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Incidence and severity of black scorch on date palms in Kuwait

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ABSTRACT

The causal agent of black scorch in date palms in nurseries, landscapes and a few plantations in Kuwait was found to be *Thielaviopsis paradoxa* and *Ceratocystis radicicola*. This is the first report of the occurrence of the disease in Kuwait. Both pathogens were isolated from all tissues of diseased trees with *C. radicicola* being more virulent and frequently found on diseased roots. The optimum growth temperature was 25° and 35°C for *T. paradoxa* and *C. radicicola*, respectively. Optimum pH for growth of both pathogens was in the 5.0–6.0 range. Poor cultural practices, particularly drought stress, had the greatest impact on trees. Seedling infections on 4–5-leaf date palms in both the greenhouse and growth chambers produced cankers, tissue necrosis, wilting, death of buds, and plant mortality. Despite the common occurrence of visible cankers in field-infected palm trees, mortality of few plants was by death of the terminal bud. Histologically, damage was restricted to the parenchyma tissue. Five cultivars from tissue cultures were screened for genetic variability in the greenhouse, and *Nabut-saif*, *Berhi* and *Kadrawi* cultivars were found to be the most susceptible.

Keywords: Ceratocystis paradoxa, Ceratocystis radicicola, Phoenix dactylifera

INTRODUCTION

Until recently, black scorch disease of date palms (*Phoenix dactylifera L.*) was not found in Kuwait (Morgan-Jones 1957, Upadhyay 1981, Kyle 1993). Presently, diseased palms are found in plantations, landscapes and nurseries. The disease syndrome was typical of infections caused by *Ceratocystis* (*Thielaviopsis*) *paradoxa* (Dade) Moreau, and *Ceratocystis radicicola* (Bliss) Moreau. Infected palm trees of all ages had one or more of the following symptoms: black scorched leaves including the rachides, cankers, trunk or heart rot and inflorescence blight. The blackish appearance of the disease is due to the abundant dark-colored conidia of the two pathogens (Hunt 1956, Upadhyay 1981). Infections can occur on any part of the palm tree and perennial cankers often develop on tree trunks. The disease may be sporadic and of minor importance in some date palm cultivated areas (Simone 1993). However, in Kuwait, the number of infected trees that have been lost or replaced since 1995 indicates a need for special measures of control. In

addition, trees with cankers near the canopy can topple over with strong winds or a heavy crop load. The anticipated cost of replacing diseased trees is significant if the disease is not properly managed. Dependence on chemical control could lead to selection pressure in the pathogen populations, leading to an increase risk of resistance, especially with C. *radicicola.* Identification of resistant date palm cultivars would ameliorate this selection pressure on the pathogen. The objective of this study was to determine the pathological effects of the isolates in naturally and artificially infected plants, cultivar susceptibility in tissue culture plants, and the effect of temperature on growth and development of *C. paradoxa* and *C. radicicola.*

MATERIALS AND METHODS

Survey and identification of pathogens

To determine the prevalence and severity of black scorch, surveys were conducted in landscapes, nurseries and orchards in the central and southern regions of Kuwait. Information on cultural practices such as fertilizer application, irrigation, pruning etc., were recorded to determine the relationship to incidence and severity of the disease. Samples were taken to the laboratory for further examination. The causal agent was isolated by washing infected tissues with sterile distilled water and disinfesting with 1.0% NaOCl for 5, 15 and 30 seconds. The tissues were rinsed twice in sterile distilled water and dried between sterile filter papers. Pieces of disinfested tissues were plated on potato-dextrose agar (PDA, Difco), malt extract agar (MEA, Difco), and corn meal agar (CMA, Difico) amended with streptomycin sulfate (300 mg/l, Sigma). Identification of pathogens was based on taxonomic features. Monosporic isolates of each pathogen were obtained from chlamydospores and conidia plated on PDA, transferred to slants and stored at 5°C in the dark.

