analysis of variance. The effects of disease and water salinity were tested by two-way analysis of variance. The combinations were compared by one-way analysis of variance and interaction effects were tested by contrast *t* tests. All treatment means were compared simultaneously by the Fisher protected test for significant differences. All analyses were performed with the SAS program (SAS Institute, Cary, NC, and release 8.01 for PC) at $P \leq 0.05$.

RESULTS

Effect of saline water irrigation and fertilizers in greenhouse tests on crown and root rot disease. Tomato transplants were inoculated with conidia of *F. oxysporum* f. sp. *radicis-lycopersici* collected from stems of naturally infected plants. Subsequent irrigation with saline water significantly increased disease severity in the inoculated tomato transplants (Fig. 1). Similarly, the percentages of severely diseased plants were 5 and 39% for plants irrigated with tap or saline water, respectively, after inoculation (Fig. 1B).

Mineral fertilization of inoculated tomato transplants further increased disease severity under irrigation with saline water (Fig. 2). For example, the percentages of diseased plants ranked 3 and 4 for disease severity in the nonfertilized plants were 0, 11, and 16% for 0, 20, and 50 mM NaCl, respectively, whereas the respective numbers for the fertilized plants were 0, 39, and 44%. Dead plants (grade 4) were observed only in pots which were both irrigated with saline water and fertilized (Fig. 2B). Significant differences were obtained ($P \leq 0.001$) for the main effects (i.e., irrigation and mineral fertilization), but no interaction between effects was recorded.

The shoots of plants that had been irrigated without fertilizer were chlorotic compared with those of plants which had been fertilized. However, the roots of the nonfertilized plants were larger in volume, root diameter, and branching (data not shown).

Fig. 1. Effect of irrigation with saline water after inoculation on **A**, crown and root rot disease severity and **B**, incidence of severely diseased tomato plants in a greenhouse experiment. Roots were dipped in a conidial suspension of *Fusarium oxysporum* f. sp. *radicis-lycopersici* and plants were transplanted to noninfested soil and irrigated with tap water (0 mM NaCl) or with saline water (20 mM NaCl). Disease assessment was based on a 0-to-4 severity scale, where 0 = no vascular discoloration or wilt and 4 = plant death. Different letters within **A** or **B** denote significant differences among treatments ($P \leq 0.05$).

Effect of irrigation with saline water on crown and root rot disease under field conditions. The first field experiment was carried out at the Zohar Experimental Station in Ein Tamar, in a field which had been irrigated with saline water ($EC = 3.2 \pm 0.1 \text{ dS m}^{-1}$) for 10 years. The tomato cultivar in this open field crop was 5656, which is highly susceptible to crown and root rot disease. First symptoms of crown and root rot disease appeared 75 days after planting (Fig. 3). High disease incidence (75%) was evident in plots which were irrigated with saline water, compared with 38% in plots which were irrigated with fresh water ($EC = 0.4 \pm 0.1 \text{ dS m}^{-1}$). Similarly, area under disease progress curve values were significantly higher in plots irrigated with saline water, and disease onset was earlier and yield was lower in plots irrigated with saline water, compared with irrigation with fresh water.

Leaf analysis was carried out 120 days after planting, to assess the effects of water salinity on mineral availability and transport in plants. The mineral content in plants irrigated with fresh water was (in percentage of dry matter) was Na = 0.20, N = 3.40, P = 0.49, and K = 3.10, and in those irrigated with saline water was Na = 0.32, N = 2.90, P = 0.39, and K = 2.00.

Soil samples were taken from the root zone of the plants at certain intervals after planting. The buildup of *Fusarium* populations in soil was faster and to a higher level under saline water irrigation (Fig. 4). At 90 days after planting, the population reached 1,500 CFU/g of soil compared with 396 in the plots which were irrigated with fresh water. The fast buildup of *Fusarium* spp. corresponds with the earlier disease onset in the plants which were irrigated with saline water (Fig. 3).

In the second experiment at the Ashalim Experimental Station in Ramat Negev, the first symptoms of crown and root rot disease appeared 80 days after planting. Essentially, the results confirmed those of the first field experiment. Thus, final disease incidence, 250 days after planting, was 12% in plants which had been irrigated with saline water compared with 4% in plants irrigated with fresh water (Fig. 5). At the end of the experiment, the remaining plants were uprooted and examined for late disease symptoms of crown and root rot (i.e., symptoms which could not be detected earlier in the season). A high incidence of late disease symptoms (50%) was evident in plots irrigated with saline water, compared

with 16% in plots irrigated with fresh water. Irrigation with saline water caused a significant reduction in yield (by 15%). Analysis of chemical leaf contents for N, P, and K were similar to these obtained in Ein Tamar experiment (data not shown).

