Effect of Soil Salinity and Water Content on Stem Rot Caused by *Phytophthora citrophthora* and Accumulation of Phytoalexin in Citrus Rootstocks

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ABSTRACT

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Both high soil salinity and water content increased the severity of stem rot caused by *P. citrophthora* in the citrus rootstocks troyer citrange, sour orange, and rough lemon. As high salinity did not stimulate growth of the pathogen in vitro, the increase in disease under saline conditions probably was not attributable to a direct effect of salt on the fungus but to a reduction in the resistance of the host. Severity of stem rot was positively correlated with relative stem water content (θ_s) as affected by soil water content but was negatively correlated with θ_s as affected by soil salinity. Therefore, although soil water content may affect disease directly through an effect on θ_s, salinity probably does not increase disease through an effect on θ_s. High salinity apparently causes reduced accumulation of the phytoalexin, 6,7-dimethoxycoumarin, and therefore increases susceptibility of plant tissues to invasion by the fungus. Concentration of 6,7-dimethoxycoumarin was not affected by soil water content.

Additional keywords: resistance mechanisms, environmental stress, stress physiology.

Phytophthora citrophthora (R.E. Sm. & E.H. Sm.) Leonian is a major pathogen of irrigated citrus in inland Australia and other semiarid regions, causing rotting of feeder roots and cankering and gummosis of the lower trunk (9,14,15). In these regions, environmental stresses occur regularly and must be considered in disease epidemiology and control. Prolonged flooding during irrigation has long been a problem leading to high incidence and severity of diseases of citrus caused by *Phytophthora* spp. (20,28), and in these regions soil salinity is an increasingly serious problem that probably also has an impact on these diseases (7,23,24). Use of resistant citrus rootstocks has been important in control of these diseases (9). As most citrus species are very sensitive to

salinity (4), the effect of salinity on the resistance of rootstocks should be determined.

A saline treatment before inoculation predisposed a susceptible cultivar of sweet orange to root rot caused by *P. parasitica* (7). The treatment did not increase severity of the disease in troyer citrange, which has some resistance to *Phytophthora* (8) and tolerance to salinity (17). However, prolonged growth of troyer citrange in saline soil increased severity of root rot compared with that in nonsaline soil. In the field in California, root rot of citrus caused by *P. parasitica* was more serious at high than low soil salinity, although the effects of salinity were difficult to separate from those of high soil water content (7).

Flooding of soil not only favors important aspects of *Phytophthora* activity (11,13,26) but may also predispose host plants to fungal invasion (5,21,23).

The mechanisms by which salinity or high soil water content predispose plants to disease caused by *Phytophthora* spp. are not understood (23). High salinity may directly enhance the growth of *Phytophthora* in plant tissues (7) or alter the physiology of the host plant, resulting in reduced resistance to fungal invasion within the plant tissues (23). Soil salinity and water content may affect susceptibility of tissues to invasion by *Phytophthora* through an effect on relative tissue water content (θ_s). Soil water content affects the rate of expansion of lesions caused by *P. cinnamomi* in *Eucalyptus marginata* Sm. through an effect on relative phloem water content of the host (31).

Salinity or flooding stress may reduce the accumulation of chemicals, such as phytoalexins, which contribute to the normal resistance of plant tissues to fungal invasion (25). Increased salinity of the rhizosphere decreased the ability of soybean to accumulate the pterocarpenoid phytoalexin, glyceollin, in the stem after inoculation with *P. megasperma* var. *sojae* (27). Accumulation of a phytoalexin, 6,7-dimethoxycoumarin (DMC), is associated with resistance of citrus rootstocks to *P. citrophthora* (1,29). The effect of soil salinity and water content on the resistance of citrus rootstocks to stem rot caused by *P. citrophthora* has not been reported and was investigated in the present study. Direct inoculation of stem bark enabled study of the effect of soil environment on the colonization phase of the disease, which is the phase most directly affected by tissue resistance. Furthermore, the effect of soil salinity and water content on accumulation of DMC at the infection site of *P. citrophthora* in the stems of citrus rootstocks was investigated.

MATERIALS AND METHODS

Plants and fungus. The citrus rootstocks chosen for the experiments were troyer citrange (*Poncirus trifoliata* (L.) Raf. × *Citrus sinensis* (L.) Osbeck), sour orange (*C. aurantium* L.), and rough lemon (*C. jambhiri* Lush.), which are resistant, moderately resistant, and very susceptible, respectively, to *P. citrophthora* (8,9). All experiments were conducted in a controlled environment room at approximately 26/22 C with 16-h light and 8-h dark photoperiod.

P. citrophthora was cultured at 25 C on 10% V8 juice agar (2), pH 6.5, inoculated into half-ripe lemon fruit, and reisolated from the leading edge of the infection to maintain pathogenicity before each experiment.

