The Effect of Solute Potential and Water Stress on Black Scorch Caused by *Chalara paradoxa* and *Chalara radicicola* on Date Palms

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ABSTRACT

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Date palm trees (*Phoenix dactylifera*) were found to be infected with *Chalara radicicola* and *Chalara* (*Thielaviopsis*) *paradoxa* in 1992. Compared with healthy palms, most of the diseased palms appeared to be drought stressed and poorly maintained in landscape settings and nurseries. Water potential studies conducted in growth chambers with 5- to 6-leaf seedling plants subjected to water stress at –2.3 MPa had relatively larger necrotic lesions that developed into cankers, death of buds, and eventually plant death. Tissue necrosis was directly related to water potential. Histological studies showed many necrotic islands of parenchyma tissue in drought-stressed infected plants. Only a few inoculated plants in the growth chamber study died without developing extensive cankers, apparently due to the invasion of the crown or terminal bud by the pathogens. In vitro studies with potato dextrose agar amended with glycerol, NaCl, and KCl to decrease the osmotic matrix-based water potential of the media (–4.25 MPa) resulted in a decrease in the radial growth, biomass, and the sporulation of *C. radicicola* and *T. paradoxa*. Solute potential of –0.35 to –1.97 MPa, however, favored the growth of both fungi. Sodium chloride had the greatest effect on the growth characteristics of both fungal species. These studies indicate that in parts of Kuwait where drought and salinity prevail, opportunistic pathogens such as *C. radicicola* and *T. paradoxa* could become aggressive and cause serious damage to date palms.

Black scorch of date palms (*Phoenix dactylifera*) is a disease of minor importance that occurs on stressed palms or senescent tree parts (5). It is caused by the fungi *Chalara radicicola* (synonym, *Chalaropsis radicicola*) and *Chalara paradoxa* (*Thielaviopsis paradoxa*), teleomorphs *Ceratocystis radicicola* and *Ceratocystis paradoxa*, respectively (2,3,13). These fungi can infect any part of the palm tree, and symptoms are often expressed as black scorched leaves, trunk rot, bud rot, or inflorescence blight (2,5). The disease has been reported in a number of areas where date palms are grown, including Saudi Arabia and Iraq (2,3,5). Although sporadic in its occurrence, black scorch has the potential to be a serious disease and has been associated with tree decline and death (5). A possible explanation for the severity of the disease in the Shatt Al-Arab region in Iraq is the prevailing changes in the levels and salinity of the water (5), but no studies were conducted to validate this assertion. In Kuwait similar environmental factors have been observed in areas with high disease incidence. Our observations indicated drought and salinity stress may influence the incidence and severity of the disease in nursery and landscape palms. In the agricultural areas of the central and southern regions, salinity of the soil and brackish water used in the irrigation of crops is a major concern. The studies reported here were conducted to investigate the influence of solute potential on the growth of the fungi and the effect of drought stress on tissue colonization and disease severity.

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MATERIALS AND METHODS

Survey and pathogen identification. Surveys were conducted on date palm trees of all ages in three plantations, eight nurseries, and four landscape settings in the central and southern regions of Kuwait from 1996 to 1998 to ascertain the severity of black scorch. Each site was inspected once a month from November through May for the presence of the disease and for new infections. Samples of rachides with leaves, stems, and roots from infected trees were examined in the laboratory. Samples from infected palms were sectioned to determine the extent of canker development and tissue damage. Stem and root samples were washed in tap water. Surfaces were disinfested by dipping pieces of the sample once in 70% ethyl alcohol and briefly passing them over a Bunsen and Logan burner flame. Pieces of tissue (approximately 5 mm) were aseptically cut and placed on potato dextrose agar (PDA), amended with 0.3 g of streptomycin sulfate per liter. Leaf samples were surface disinfested using 10% sodium hypochlorite (NaOCl) solution. Monosporic cultures from conidia characterized as *C. radicicola* and *T. paradoxa* cultured on PDA and malt extract agar (MEA) were used for pathogenicity, solute potential, and drought stress studies. Verification of *T. paradoxa* and *C. radicicola* as the cause of black scorch and the effect of the disease on palms were determined by greenhouse and growth-chamber inoculations using 5 to 6 leaf seedling plants. Inoculations were done on the third leaf by excising 5 mm from the leaf tips and dipping the cut surfaces in a spore suspension (10^6 spore/ml) for 3 s. Two sets of 12 plants were inoculated with either *T. paradoxa* or *C. radicicola*. Each set was placed in a greenhouse or growth chamber. Six control plants for each set of treatment were dipped in sterile distilled water without fungal spores. Inoculated plants were placed in a 30ºC greenhouse and in a growth chamber at 24ºC, 70% relative humidity (RH) and a photoperiod of 13 h at 120 W/m^2 of combined incandescent and cool-white fluorescent light. Isolations were made from lesions to verify the presence of each pathogen after 4 weeks.