Growth of tomato plants under saline water irrigation in the greenhouse. Irrigation of tomato transplants with saline water (20 mM NaCl, which is considered as a moderate salinity level) did not inhibit plant development (Fig. 6). At higher salt concentrations, root volume and shoot fresh weight were reduced by 24 and 66%, respectively, at 100 mM NaCl, relative to tap water. At 200 mM NaCl, root volume was reduced by 38% and shoot fresh weight by 99%, relative to tap water. Irrigation with saline water increased chlorophyll counts by 18% at 70 mM NaCl; however, at 200 mM NaCl, counts were lower by 10% compared with tap water.

Pathogen growth and development on salt-amended medium. Growth of *F. oxysporum* f. sp. *radicis-lycopersici* in culture was not adversely affected when the concentration of NaCl or KCl was at or below 100 mM (Fig. 7). Only when the salt concen-

Fig. 3. Effect of water salinity on **A**, incidence of crown and root rot disease, **B**, area under disease progress curve (AUDPC), and **C**, tomato cumulative yield (kg m^{-2}) at the Zohar Experimental Station in Ein Tamar. The plants were irrigated with either fresh water (electrical conductivity $[EC] = 0.4 \pm 0.1 \text{ dS m}^{-1}$) or saline water ($EC = 3.2 \pm 0.1 \text{ dS m}^{-1}$). Different letters within **A**, **B**, and **C** denote significant differences among treatments ($P \leq 0.05$).

Fig. 2. Effect of mineral fertilization and water salinity on **A**, crown and root rot disease severity and **B**, incidence of severely diseased tomato plants inoculated with *Fusarium oxysporum* f. sp. *radicis-lycopersici*. After inoculation, plants were planted in noninfested soil and irrigated with 0 mM NaCl (tap water) or saline water at 20 or 50 mM NaCl, with or without fertilizer. Disease assessment was based on a 0-to-4 severity scale, where 0 = no vascular discoloration or wilt 4 = plant death. Vertical bars are standard deviation for each column. Statistical analysis of the main effects show a significant effect of water salinity ($P \leq 0.0055$) and mineral fertilization ($P \leq 0.0003$) on disease severity, but no interaction was evident ($P \leq 0.0666$).

Fig. 4. Effect of water salinity on buildup of *Fusarium* population in the root zone in soil during tomato crop at the Zohar Experimental Station in Ein Tamar. The plants were irrigated with either fresh water (electrical conductivity $[EC] = 0.4 \pm 0.1 \text{ dS m}^{-1}$) or saline water ($EC = 3.2 \pm 0.1 \text{ dS m}^{-1}$). Vertical lines indicate standard deviation.

trations exceeded 200 mM was colony area significantly reduced. Germination of conidia from infected stems was not affected (95% viability) when they were incubated in a 20 mM NaCl salt solution for 10 min (data not shown).

Pathogen survival in soil with saline water irrigation. Survival in soil of conidia of *F. oxysporum* f. sp. *radicis-lycopersici* originating from naturally infected stems under saline water irrigation to drainage (up to 100 mM NaCl) was similar to that in soil irrigated with tap water (Fig. 8). Their population was reduced with all treatments to $\approx 20\%$ of their initial numbers after 21 days.

In a similar experiment, a soil 2 sample, which naturally contains chlamydospores (13), was irrigated with either tap or saline water (up to 80 mM NaCl). The population of *F. oxysporum* f. sp. *radicis-lycopersici* after 60 days of incubation was reduced from 3,500 to $\approx 3,000$ CFU/g of soil with all treatments, without significant differences (data not shown). Thus, survival of chlamydo-

spores under natural conditions, namely, when exposed to microbial activity, was not significantly affected by irrigation with saline water at the tested levels, similar to survival of conidia from stem in the soil, the other type of natural inoculum.

Effect of saline water irrigation on plant predisposition to disease. To assess the effect of a previous irrigation with saline water on predisposition to disease, noninoculated tomato plants were irrigated with either tap or saline water and afterward removed, their roots washed and inoculated, and transplanted to a new soil where all plants were irrigated with tap water. Plants which were preirrigated with saline water were more severely diseased than those preirrigated with tap water (77 versus 38%) (Fig. 9A). Additional irrigation with saline water after inoculation increased disease severity in plants which had been preirrigated with fresh water but it barely increased disease in plants which previously had been irrigated with saline water (Fig. 9B).