Soil. The toomuc sandy loam soil (sand 68%, silt 14%, clay 18%, organic matter content 6%, cation exchange capacity 5.8
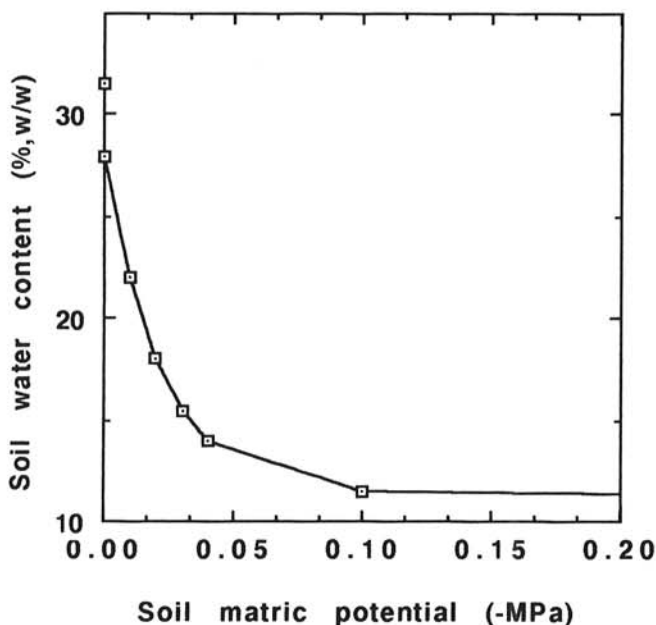

Fig. 1. Relationship between water content and matric potential of the sandy loam soil used in all experiments.

meq 100 g^{-1} dry soil) (18) used in the pot experiments was passed through a sieve with a mesh size of 1.5 cm. The relationship between soil water content and matric potential (φ_m) of the soil is shown in Figure 1. Soil water contents at saturation (28%, w/w, $\varphi_m = 0$ MPa), field capacity (18%, w/w, $\varphi_m = -0.02$ MPa), and permanent wilting point of citrus (9–10%, w/w, $\varphi_m < -0.1$ MPa) were determined. The soil was steam-pasteurized at 80 C for 1 h, and basal rates of all macronutrients and micronutrients were added to ensure that nutrient deficiencies did not occur. Soil pH was adjusted with lime to 6.5. A sample of amended soil was analyzed for Na$^+$, K$^+$, Ca^{2+}, and Mg^{2+} content by atomic absorption spectrophotometry of soil extracts prepared by shaking the soil with distilled H$_2$O. Ion concentrations were Na$^+$, 1.4; K$^+$, 1.15; Ca^{2+}, 7.1; and Mg^{2+}, 2.17 meq 100 g^{-1} dry soil. The soil was then air dried to approximately 1% soil water content and brought to the required soil water contents and salinities with deionized distilled H$_2$O or appropriate NaCl solutions. Soil salinities and water contents were maintained by watering pots with deionized water at the same time each day to maintain the predetermined weights of the pots. Water was added through two perforated plastic tubes inserted in the soil, and a 2.5-cm-thick layer of alkathene beads (ICI Australia, Melbourne) was added to the soil surface in each pot to minimize evaporation from the soil. Water content in soils kept at 12 and 15% decreased by less than 1%, and water content in soils at 18, 22, and 28% decreased by 1–1.5% between waterings.

Effect of soil salinity and water content on stem rot. Twenty-month-old plants were removed from pots and the roots were washed. The plants were then grown in soil in 3-L pots and given water and salinity treatments in a factorial design with three concentrations of NaCl in the soil solution (12.5, 30, or 60 mM) and three soil water contents (12, 18, or 28%, w/w), with three replicates per treatment. There was no evidence of stress of seedlings due to transplant shock. Plants transferred into dry soil (12% water content) were initially transferred into soil at 15% water content; after equilibration for 3 days, this had declined to 12%. The salinity levels used in these experiments were comparable to those that occur in citrus-growing regions in inland Australia, particularly in the periods between irrigations (S. Sykes, *personal communication*).

Three days after transplanting, the stems were inoculated with a piece of shoot infected with *P. citrophthora*. Inoculum was prepared by surface-sterilizing 5-mm-long segments from young shoots of sour orange, placing them on the edge of a 3-day-old colony of *P. citrophthora* growing on 10% V8 juice agar, and incubating them with the fungus for 5 days at 25 C. Stems were inoculated approximately 25 cm above the soil by inserting an infected shoot segment into a 3-mm-wide cut in the bark, which was then bound with Parafilm (American National Can, Greenwich, CT). A noninfected shoot segment was inserted into a cut in the bark of each control plant.

The degree of stem rot was measured 10 days after inoculation as the surface area of the elliptical zone of necrotic tissue. The effects of soil salinity and soil water content separately on stem rot were confirmed in two experiments as described below.

Effect of soil salinity on stem rot. Twenty-month-old plants were transplanted as described above into pots containing soil at field capacity with four different concentrations of NaCl (12.5, 30, 45, or 60 mM). Treatments were replicated four times. Three days after plants were transplanted, the stems were inoculated as described above.

Leaf water potential (φL) was measured with a pressure vessel (22) 5 and 10 days after inoculation. Two leaves from each plant, sampled from just below the area of stem necrosis, were measured and the data averaged. Usually, the two measurements varied by no more than 0.1 MPa.

The amount of stem rot was measured 10 days after inoculation by excision and determination of the fresh weight of necrotic tissue. Excised pieces of necrotic tissue were kept wrapped in moist tissue paper in a petri dish for at least 4 h to ensure that all tissue samples had a consistent water content when weighed. This was a more accurate measurement of the extent of necrosis

than the surface area of necrotic bark because the internal necrosis frequently was more extensive than the necrosis visible on the surface. At the same time, the relative tissue water content (θ_s) (3) of a piece of stem tissue (5 cm long) excised from just below the area of necrotic tissue was estimated from the formula: $\theta_s = $ (fresh weight − dry weight)/(turgid weight − dry weight) × 100%, in which fresh weight is the weight of the tissue immediately after excision, dry weight is the oven-dry weight of the tissue, and turgid weight is the weight of the tissue after immersion in sterile distilled H_2O for 2 days. Relative tissue water content was determined for a single piece of stem tissue per plant.