Temperature and pH studies

Optimal temperature and pH were determined, so that pathogenicity studies could be conducted at the optimum growth rate of the isolates. Five mm-diameter plugs from the margins of 3-day-old cultures on PDA were transferred near an inside edge of a Petri plate containing 20 ml of PDA. A single plug was transferred to each plate. Cultures were incubated at 20, 25, 30, 35, 40 and 45°C. Hyphal extensions were measured for 5 days from a baseline drawn after 24 h. The study was duplicated with four replicates for each treatment. Similarly, the pH of PDA was adjusted incrementally from 3.0 to 11.0 with 1.0 M HCl or NaOH.

Pathogenicity study

Experiments were conducted in both the growth chamber and greenhouse to determine differences in virulence among isolates of *T. paradoxa* and *C. radicicola* obtained from the surveys. Seedlings (cv *Berhi*) at the 4–5-leaf stage were potted in a medium consisting of sandpeat mixture (3:1). Plants were fertilized twice with a water soluble fertilizer (N:P:K, 10:10:10) prior to inoculation.

The inoculum was prepared from 3-day-old isolates grown in the dark on PDA at 24°. Conidia were dislodged with a sterile bent glass rod and then suspended in sterile distilled water. The spore suspension was filtered through two layers of cheesecloth and suspension was adjusted to 1.0×10^6 spores/ml with a hemocytometer. Inoculation was done by two methods. The first involved the excision of the terminal 10 mm of leaf tips followed by dipping the cut surface into the spore suspension for five seconds. With the second method, an entire leaf blade was excised and a droplet of spore suspension was placed on the cut petiole surface. Inoculated plants were placed in a growth chamber at 24°C and 70% RH. The photoperiod was 13-hr at 120 W/m^2 of combined incandescent and cool-white fluorescent light. Each treatment was replicated four times and sterile distilled water was used as uninoculated control. Treatments were evaluated 35 days after inoculation (DAI) on the basis of number of plants infected.

Five cultivars, *Berhi*, *Seavi*, *Nabut-Saif*, *Kadrawi* and *Sukkary* were screened for resistance to the two fungal pathogens. Approximately two-year-old palm cultivars from tissue cultures were used. Inoculation was done on the median rachis of each tree by placing a 2 mm diameter culture in a wound of the same size. Wounds were wrapped with parafilm for 24 hrs to avoid desiccation of the mycelium. Cultivars were evaluated 7 and 21 DAI for disease incidence and lesion size. Tree mortality was assessed 4 months after inoculation.

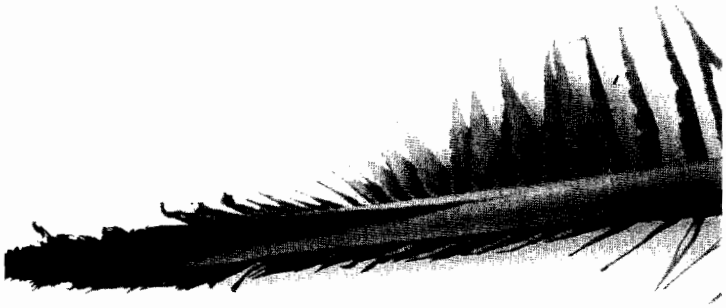
Histological studies

Leaf and petiole samples from inoculated and naturally infected plants were fixed in a mixture of formalin, acetic acid, and ethanol (95:5:5, v/v) and dehydrated in a *t*-butyl alcohol or ethanol series, 50–95%, before embedding in paraffin (Tissueprep, melting point = 61°C, Fisher). Sections obtained from the embedded tissue were 10–20 μm thick. Histochemical stains used were sudan black for suberin and toluidine blue (Fisher) for general morphological features. Microscopic examination of sections was done with a light microscope (Karl Zeiss, Optun 3) and observations photo-documented on Kodak color film (35 mm, ISO-100).

RESULTS

Survey and Isolate Identification

The survey revealed that black scorch on date palms was primarily found in isolated pockets of landscape settings in Kuwait. In nurseries and one plantation in Rabya the disease was quite prevalent. About 6.0% of the palms in the plantation were infected. Young, neglected and recently transplanted palm trees in the landscape had the most severe symptoms. Symptoms of natural infections associated with black scorch are shown in Fig. 1. Infected trees of all ages showed at least one of the four characteristic symptoms, i.e., heart or trunk rot, black scorched leaves, bud rot and inflorescence rot. Foliar symptoms were most common in nursery plants. In the field-infected plants, leaves often wilted and infected roots were necrotic or had reddish-brown lesions on internal tissues. Stem infections resulted in trunk rot or cankers and appeared mostly to have been initiated through wounds on rachides created during the pruning of fronds (Fig. 1B). *T. paradoca* and *C. radicicola* were routinely isolated from diseased samples, particularly from