Effect of solute potential on growth and development of *T. paradoxa* or *C. radicicola*. In vitro studies were conducted to compare the response of *C. radicicola* and *T. paradoxa* to osmotically lowered water potential on PDA and potato dextrose broth (PDB). The solute potentials of the media (1.15, 1.97, 2.79, 3.41, 4.25 MPa) were adjusted by incorporating sodium chloride, potassium chloride, mannitol, or glycerol and determined with a vapor pressure osmometer (VAPRO, model 5520, Wescor, Logan, UT) prior to sterilization. Plugs of cultures 5-mm in diameter were taken from margins of 4-day-old cultures and placed in petri dishes with 20 ml of PDA or flasks with 150 ml of PDB. PDA cultures were placed in plastic bags and incubated at 25ºC for 5 days in the dark. Similarly, PDB cultures were incubated at 25ºC, in the dark on a rotary shaker. Radial growth measurements were taken daily for 5 days because of the rapid growth of the fungal species. Dry weight

and sporulation data were also recorded after 5 days. The experiment was done twice with three replications for each of the five solute potential concentrations.

Effect of drought stress on invasion of date palm tissues by *T. paradoxa* or *C. radicicola*. Tissue-cultured date palms at the 5- to 6-leaf developmental stage of the cultivar Berhi were used in growth chamber studies. Plants were grown in 1.2-liter black plastic pots under 25% shading net. The soil mix was 80% local sand and 20% peat (4:1 wt/wt). The soil moisture of all the plants was at field capacity when they were placed in a growth chamber at 24ºC, 70% RH, and a photoperiod of 13 h at 120 W/m^2 of combined incandescent and cool-white fluorescent light. Water was withheld from all plants except those selected to receive water as a treatment. Using a pressure bomb apparatus (model 3000, Soil Moisture Equip. Corp., Santa Barbara, CA), the water potential of the drought-stressed plants was determined to be –2.30 Mpa, and that of adequately watered plants was –0.30 MPa at the time of inoculation.

The treatments were drought-stressed plants inoculated with either pathogen, adequately watered plants inoculated with either pathogen, drought-stressed plants inoculated with sterile distilled water, and adequately watered plants inoculated with sterile distilled water. The plants were inoculated with a spore suspension (10^6 spores per ml) of either *C. radicicola* or *T. paradoxa* by excising the whole leaf blade and placing a droplet of spore suspension on the cut petiole surface. Plants were placed back in the growth chamber and not watered for 5 days, thereafter 100 ml of water was added to each plant to ensure that the water potential for drought-stressed plants remained–1.5 MPa or lower and that of unstressed plants at –0.5 MPa or higher. Watering was continued every 3 to 4 days to ensure that drought-stressed plants did not wilt.

The experiment was done three times with four replicates per treatment. The areas of the cankers that developed were determined 35 and 56 days after inoculation (DAI). Percent disease area of infected plants was estimated based on a computer software program (Distrain, USDA, Beltsville, MD).

Samples from inoculated plants were examined visually and with a light microscope to determine the extent of tissue destruction at the end of the study. Sections of infected tissue were fixed in a mixture of formalin, acetic acid, and ethanol (95:5:5, vol/vol), dehydrated in 50 to 95% ethanol series and embedded in paraffin wax (Tissueprep, melting point = 61ºC, Fisher Scientific, Springfield, N.J.). Sections obtained from the embedded tissue were 10 to 15 µm thick. Histochemical stains used were sudan black for suberin and toluidine blue for general morphological features.