This experiment was conducted twice, and results of the second experiment are presented.

Effect of soil water content on stem rot. Twenty-month-old plants were transplanted as described above into pots containing soil with five different soil water contents (12, 15, 18, 22, or 28% w/w). The concentration of NaCl in the soil solution was 12.5 mM. Three days after plants were transplanted, the stems were inoculated as described above. The treatments were replicated three times.

Leaf water potential was measured with a pressure vessel 5 and 10 days after inoculation as described above. The degree of stem rot was measured 10 days after inoculation by excision and determination of the fresh weight of necrotic tissue. At the same time, θ_s for a piece of stem tissue (5 cm long) excised from just below the infected region on each plant was determined.

This experiment was conducted twice and the results of the second experiment are presented.

Effect of salinity on growth of *P. citrophthora* in vitro. Growth of *P. citrophthora* was measured as dry weight increments in broth containing various concentrations (1, 5, or 10%) of V8 juice cleared by centrifugation for 10 min at 2,000 g. The effects of two concentrations of Ca^{2+} in 10% cleared V8 juice broth were also compared. The Ca^{2+} concentrations (measured by atomic absorption spectrophotometry of ashed media) were 0.12 meq L^{-1} in unamended medium and 1.0 meq L^{-1} in medium that was stirred with excess $CaCO_3$ and centrifuged at 2,000 g for 10 min to remove undissolved $CaCO_3$. Eight concentrations of NaCl in each medium were prepared in 100-ml Erlenmeyer flasks. A 5-mm disk of Miracloth (Calbiochem Co., La Jolla, CA) bearing 7-day-old mycelium was transferred into each flask and incubated at 25 C for 10 days. The mycelium was removed from the medium, separated from the Miracloth, and washed carefully with distilled H_2O. Mycelial mats were dried at 70 C for 3 days and then weighed.

The colony diameter and amount of sporulation of the fungus were measured in plastic petri dishes (9 cm in diameter) containing 5% cleared V8 juice agar medium (pH 6.5) with no added Ca^{2+} and eight concentrations of NaCl (ranging from 8 to 200 mM NaCl). A disk of Miracloth bearing mycelium of the fungus was transferred into the center of each dish. Treatments were replicated three times. Cultures were incubated for 7 days at 25 C. The diameter of each colony along eight axes through the center of the colony was measured daily and marked on the base of the petri dish. At each mark a sporangial count was made daily in a single field of view at 40× magnification. These experiments were conducted twice, and the results of the second set of experiments are presented.

Effect of soil salinity and water content on the accumulation of DMC in necrotic stems. Twenty-month-old plants were transplanted as before into pots containing soil with two concentrations of NaCl (12.5 or 60 mM). Three days later, the stems were inoculated as described above. The treatments were replicated four times. Leaf water potential was measured with a pressure vessel 5 and 10 days after inoculation.

Ten days after inoculation, the extent of stem necrosis was measured as before and the excised necrotic tissue was extracted for DMC as described below. At the same time, θ_s, and Na^+ and Cl^- concentrations of stem pieces (approximately 2 cm long for each measurement) from just below the necrotic region were measured. Na^+ concentration was measured with a flame photometer (Corning 400, Halstead, Essex, England) and Cl^-

concentration with a chloride meter (Corning-EEL 920, Halstead, Essex, England). The experiment was conducted twice, and results of the first experiment are reported. DMC concentration in necrotic tissue was also determined following inoculation of seedlings subjected to different soil water contents maintained as described above.

Extraction and quantification of DMC. The method used to quantify DMC was similar to that of Sulistyowati et al (29). A quantity of necrotic bark tissue (approximately 20–600 mg, depending on the extent of necrosis) from the stem of each plant was ground in 3 ml of distilled H_2O with a mortar and pestle and the homogenate incubated for 2 h at 40 C. Ethylacetate (EtOAc) (2.5 ml) was then added and samples (200–400 μl) of the EtOAc fraction resolved by thin-layer chromatography (silica gel 60, F_{254}, 0.2 mm thick; E. Merck, Darmstadt, Germany). Ascending chromatograms were developed in toluene:EtOAc (1:1, v/v) and visualized under UV light (360 nm). DMC gave a single blue fluorescent spot at $R_F = 0.4$, and this was removed and dissolved in MeOH (usually 5–10 ml). Fluorescence was determined in 10-mm cuvettes in a spectrophotofluorimeter (Perkin-Elmer 3000, Norwalk, CT) at an emission wavelength of 427 nm and excitation wavelength of 341 nm. DMC was quantified by reference to a standard curve determined with authentic DMC (Aldrich Chemical Co., Milwaukee, WI).

Data analysis. Analysis of variance and regression and correlation analyses were performed with Superanova (Abacus Concepts Inc., Berkeley, CA).

RESULTS

Approximately 1 wk after exposure to 60 mM NaCl in the soil solution, all seedlings showed symptoms of salt toxicity, although symptoms developed faster in rough lemon and sour orange than in troyer citrange. These symptoms included leaf scorch (initially at the tip of leaves on rough lemon and sour orange and on the tip of the central leaflet of troyer citrange) and leaf folding, followed by leaf abscission. Symptoms developed 2–3 days later in seedlings exposed to 45 mM NaCl than in seedlings exposed to 60 mM NaCl.