Fig. 5. Effect of water salinity on **A**, incidence of crown and root rot disease and **B**, tomato yield (kg m^{-2}) at the Ashalim Experimental Station in Ramat Negev. The plants were irrigated with either fresh water (electrical conductivity $[\text{EC}] = 1.2 \pm 0.1 \text{ dS m}^{-1}$) or saline water ($\text{EC} = 4.6 \pm 0.1 \text{ dS m}^{-1}$). Plants showing typical disease symptoms were recorded and uprooted until 250 days after planting. All remaining plants then were uprooted and assayed for late symptoms of disease. Different letters within **A** and **B** denote significant differences among treatments ($P \leq 0.05$).

Fig. 6. Effect of water salinity on development parameters of tomato plants. Plants were irrigated with tap water (0 mM NaCl) or saline water (10 to 200 mM NaCl) for 28 days. Shoot fresh weight represents weight additional to that of the plants on the day of planting. Vertical bars are standard deviation for each value. Arrows indicate the NaCl concentration at which the tested parameter is significantly different from tap water (except for the last measurement by SPAD) according to *t* test ($P \leq 0.05$).

Fig. 7. Effect of KCl and NaCl concentration (mM) added to Czapek agar medium on relative growth of *Fusarium oxysporum* f. sp. *radicis-lycopersici* after 96 h of incubation in the dark at 28°C. Regression line expresses colony development versus salt concentration. Growth in the nonamended control was considered to be 100% (horizontal line).

Fig. 8. Decline in conidial population of *Fusarium oxysporum* f. sp. *radicis-lycopersici* (naturally produced on tomato stems) added to soil and irrigated with saline water (0 to 100 mM NaCl). Initial population of conidia was $\approx 10^4$ per gram of soil. Results are expressed as percentage of initial during 28 days of incubation at 25°C. Standard deviation (SD_{max}) at the final data point was 8% of initial. No significant differences were found between treatments ($P \geq 0.05$).

DISCUSSION

The incidence and severity of crown and root rot disease of tomato were significantly increased under irrigation with saline water, as observed under both controlled and field conditions. Moreover, the pathogen buildup at the root zone in soil was faster, the disease onset came earlier, and yield was significantly reduced under saline water irrigation. The increase in disease incidence and severity following irrigation with saline water was validated in field experiments with high inoculum density. The field plots had been irrigated continuously with saline water for the previous 10 years. The long-term irrigation with saline water, as well as frequent cropping of tomato, appears to be responsible for the increase in disease incidence in these fields. Increased disease incidence following irrigation with high-salinity water had been reported with a variety of soilborne pathogens, including pathogenic forms of *Fusarium* (3,17,21,22,25,35,37–39). It will be interesting to study, in the future, the effect of salinity on diseases in salt-tolerant cultivars.

Mineral fertilization of plants irrigated with saline water further increased the severity of crown and root rot disease (Fig. 2). Woltz et al. (39) and Jones et al. (17) found that mineral fertilization with $(\text{NH}_4)_2\text{SO}_4$, irrigation with saline water (NaCl), and an acidic environment ($\text{pH} < 4$) enhanced disease severity of tomato. Low concentrations of NO_3^- fertilization (39 to 79 mg liter⁻¹) reduced disease severity of crown and root rot in hydroponically grown tomato; however, high concentrations of fertilizer, >100 mg liter⁻¹, increased disease severity (8). In our system, a level of NO_3^- at 85 mg liter⁻¹, using commercial fertilizer, was sufficient to increase disease incidence and severity. The availability and absorption of minerals by plants differ in saline environments compared with nonsaline conditions (6). Irrigation of tomato plants in noninfested soil with saline water (20 mM NaCl) did not affect plant development; however, at higher concentrations (>100 mM NaCl), plant growth was adversely affected (Fig. 6).

The increase in disease incidence and severity following irrigation with saline water can be attributed to an effect on one or more of the biotic components involved in disease: pathogen, microbial antagonistic activity, and host resistance or susceptibility. Moreover, salinity may affect physical-chemical properties of the soil (e.g., aggregation and aeration) and these in turn may affect the aforementioned biotic components. Various studies have shown that high salinity can affect one or more of these biotic components. In the present study, neither growth in culture of *F. oxysporum* f. sp. *radicis-lycopersici* (Fig. 7) nor conidial germination were affected by water salinity at 100 mM NaCl, indicating that this pathogen tolerates a high salinity level, even levels at which crop growth is damaged. Thus, there is no evidence that high salinity stimulates this pathogen, although the effect on virulence (29) was not tested. Tolerance of soil fungi to high salinity levels has been demonstrated in other studies (2,21, 28,30). In this study, the survival in soil of two types of natural inoculum, chlamydospores and conidia, produced on stems (Fig. 8) was not affected by high salinity. Survival in natural soil is connected directly with microbial activity. Thus, a possible effect of saline water via increased plant susceptibility to the pathogen, the third biotic component, should be considered.