Experimental design and data analyses. Experiments on pathogenicity and drought stress were set up in a complete randomized design, and each treatment unless otherwise stated was replicated four times. Analyses of variance were computed with MSTAT-C statistical analysis software (Michigan State University, Lansing, MI) for data obtained from solute potential and drought stress studies and Duncan's multiple range test used for separation of means. Linear regression of increase in radial growth of mycelia against time (days) was used to determine the growth rates of the *C. radicicola* and *T. paradoxa*.

RESULTS

Survey and pathogen identification. Date palm trees with typical black scorch symptoms were found in the nurseries, one plantation in Rabya, and landscape settings included in the survey. Approximately 2.0 and 6.0% of palms in nurseries and the plantation, respectively, were infected.

Isolation rate of both *T. paradoxa* and *C. radicicola* were 88.0, 76.5, and 56.5% from root, stem, and leaf samples, respectively. No variations in morphological or cultural characteristic were observed between single spore cultures of each pathogen. Mycelia of *C. radicicola* and *T. paradoxa* on PDA and MEA appeared grayish at first with abundant floccose aerial mycelia. Centers of colonies turned greenish within 2 days and were entirely dark green in 7 to 10 days. The endoconidiophores were hyaline to pale brown, elongated, urn-shaped, thin-walled, and septate. The endoconidia were cylindrical to ellipsoid, hyaline to brown, in chains and borne on side branches of hyphae. Chlamydospores of *T. paradoxa* were smooth, thick-walled, brown, and in chains. The chlamydospores of *C. radicicola,* however, were solitary, with thick, smooth to slightly ornate walls. The growth rates on PDA of *C. radicicola* and *T. paradoxa* were 12.5 and 14.3 mm/day, respectively.

Infected trees of all ages showed at least one of the four characteristic symptoms: heart or trunk rot; black scorched leaves; bud rot; and inflorescence rot. Foliar symptoms were common on nursery plants. Young and recently transplanted ornamental date palm trees were severely affected in landscape settings. Stem infections appeared to have been initiated through wounds on rachides created during pruning of fronds and resulted in trunk rots or cankers. In the field, leaves were usually free from fungal invasion until the top of the palm began to wilt. Infected terminal buds and the surrounding tissues of palms in the field in a few cases were infested with fly maggots, causing the rapid death of the entire palm tree. Also palm trees with large cankers toppled over in high winds or with a heavy fruit crop. The pattern of stem colonization from natural infections in the field was typical of plants predisposed to drought. The bark and its adjacent tissues were extensively colonized and damaged, with much less invasion of the inner older tissues. Isolation from root samples usually yielded the presence of only *C. radicicola*. The teleomorph of *C. radicicola* was observed on the roots of a dead tree in the plantation, but the teleomorph of *T. paradoxa* was not seen on any of the samples nor in culture. *Diplodia* sp. and *Fusarium* sp. were also isolated from a few samples, particularly those from trees in landscapes. Pathogenicity study in the growth chamber yielded 90% infected palms with *T. paradoxa* and 100% with *C. radicicola*. In the greenhouse, the percent infection was 80.0 and 95.0% for *T. paradoxa* and *C. radicicola*, respectively.

Effect of solute potential on growth and development of *T. paradoxa* or *C. radicicola*. Radial growth of *T. paradoxa* and *C. radicicola* was greatly affected by the concentration of NaCl in the media. Optimum radial growth for both *T. paradoxa* and *C. radicicola* was at –1.15 MPa with PDA adjusted with NaCl below which there was a steady decline in growth (Table 1). With KCl- and glycerol-amended media the radial growth followed a trend similar to that with NaCl, although growth declined more slowly with decreasing solute potential. The optimum growth for both fungi was between –1.15 and –1.97 MPa for KCl and glycerol (Table 1). Mannitol did not effect radial growth of either fungus in vitro even at –4.25 MPa, (data not shown) and was not used in biomass and sporulation studies. Radial growth reduction for both fungi was slightly greater than 60% with NaCl, less than 50% with KCl and about 30% for glycerol (Table 1). The biomass (dry weight) of both pathogens in PDB adjusted media with NaCl was greatly reduced between –2.79 and –4.25 MPa. The reduction in dry weight at –4.25 MPa was 81.9 and 89.2% for *T. paradoxa* and *C. radicicola*, respectively. The decrease in dry weight with KCl occurred at –4.25 MPa. Glycerol had minimal effect on dry weight of both species with a decrease in solute potential. Sporulation of both fungal species was significantly reduced ($P = 0.05$) with a decrease in solute potential with all three solutes (Table 1). The reduction in sporulation by KCl and glycerol was much less than that of NaCl. Sporulation was reduced by 50% or more at –1.15, –1.97, and –2.79 MPa with NaCl, KCl, and glycerol, respectively.