When experiments were repeated, results were similar among experiments allowing for variation in seedling age and pathogenicity of the isolate of *P. citrophthora*.

Effect of soil salinity and water content on stem rot. Higher levels of soil salinity and water content both caused significantly greater ($P < 0.01$) severity of stem rot in rough lemon and sour orange than lower levels (Table 1). In all treatments, the severity of stem rot was greater in rough lemon than in sour orange. Inoculation did not cause necrosis in stems of troyer citrange, which was excluded from the analysis. A significant ($P < 0.05$) interaction occurred between salinity and soil water content. Regression analysis showed that an increase in salinity caused a significantly greater increase in disease severity in rough lemon in wet soil than in moist ($P < 0.05$) or dry ($P < 0.01$) soil.

Effect of soil salinity on stem rot. The severity of disease was significantly different ($P < 0.01$) among the three species at all salinity levels (Fig. 2). A significant ($P < 0.01$) linear regression of stem rot on salinity occurred for all rootstock species. Relative stem water content was negatively related to soil salinity. Highly significant ($P < 0.001$) regressions of θ_s on salinity occurred for all species (troyer citrange, $Y = 83 − 0.28X$, $r^2 = 0.91$; sour orange, $Y = 84 − 0.22X$, $r^2 = 0.93$; rough lemon, $Y = 86 − 0.24X$, $r^2 = 0.91$). Relative stem water content of troyer citrange was significantly ($P < 0.05$) lower than that of sour orange and rough lemon at all salinity levels. The extent of stem necrosis in the three species was negatively correlated ($P < 0.01$) with relative stem water content induced by different NaCl treatments (Fig. 3). Disease was more severe on rough lemon than sour orange or troyer citrange at any given θ_s, particularly at low levels of θ_s. Relative stem water content was highly correlated with φL ($r^2 = 0.94$).

Effect of soil water content on stem rot. Severity of stem rot at high soil water content was increased more for rough lemon

TABLE 1. Effect of soil salinity and water content (w/w) on the area of stem necrosis (cm^2) caused by *P. citrophthora* in sour orange (So) and rough lemon (R1) rootstocks

Salinity (mM NaCl)	Soil water content					
	So			R1		
	12%	18%	28%	12%	18%	28%
12.5	0.6 ± 0.1[a]	3.2 ± 0.2	4.0 ± 0.1	2.8 ± 0.2	3.6 ± 0.1	4.6 ± 0.1
30.0	2.6 ± 0.5	4.0 ± 0.5	5.5 ± 0.4	5.0 ± 0.1	6.9 ± 0.1	10.1 ± 1.4
60.0	4.6 ± 0.8	6.1 ± 0.8	8.9 ± 2.3	7.3 ± 0.3	9.8 ± 1.2	15.3 ± 1.4

Source	df	Analysis of variance
		MS
Species (A)	1	110.1[**][b]
Salinity (B)	2	138.4[**]
Soil water content (C)	2	82.7[**]
A × B	2	11.9[*]
A × C	2	3.2
B × C	4	5.7[*]
A × B × C	4	2.8
Residual	36	2.04

[a] Mean of three values ± standard error.
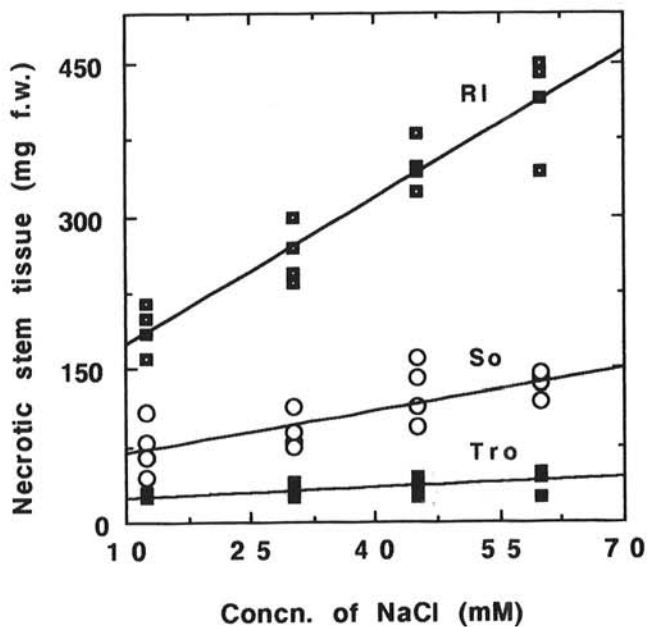[b] *F* test significant at $P = 0.05$[*] or 0.01[**].