(A)

(B)

Fig. 1. Symptoms of black scorch observed on *Phoenix dactylifera* (A) leaves, (B) tree trunk as a canker and (C) inflorescence as a blight.

samples collected early in the year. The isolation rate was 88.0% from root samples, 76.5% from stems and 56.5% from leaf samples. Isolation from root samples were frequently *C. radicicola.* Perithecia of *C. radicicola* were found only on the roots of a dead tree in one plantation. *Ceratocytis paradoxa* (teleomorph of *T. paradoxa*) was not observed on diseased plant tissues or in culture. *Diplodia* species and *Fusarium oxysporum* f. sp. *albedinis* and unidentified nematodes were also isolated from a few samples collected from trees in the field.

Cultures of the two fungal pathogens were similar. Hyphae on media appeared hyaline and, in time, grayish with abundant floccose aerial mycelia. Growth rates of the 2 isolates were significantly different, as shown in Fig. 2. Within two days, centers of the cultures turned greenish with the margins remaining grayish. The entire colony turned dark green to black in 6 to 10 days. Conidiophores were hyaline to pale brown, elongated, urn-shaped, thin-walled, and septate. The conidia were cylindrical to ellipsoidal, hyaline at first then brown, borne in chains on short-sided branches of hyphae. Chlamydospores of *T. paradoxa* were smooth, thick-walled, brown and also in chains (Fig. 3A). The chlamydospores of *C. radicola* were solitary, with thick, smooth to slightly ornate walls (Fig. 3B).

Temperature and pH studies

The optimum growth temperature in culture for isolates of *T. paradoxa* was 25°C while *C. radicicola* was 35°C (Fig. 2) and the mycelial extension rates were 14 and 12.5 mm/day, respectively. Both fungi grew minimally at 40°C but not at 45°C. The isolates also grew optimally at a pH range of 5.0–6.0 (data not shown). With an increase or decrease in pH of the culture medium outside this range, growth of the isolates declined rapidly.

Pathogenicity study

The inoculation methods of excising leaf tips and whole leaf blades resulted in infections of either the remainder of the leaf or leaf petiole. Dark-brown, elongated lesions developed on leaf blades 35 DAI. Invasion of leaf blades were much slower than those of petioles. The lesions that developed between leaf veins were usually surrounded with a diffused halo. Lesions on the petiole caused a few leaves to shrivel or wilt within 2–3 weeks. Mean lesion size on petioles caused by *C. radicicola* was significantly greater than those of *T. paradoxa* (Fig. 4).

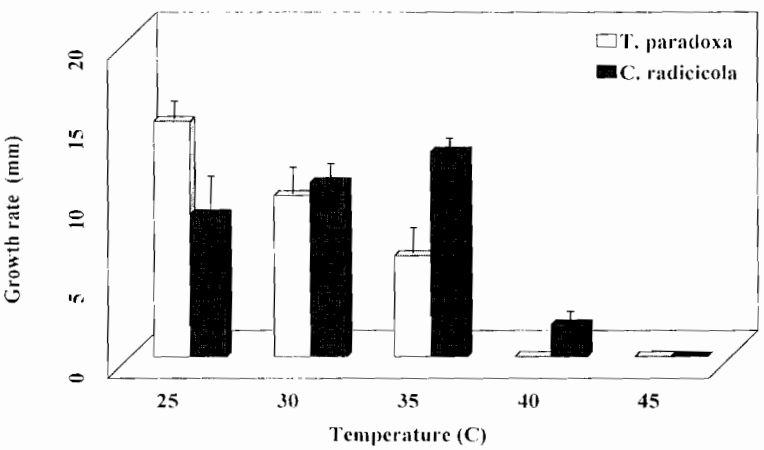


Fig. 2. The effect of temperature on the growth rate of *T. paradoxa* and *C. radicicola* on PDA. Each point represents the mean of duplicated study with four replicates. Bars represent standard error, $P = 0.05$.