Effect of drought stress on invasion of date palm tissues by *T. paradoxa* or *C. radicicola*. Necrotic lesions or cankers on rachides and stems on adequately watered palms were larger compared with lesions on palms under drought stress during the first 35 days after inoculation (Table 2). At the end of the study, the size of cankers caused by the two pathogens on drought-

stressed plants was not significantly different (P = 0.05) (Table 2). Nevertheless, a significant differences in canker size were caused by either *T. paradoxa* or *C. radicicola* at –0.3 and –2.3 MPa (P = 0.05).

Examination of sections of rachides and stem tissues revealed numerous necrotic islands of tissue distant from canker margins in drought-stressed palms. When all necrotic tissues were estimated as total disease area (percent necrotic tissue), there was much greater tissue damage on palms under drought stress than those under adequate water treatment (P = 0.05) (Table 2).

Microscopic observations of infected palms showed intercellular spaces of infected tissues were filled with mycelia and spores of *C. radicicola* and *T. paradoxa* prior to tissue necrosis. Necrosis was limited to the cortical regions, leaving the surrounding fibrous sclerenchyma and vascular tissues intact. As the infection progressed masses of chlamydospores were seen in and around the xylem vessels at least 3 to 5 cm in advance of the necrotic tissues.

Eight percent of the Berhi palms under water stress died 56 DAI with *C. radicicola*, none with *T. paradoxa* nor with uninoculated plants. *C. radicicola* was isolated from apical tissues of dead plants. Plant death was caused by the invasion of the terminal bud or crown of palms.

DISCUSSION

Plant pathogens in semi-arid conditions such as Kuwait are probably adapted or tolerant to drought stress or decrease in water potential. This study showed that the two pathogen species that cause black scorch can tolerate a decrease in water potential. Except for sporulation, which was greatly reduced at –1.97 MPa, both *C. radicicola* and *T. paradoxa* grew at solute potential between –1.15 and –3.41 MPa, even with NaCl. Regardless of the solute, the optimum growth (radial growth and biomass) for the two fungal species was between –1.15 and –1.97 MPa. These results are similar to those reported by Shokes et al. (10), and Olaya et al. (9), with the fungus *Macrophomina phaseolina*. The radial growth, biomass, and sporulation curves of the two fungi in this study were similar in all three solutes with glycerol having the least effect.

The effect of solute potential on the two fungi in this study may be attributed to the nature of the three compounds. The uptake of ions of these compounds by the fungal cells may be for the maintenance of turgor and physiological functions. The growth measurements of these fungi in response to KCl and glycerol in this study are quite similar to those reported elsewhere (7,9), although those studies were done with different fungi. The influence of KCl and glycerol may be due to fact that K^+ ions and glycerol accumulate easily in microbial cells and serve as compatible solutes of low toxicity in the cytoplasm of fungi (1,4,6). Polyols such as glycerol and mannitol are known to function as compatible solutes and are accumulated by xerotolerant filamentous fungi (1,6,8). The two species of fungi appear to withstand these compounds in that capacity.

In vivo studies also showed that both *C. radicicola* and *T. paradoxa* colonized palm tissues under drought stress at –2.3 MPa. At the end of the study canker size and percent necrotic tissue were greater in drought-stressed palms inoculated with either *C. radicicola* or *T. paradoxa*. These results are similar to those reported elsewhere (11,12). Shoenewiess (11), observed that stems of plants with water potential more negative than 13 bars (predisposition threshold) were greatly colonized by plant pathogens. Plants in this study (at –2.3 MPa) were in a state of reversible or elastic predisposition to fungal colonization based on Schoeneweiss (11) categorization of predispostion. Drought stressed plants are more likely to experience changes in osmotic potential or cell turgor which reduced plant vigor. Increased susceptibility to pathogen invasion is a consequence of reduction in vigor. Palms at –2.3 MPa experienced changes in osmotic potential and vigor thus, *C. radicicola* and *T. paradoxa* were able to infect weakened palms, resulting in greater tissue damage. It may be that both the weakening of the host's defenses and stimulation of the growth of the pathogens in drought-stressed palms were operative in this study.