Fig. 2. Relationship between soil salinity and the amount of necrotic stem tissue (milligrams fresh weight) induced in three citrus rootstocks (troyer citrange, Tro, $Y = 22 + 0.31X$, $r^2 = 0.39$; sour orange, So, $Y = 56 + 1.4X$, $r^2 = 0.59$; rough lemon, R1, $Y = 127 + 4.8X$, $r^2 = 0.90$) following inoculation with *Phytophthora citrophthora*.
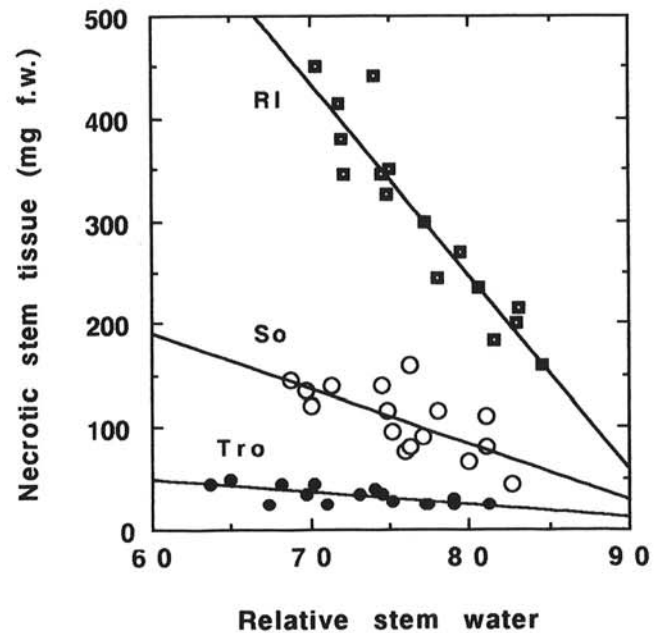

Fig. 3. Relationship between relative stem water content (θ_s) as determined by different soil salinities and the amount of necrotic tissue (milligrams fresh weight) induced in the stems of three citrus rootstocks (troyer citrange, Tro, $Y = 119.3 - 1.2X$, $r^2 = 0.48$; sour orange, So, $Y = 510 - 5.3X$, $r^2 = 0.47$; rough lemon, R1, $Y = 1760 - 18.9X$, $r^2 = 0.89$) following inoculation with *Phytophthora citrophthora*.

than for sour orange and troyer citrange (Fig. 4). Severity of stem rot had a significant linear relationship with soil water content in troyer citrange ($Y = -2.1 + 1.8X$, $r^2 = 0.70$) and sour orange ($Y = 36.6 + 1.5X$, $r^2 = 0.30$). The regression slopes for these two species were not significantly different ($P < 0.05$). For rough lemon, however, the relationship was described more accurately by a cubic ($Y = 850 - 155X + 9.3X^2 - 0.16X^3$, $r^2 = 0.96$) than by a linear equation; a larger increase in disease occurred when soil water content was raised from 15 to 22% than when raised from 12 to 15%. Relative tissue water content was significantly ($P < 0.001$) related to soil water content for all species (troyer citrange, $Y = 62 + 0.90X$, $r^2 = 0.91$; sour orange, $Y = 64 + 0.92X$, $r^2 = 0.93$; rough lemon, $Y = 65 + 0.97X$, $r^2 = 0.94$). Troyer citrange had a significantly ($P < 0.05$) lower θ_s than sour orange and rough lemon at 12.5, 18, and 28% soil water content. The amount of necrotic stem tissue was positively correlated with θ_s for all three species (Fig. 5). The effect of θ_s on stem necrosis was greater for rough lemon

the other species. The relative susceptibility of rough lemon was not clearly evident until θ_s exceeded approximately 80%. Again θ_s was highly correlated with φL ($r^2 = 0.93$).

Effect of NaCl on growth of *P. citrophthora* in vitro. High concentrations of NaCl inhibited vegetative growth of the fungus in culture, particularly in the weaker V8 juice broth (1 or 5%) to which no extra Ca^{2+} had been added (Fig. 6). In the stronger media (10% V8 juice), inhibition of fungal growth occurred only at NaCl concentrations greater than 100 mM. Inhibition of fungal growth by NaCl was not significant in the media containing added Ca^{2+}. Radial growth and sporulation of the fungus on agar medium were inhibited by NaCl concentrations greater than 50 mM without supplemental Ca^{2+} (Figs. 7 and 8). In the presence of extra Ca^{2+}, concentrations of NaCl up to 200 mM stimulated sporulation and caused only slight inhibition of radial growth of mycelium.

Effect of soil salinity and water content on the accumulation of DMC in infected stems. The concentration of DMC that accumulated in necrotic bark of all three species was significantly ($P < 0.01$) less at high than at low salinity (Fig. 9). However, the difference in DMC concentration between the two salinity levels was particularly great in rough lemon.

Concentrations of Na^+ and Cl^- in the stems were increased at high soil salinities (Table 2). Concentrations in troyer citrange increased less than in sour orange, and rough lemon.

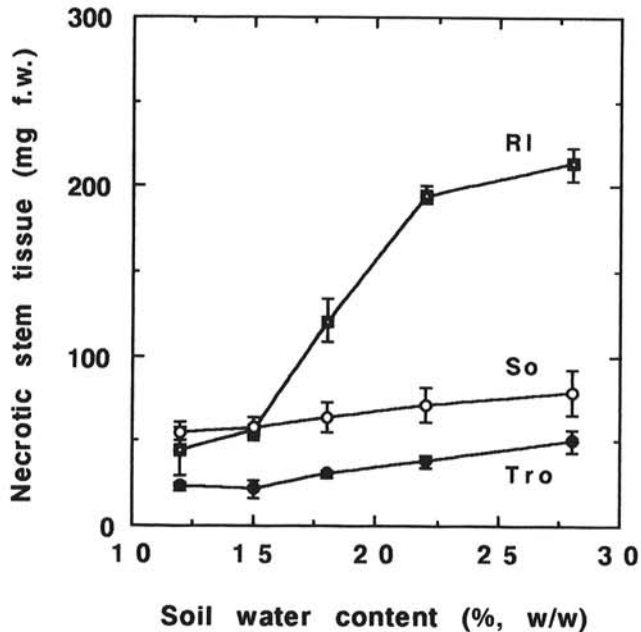Soil water content had no significant effect on DMC accumulation in the stems of the three species.