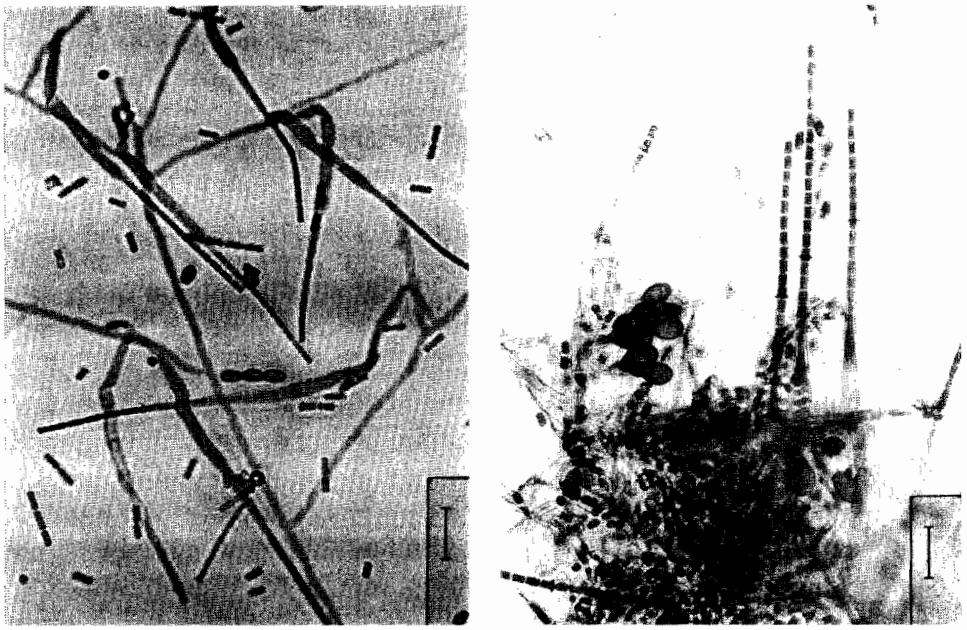


Fig. 3. Hyphae, conidiophores, and spores types (×1000) of (A) *T. paradoxa* and (B) *C. radicicola* on PDA.

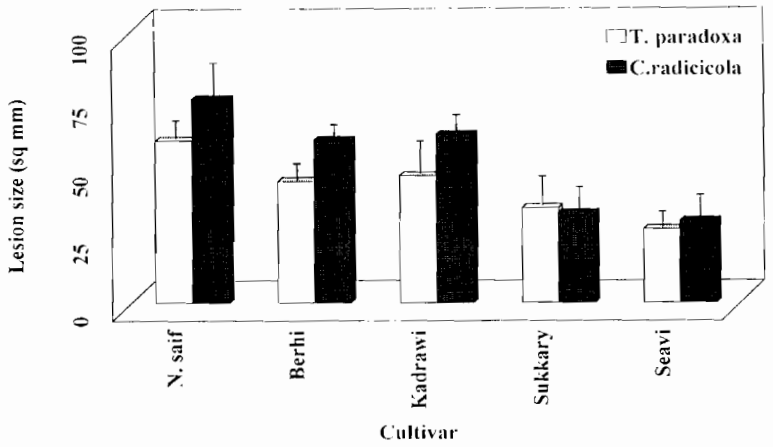


Fig. 4. Susceptibility of five date palm (*Phoenix dactylifera*) cultivars to black scorch caused by *C. paradoxa* and *C. radicicola*, ranged from susceptible (*Nabut-saif*) to moderately resistant (*Seavi*). Each point represents the mean of four replicates. Bars represents standard error, $P = 0.05$.

Histologically, parenchymatous tissue was readily colonized and severely damaged. Mycelia and spores of the fungi were also seen in the sclerenchyma cells surrounding vascular tissues and occasionally in xylem vessels. As lesions turned from brown to black, numerous chlamydospores were found intermingled with necrotic tissues. All cultivars inoculated with either pathogen developed brown lesions surrounded by water-soaked cells. Among highly susceptible cultivars, lesions around the inoculated sites caused leaves to wilt in 2 weeks. There was a significant difference in lesion size among cultivars 21 DAI (Fig. 4). *Seavi* developed the smallest lesions with no further lesion expansion 21 DAI. Susceptible cultivars to *C. radicicola* were *Birhi*, *Kadrawi* and *Nabut-saif*. *Nabut-saif* was the most susceptible to *T. paradoxa while Birhi*, *Kadrawi* and *Sukkary* possessed intermediate resistance.