This study has a practical implication for the "greenery program" for Kuwait. Irrigation water is to a large extent brackish with salinity of 3,500 ppm or even higher. The salinity of this irrigation water could lead to opportunistic pathogens becoming more aggressive or could predispose healthy plants to these pathogens. We recommend the use of less saline water or cleaner water for irrigation, planting palm cultivars that are salt tolerant, or resistant to *C. radicicola* and *T. paradoxa*. Irrigation in the hot and dry months or after transplanting is

Table 2. Average canker size and percentage necrotic tissue of *Phoenix dactylifera* subject to drought stress and inoculated with *Thielaviopsis paradoxa* and *Chalara radicicola* in a growth chamber

Treatment	Canker size (cm^2) 35 DAI	Canker size (cm^2) 56 DAI	Percent necrotic area 56 DAI
T. paradoxa at –0.3 MPa	45.0 a[z]	120.4 b	7.5 c
T. paradoxa at –2.3 MPa	36.5 b	124.3 ab	15.1 b
C. radicicola at –0.3 MPa	33.0 b	115.0 b	10.2 bc
C. radicicola at –2.3 MPa	30.2 b	130.2 a	22.5 a
Uninoculated at –0.3 MPa	4.0 c	4.0 c	0.0 d
Uninoculated at –2.3 MPa	6.0 c	6.0 c	0.0 d

[z] Each value represents the mean of 24 lesions or cankers pooled from three separate studies 35 and 56 days after inoculation (DAI). Mean values in the same column followed by the same letter are not significantly different at P = 0.05 based on Duncan's multiple range test.

Table 1. Radial growth on potato dextrose agar, biomass, and sporulation in potato dextrose broth of *Thielaviopsis paradoxa* and *Chalara radicicola* at different solute potentials using NaCl, KCl, and glycerol, 5 days after inoculation at 24ºC

	Solute	Radial growth (mm)[y]			Biomass (mg)[y]			Sporulation (×100)[y]		
Pathogen	Potential (–MPa)	NaCl	KCl	Glycerol	NaCl	KCl	Glycerol	NaCl	KCl	Glycerol
T. paradoxa	0.35	98 ab[z]	98 a	98 ab	460 a	460 b	460 a	845 a	845 a	845 a
	1.15	105 a	112 a	125 a	450 a	465 b	451 a	350 b	542 b	572 b
	1.97	95 b	101 a	120 a	422 a	481 ab	438 ab	161 c	430 c	450 c
	2.79	73 c	89 ab	113 a	224 b	510 a	462 a	102 cd	381 d	390 d
	3.41	50 d	83 ab	90 ab	119 c	453 b	415 b	66 d	165 e	273 e
	4.25	36 e	60 b	77 b	45 d	401 c	404 b	37 d	56 f	226 f
C. radicicola	0.35	90 a	90 b	90 b	419 a	419 b	419 ab	650 a	650 a	650 a
	1.15	101 a	97 a	105 a	392 b	435 ab	410 bc	403 b	408 b	415 b
	1.97	93 a	95 a	110 a	401 b	440 ab	428 ab	178 c	250 c	302 c
	2.79	70 b	80 bc	90 b	262 c	457 a	433 a	82 d	160 d	294 d
	3.41	58 b	72 c	71 c	185 d	350 c	390 cd	48 e	102 e	244 e
	4.25	40 c	56 d	62 c	85 e	338 c	379 cd	19 f	39 f	196 f

[y] Number of plates per value was 6.
[z] Mean values in the same column followed by the same letter are not significantly different at P = 0.05 based on Duncan's multiple range test.

essential for the prevention of this disease. Currently, date palm cultivars in Kuwait are being screened for resistance to the two pathogen species. Mycostop, a biofungicide, and broadspectrum fungicides are also being evaluated for efficacy in the control of the two fungi.

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