DISCUSSION

Predisposition of a citrus species to stem rot caused by *P. citrophthora* in saline soils is probably related to the sensitivity of the species to salt damage. The development of symptoms of salt toxicity and the rate of accumulation of Na^+ and Cl^- in stems of seedlings exposed to high salinity were evidence that troyer citrange was more salt tolerant than sour orange and rough lemon, as found by others (17,19). High salinity caused a significant increase in stem rot severity in the salt-sensitive species

Fig. 4. Relationship between soil water content and the amount of necrotic stem tissue (milligrams fresh weight) induced in stems of three citrus rootstocks (troyer citrange, Tro; sour orange, So; rough lemon, R1) following inoculation with *Phytophthora citrophthora*. The values shown are the means of three replicates with associated standard errors.
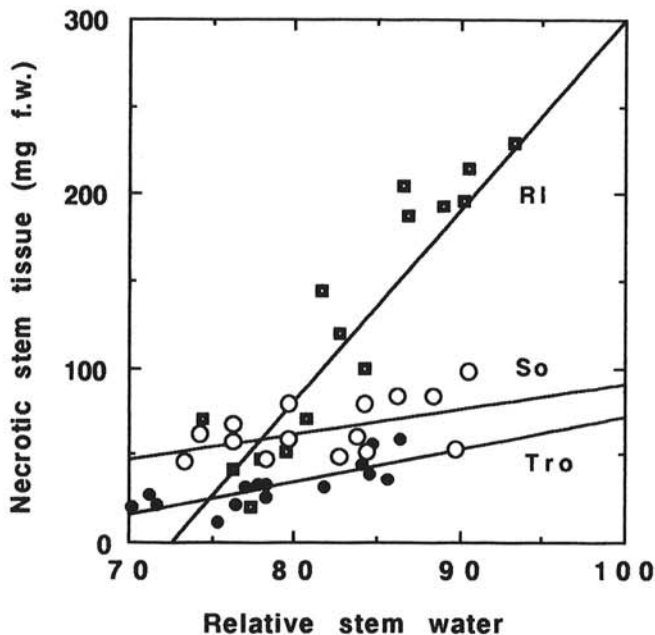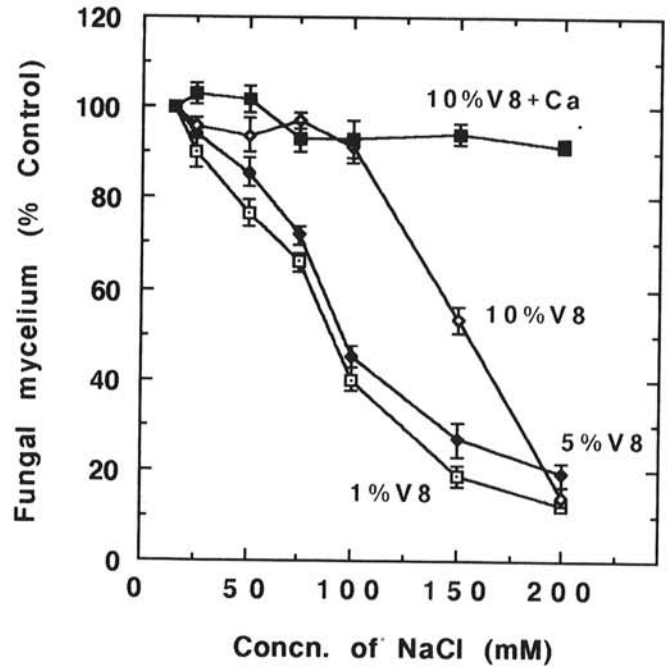
Fig. 6. Effect of NaCl, V8 juice, and Ca^{2+} concentration on production of mycelium (% Control-15mM NaCl) of *Phytophthora citrophthora* over 10 days (□, 1%; ◆, 5%; ◇, 10%; ■, 10% V8 juice plus Ca^{2+}). Values shown are the means of three replicates with associated standard errors.