We demonstrated an effect on the plant's predisposition to disease. A short exposure of 14 days to high salinity (in the absence of pathogen) was sufficient to render the plants significantly more susceptible to subsequent inoculation with the pathogen (Fig. 9A). A subsequent irrigation with fresh water did not alleviate the increased susceptibility, indicating that the salinity stress is a "plastic strain" (i.e., it is irreversible) (34). Additional exposure to high salinity after inoculation, thereby exposing both the pathogen and the soil organisms to high salinity, did not further increase disease severity (Fig. 9B); this result emphasized the importance of the effect of salinity on plant susceptibility or resis-

tance. Increased susceptibility of plants exposed to disease by high salinity has been reported with various pathogens. Salinity stress increased *Phytophthora* root rot in chrysanthemum (21,22). The increased root rot was attributed directly to the effect of salinity on the host without any concomitant salinity effect on the pathogen. The salinity stress was regarded as a predisposing factor. Salinity increased *Phytophthora* root rot in tomato, even though it reduced zoospore release and motility (36). Salinity increased the incidence of *Phytophthora* root rot of citrus (3), *Pythium* blight in *Agrostis* spp. (30), and *Phytophthora capsici* in chile pepper (33), and those increases were attributed to an effect on plant susceptibility.

High salinity may affect plant physiology via morphological, anatomical, metabolic, and biochemical changes, such as water relations; number and size of stomata; stem, leaf, root, and membrane structure; photosynthesis; protein synthesis; lipid metabolism; thickening of the cuticle; ion homeostasis and membrane function; water uptake and transport, salt accumulation; metabolic pathways; and synthesis of osmolytes, enzymes, and nucleic acids (1,5,15,20,26,27). The above changes inflicted on the plant by high salinity might be associated with increased susceptibility to the pathogen. Salinity may lead to decrease in other nutrient ions in the tissues (1,26), including K^+ , which frequently is connected with resistance to pathogens. Salinity imposes on the plant osmotic stress, lowered water potential, and water deficit (1,24), which may further enhance wilt severity. Another factor to consider is the effect of salinity on root exudates (1), which are a crucial factor in the early stages of infection by a soilborne pathogen.

Increased disease incidence with increased salinity is not a general rule. On the contrary, some diseases are suppressed by high salinity, including *Fusarium* wilt of date palm under irrigation with water having an $\text{EC} = 20 \text{ dS m}^{-1}$ (4), *Rhizoctonia* root and crown rot of table beets (9), and *Fusarium* crown and root rot of asparagus, apparently due to increasing Mn levels in the root cells, which induces disease resistance (12). Application of NaCl did not affect the final disease incidence (but delayed onset of disease symptoms) in cyclamen infected with *F. oxysporum* f. sp. *cyclaminis* (10).

Fig. 9. Effect of irrigation with saline or tap water before or after inoculation with *Fusarium oxysporum* f. sp. *radicis-lycopersici* of tomato plants on crown and root rot disease severity and incidence of severely diseased plants. Before inoculation, plants were irrigated with tap water (0 mM NaCl) or saline water (20 mM NaCl). After inoculation, plants were planted in noninfested soil and irrigated with **A**, tap water (0 mM NaCl) or **B**, saline water (20 mM NaCl). Disease assessment was based on a 0-to-4 severity scale, where 0 = no vascular discoloration or wilt and 4 = plant death. Vertical bars are standard deviation for each column. Statistical analysis of the main effects shows a significant effect of water salinity before ($P \leq 0.0153$) and after ($P \leq 0.0284$) inoculation on disease severity, but no interaction was evident ($P \leq 0.0842$).

Our findings concerning the interaction of crown and root rot disease of tomato with salinity correspond with studies in other pathosystems and indicate that the use of high-salinity water for irrigation is harmful from a phytopathological perspective extending beyond its direct damage to the plant. Thus, high salinity may adversely affect plant health in a very broad sense, an aspect which needs to be more thoroughly considered.

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