DISCUSSION

Based on pathogenicity and taxonomy of *Ceratocystis* (Hunt, 1956, Morgan-Jones 1957, Upadhyay 1981, Kile 1993), *C. paradoxa* and *C. radicicola* were causal agents of black scorch of date palms in Kuwait. Both species have been reported as pathogens on a number of monocots, including date palms (Morgan-Jones 1957, Upadhyay 1981, Kile 1993). Only the anamorph of *C. paradoxa* was found in the field and in culture, but the perithecia of *C. radicicola* were found on host roots but not in culture. The occurrence of black scorch in combination with *Diplodia*, *Fusarium* and nematode species isolated from samples could lead to disease complexes that may have devastating effects on the longevity of date palms in Kuwait.

Black scorch symptoms were found on all tissues of date palm trees. *C. radicicola* was isolated mostly from root samples. *C. radicicola* is probably more adapted as a soil inhabitant or invader because it forms much thicker and larger chlamydospores which increases the potential of survival. Black scorch was severe on 30-year-old trees (6.0% of palms infected) in one plantation in Rabya despite being well-managed. A plausible explanation for the large number of infections in this plantation is that the pathogens may have been spread by pruning activities through contaminated pruning tools and wounds on pruned rachides that were portals of entry. In landscapes, palms were also found with severe symptoms; apparently most of them were recent transplants and poorly maintained. Many of these transplanted trees were probably infected before planting and lack of maintenance coupled with drought stress contributed to the incidence and severity of the disease. In the early 1990s many transplanted trees were bought from neighboring countries with reports of black scorch on palms. Diseased palms in nurseries probably originated from vegetatively propagated offshoots obtained from infected trees. Close plant spacing and overhead sprinkler irrigation in the nurseries might have created a favorable microclimate for the pathogens.

Histological studies showed that *C. radicicola* and *T. paradoxa* damaged parenchymatous and lightly lignified tissues. However, resident mycelia and spores of both fungi were observed in xylem vessels. Thus, the pathogens may be protected in islands of necrotic tissue or in xylem tissue from ambient summer heat which exceeds 45°C. Among cultivars screened, *seavi* appeared moderately resistant to both species of the fungus. More cultivars, particularly those from other countries need to be screened for resistance to this disease.

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حدوث مرض اللفحة السوداء وشدته على نخيل البلح في الكويت

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خلاصة

أظهر تشخيص إصابة نخيل البلح باللفحة السوداء في البيوت النباتية والأراضي الحقلية أن مسبب المرض هو فطر ينتمي إلى ثايلافيوبسس بارادوكسا Thielavopsis paradoxa وسيراتوسيستس راديكولا Ceratocystis radicola . ويعتبر هذا التقرير أول تسجيل للمرض في الكويت . فقد تم عزل الفطرين المسببين للمرض من جميع أنسجة النبات المصاب مع تأكيد شدة إصابة الجذور بفطر سيراتوسيستس راديكولا. كما تم تسجيل 25 و 35 درجة مئوية كأفضل درجات الحرارة لنمو ثايليفيوبسس وسيراتوسيستس على التوالي ، ويتراوح تركيز أيون الهيدروجين بين 5-6 . إضافة إلى ذلك فإن سوء ممارسات الزراعة ، وعلى وجه الخصوص الجفاف الحاد له أكبر الأثر في إضعاف مقاومة الأشجار.

تم تحديد تطور أعراض المرض على نبتات صغيرة داخل البيوت النباتية من تقرح تآكلي وتنخر الأنسجة وموت البراعم ثم القضاء التام على النبتة . كما أظهرت الدراسة التشريحية أن التدمير النسيجي اقتصر على النسيج البرانشيمي . وأظهر اختبار مقاومة المرض في خمسة سلالات بالتقنية النسيجية أن السلالات نبوت وسيف وبرحي وخضراوي هي الأقل مقاومة.