Fig. 5. Relationship between relative stem water content (θ_s) as affected by different soil water contents and the amount of necrotic stem tissue (milligrams fresh weight) induced in three citrus rootstocks (troyer citrange, Tro, $Y = -115 + 1.9X$, $r^2 = 0.64$; sour orange, So, $Y = -55.1 + 1.5X$, $r^2 = 0.26$; rough lemon, R1, $Y = -850 + 11.7X$, $r^2 = 0.86$) following inoculation with *Phytophthora citrophthora*.
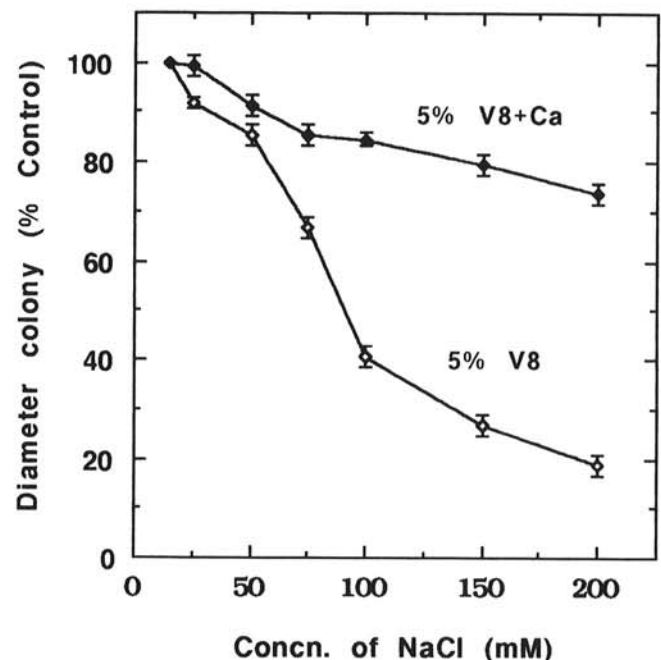
Fig. 7. Effect of NaCl and Ca^{2+} concentration in V8 juice agar medium on colony diameter (% Control-15 mM NaCl) of *Phytophthora citrophthora* over 7 days (◇, 5% V8 juice; ◆, 5% V8 juice plus Ca^{2+}). Values shown are the means of three replicates with associated standard errors.

rough lemon, whereas the more salt-tolerant species troyer citrange and sour orange largely retained resistance at high salinity. Similar results were reported for citrus root rot caused by *P. parasitica* (7). Salinity did not stimulate vegetative growth of *P. citrophthora* in culture, and so the increase in disease associated with increasing salinity apparently was not attributable to a direct effect of salt on the fungus but rather to a reduction in the resistance of the host tissues.

High salinity decreased the accumulation of the phytoalexin, DMC, in the necrotic tissue of the three citrus rootstocks tested, and this could account in part for the reduced resistance of plant tissues at high salinity. These observations are consistent with a report that high salinity decreased the accumulation of glyceollin in the stem of soybean after inoculation with *P. m. sojae* (27).

The effective concentration of DMC that caused 90% inhibition (EC_{90}) of the growth in culture of the isolate of *P. citrophthora* used in these experiments is 0.57 mM (29). The concentration of DMC that accumulated 10 days after inoculation in sour orange and troyer citrange at high salinity remained greater than the EC_{90}, but in rough lemon the concentration was much less than this level. This could account for the fact that high salinity promoted stem rot to a greater extent in rough lemon than in sour orange and troyer citrange. Although the concentrations of DMC in troyer citrange and sour orange at high salinity were similar, troyer citrange retained resistance to *P. citrophthora* to a greater extent than sour orange. At normal salinity levels, DMC accumulates faster in troyer citrange than in sour orange and rough lemon (29). The concentration of DMC reached the EC_{90} within 1, 5, and 8 days after inoculation in troyer citrange, sour orange, and rough lemon, respectively, and probably a similar situation occurs at high salinity. The rate of accumulation of toxic concentrations of phytoalexin is likely to be more important in determining disease severity than the final concentration attained in necrotic tissue.

The mechanism by which high salinity reduces accumulation of DMC is not known. Although excessive salt concentration in the plant reduces accumulation of DMC, other physiological processes in the plant may be affected (16), which may also contribute to reduced resistance to fungal invasion (23). Salinity stress could disrupt the permeability of cell membranes within the stem tissues (10), which would allow increased leakage of
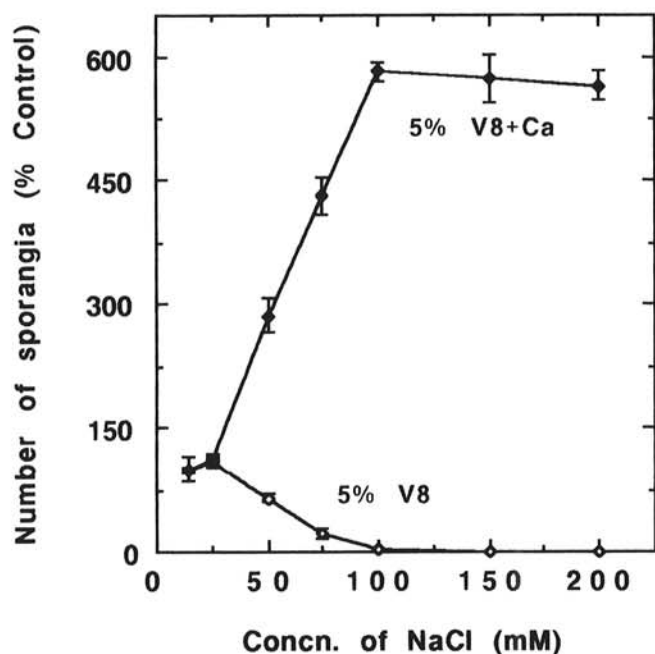
nutrients into intercellular spaces and stimulate growth of the pathogen. Chrysanthemum exposed to a pulse of salinity gave none of the microscopically observable responses, such as aggregation of nuclei and cytoplasm and formation of wall appositions adjacent to the point of hyphal penetration, normally seen in resistant, nonstressed plants (30).

The NaCl concentrations that occurred in the plant tissues in these experiments would not have inhibited the fungus significantly. All isolates of *Phytophthora* used in another study (6) formed sporangia and zoospores in soils with a salinity level equivalent to the 60 mM NaCl used as the high salinity treatment in the present experiments. In our study, as long as Ca^{2+} concentration in the medium was high, growth of *P. citrophthora* was not significantly inhibited at NaCl concentrations as high as 200 mM.

High soil water content also predisposed stems to invasion by *P. citrophthora*, presumably through an effect on θ_s, as has been reported for *P. cinnamomi* in *E. marginata* (31). The positive correlation between θ_s (as affected by soil water content) and the amount of stem rot was particularly strong in the susceptible species, rough lemon. In fact, the susceptibility of the citrus species to *P. citrophthora* was strongly correlated with their respective θ_s at any given soil water content or salinity. The lower θ_s of troyer citrange compared with sour orange and rough lemon could contribute to the resistance of troyer citrange to *P. citrophthora*. At normal soil salinity (12.5 mM NaCl), rapid development of stem rot in rough lemon did not occur until θ_s exceeded about 80%, which was equivalent to a leaf water potential of −1.6 MPa. Expansion of lesions caused by *P. cinnamomi* in *E. marginata*
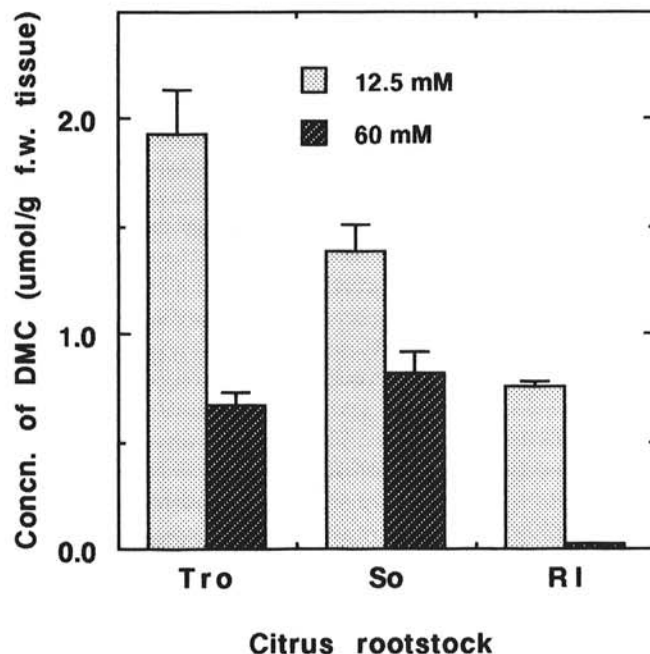
Fig. 9. Effect of NaCl concentration in the soil solution on the concentration of DMC accumulated in necrotic stem tissue (fresh-weight basis) in three citrus rootstocks (troyer citrange, Tro; sour orange, So; rough lemon, Rl) following inoculation with *Phytophthora citrophthora*. Values shown are the means of four replicates with associated standard errors.

Fig. 8. Effect of NaCl and Ca^{2+} concentration in V8 juice agar medium on production of sporangia (% Control-15 mM NaCl) by *Phytophthora citrophthora* over 7 days (◇, 5% V8 juice; ◆, 5% V8 juice plus Ca^{2+}). Values shown are the means of three replicates with associated standard errors.

TABLE 2. Effect of soil salinity on the concentration of Na^+ and Cl^- ions (meq g^{-1} fresh weight tissue) accumulated in stems of citrus rootstocks

| | Salinity (mM NaCl) | | | |
| | Na^+ | | Cl^- | |
Rootstock species	12.5	60	12.5	60
Troyer citrange	6.3 ± 1.5[a]	23.3 ± 2.9	2.0 ± 0.3	15.8 ± 1.9
Sour orange	3.9 ± 1.6	28.5 ± 2.4	2.8 ± 0.4	21.3 ± 0.9
Rough lemon	7.5 ± 1.2	48.2 ± 3.1	1.3 ± 0.3	22.8 ± 1.8

[a] Mean of three values ± standard errors.

was rapid at relative phloem water contents above 80%, which was equivalent to a phloem water potential of −1.0 MPa, and lesion expansion following trunk inoculation with *P. cinnamomi* was minimal at twig water potentials less than −2.0 MPa (31). Leaf water potential is often more negative than stem water potential (12). Growth of *P. citrophthora* in cleared V8 juice broth with no added Ca^{2+} and amended with NaCl was decreased at lower water potential of the medium and was inhibited by more than 80% at a water potential of −1.6 MPa (Sulistyowati, *unpublished data*). Thus, the soil water contents used in this study could have affected fungal growth in the stem through an effect on tissue water status, although other factors may have been involved also.

Changes to θ_s and φL caused by high salinity probably were not involved in the effect of salinity stress in predisposing citrus rootstocks to stem rot caused by *P. citrophthora*. High levels of salinity caused a reduction in θ_s and φL and therefore should have caused a reduction in disease severity if tissue water content was the only factor influencing disease. The resistance of plant tissues to invasion by *P. citrophthora* apparently is greatly affected by the reduction in the resistance response of the tissues and not by the reduction in the water content of the tissues caused by high salinity. An interaction between soil salinity and water content in predisposing stems to the disease occurred in rough lemon: the effect of salinity treatment in predisposing stems to the disease was greater at high than at low soil water content. Resistance of the stem tissues to fungal invasion is probably influenced by the combined effect of soil salinity and water content on the physiology of the tissues.

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