

Pathogenicity of *Bacillus circulans* to Seedlings of Date Palm (*Phoenix dactylifera*)

J. V. LEARY, Associate Professor, and W. W. C. CHUN, Graduate Research Assistant, Department of Plant Pathology, University of California, Riverside 92521

ABSTRACT

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When inoculated to seeds of eight date palm cultivars, *Bacillus circulans* isolate B02-3, pathogenic to date palm tissue cultures, significantly reduced the germination frequency of three cultivars and the fresh weights and lengths of seedlings of four cultivars. A significant number of inoculated seedlings of those four cultivars showed disease symptoms consisting of necrosis that progressed down the cotyledon, followed by wilting.

Members of the genus *Bacillus* have seldom been reported to be plant pathogens, although there have been reports of the association of *Bacillus* species with healthy plants (1,6). A recent report by Hosford (3), however, provides evidence that *B. megaterium* de Bary pv. *cerealis* is the pathogenic agent of white blotch disease of wheat. In addition, we recently demonstrated that *B. circulans* Jordan isolated from date palm tissue cultures and from healthy offshoots obtained from the field was pathogenic to callus and meristem cultures of date palm (4). The bacterium produced necrosis and subsequent destructive soft rot of the callus and meristem tissues. *B. circulans* was the only microorganism isolated from the tissue culture samples and, when inoculated to callus cultures, caused destruction of the cultures of the cultivars Barhee, Deglet Noor, and Empress.

B. circulans was consistently isolated in low numbers from the heart tissue, vegetative bud meristem, shoot primordia, young branch tract, and mature fronds of excised healthy palm offshoots (4). Because the tissue cultures were derived from apparently healthy palm offshoots,

it appeared that the *B. circulans* that destroyed those tissue cultures was already present, although no symptoms were apparent.

Neither *B. circulans* nor any other *Bacillus* species has been reported as a pathogen on *Phoenix dactylifera* L. However, the destructive effect of *B. circulans* on date palm tissue cultures and the isolation of *B. circulans* from healthy date palm offshoots indicated that *B. circulans* might be a potential pathogen of date palms that had been overlooked for any number of reasons. In this paper we present evidence that *B. circulans* is also capable of causing disease of date palm seedlings in the greenhouse and therefore may be a true pathogen of date palms.

MATERIALS AND METHODS

Strains and media. B02-3, a strain of *B. circulans* isolated from a diseased callus culture of date palm, was used for all pathogenicity tests. The cultures were grown from a single colony in liquid complete medium (CM) containing 10 g of casein hydrolysate, 5 g of yeast extract, and 4 g of K_2HPO_4 per liter of water to which 1% filter-sterilized glucose (CM + G) was added after autoclaving (4).

Pathogenicity tests. Strain B02-3 was grown in CM + G at 25 C until cell

density was approximately 10^8 cfu/ml, or about 72 hr. The cultures were pelleted and washed once with sterile distilled water, and the pellets were resuspended in sterile distilled water to a density of approximately 10^6 cfu/ml. Date palm seeds were soaked in the bacterial suspension for 1 hr, planted without further treatment into steamed U.C. planting mix (5) in 10-cm fiber pots at one seed per pot, and maintained on a greenhouse bench for a minimum of 4 mo. The pots were watered twice daily with a one-quarter-strength Hoagland's solution. The temperature of the greenhouse varied ± 15 C during the 4- to 5-mo period, but the amount of variation was similar during each of the three repetitions of the pathogenicity test.

Controls consisted of date palm seeds of the same cultivars soaked for 1 hr in sterile distilled water. These were subsequently treated in a manner identical to that of the inoculated seeds. Only in the third and largest repetition of the pathogenicity test was the number of control seeds equal to that of the inoculated seeds.

Pathogenicity measurements. After the incubation period, germination counts were made, the germinated seedlings were harvested, and the fresh weights and seedling lengths were determined.

Sections of the cotyledon, crown, and root of diseased and healthy seedlings were removed, surface-sterilized in 1% sodium hypochlorite for 10 min, rinsed in sterile water in wells of Coors color reaction spot plates, ground with a glass rod, and allowed to stand for 30 min. Samples of the water were streaked onto CM and CM + G and incubated at 25 C for 96 hr.

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Table 1. Reduction in germination of seeds of date palm (*Phoenix dactylifera*) and growth of seedlings inoculated with *Bacillus circulans* isolate B02-3

Cultivar ^a	Seeds planted (no.)		Seeds germinated (%)		Mean fresh weight (g)		Mean length (cm)	
	Inoculated	Noninoculated	Inoculated	Noninoculated	Inoculated	Noninoculated	Inoculated	Noninoculated
Amir Hajj	37	35	72	80	4.8	5.2	35.5	36.5
Barhee	79	54	59 ^b	85	3.7 ^{*c}	4.7	33.4 [*]	37.4
Dayri	58	51	68	82	4.4 [*]	5.9	29.4 [*]	37.0
Deglet Beida	63	52	46 ^b	90	4.5 [*]	6.4	33.6 [*]	38.3
Deglet Noor	82	47	55	54	4.3	4.0	36.8	35.6
Empress	60	52	85	80	3.7 [*]	4.5	32.8 [*]	37.3
Khdraway	69	52	55	58	4.2	4.2	33.9	35.2
Medjool	41	34	36 ^b	64	5.0	5.4	37.2	37.2

^aData for Amir Hajj and Medjool are from one experiment; data for other cultivars are combined from three experiments.

^bSignificantly different from noninoculated based on tests of 95% confidence limit.

^c* = *F* test values significantly different from those of noninoculated at *P* = 0.01.

Ungerminated seed were removed from the pots after 4–5 mo, washed with sterile distilled water, surface-sterilized in 1% sodium hypochlorite, and soaked in sterile distilled water overnight. Samples of the water were streaked onto CM and CM + G and incubated at 25 C for 96 hr.

The bacterial colonies that developed were examined for their morphological characteristics by phase contrast microscopy, stained for the Gram reaction, and characterized by routine biochemical tests (7).

RESULTS AND DISCUSSION

The combined results of three pathogenicity evaluations are presented in Tables 1 and 2. *B. circulans* isolate B02-3 significantly reduced the percentage of

germination of three cultivars and significantly decreased the fresh weights and lengths of the seedlings produced by the inoculated seeds of four cultivars (Table 1).

Seedlings of the four cultivars in which growth was suppressed appeared to be diseased. Tips were necrotic, and the necrosis progressed downward in 21–30 days until the entire cotyledon was involved (Fig. 1). Symptoms were not observed in any of the control seedlings of the eight cultivars over the 5-mo period in any of the three pathogenicity tests.

B. circulans was the only micro-organism recovered in relatively high numbers from the root, crown, and cotyledon of all seedlings showing

symptoms (Table 2). No other bacteria or filamentous fungi were recovered even though the conditions were nonselective. In no case was *B. circulans* recovered from seedlings that did not show symptoms.

The pathogenicity of *B. circulans* to the seedlings corresponds closely with the pathogenicity on date palm tissue culture reported previously (5). One of the cultivars in which germination was significantly reduced (Barhee) and two of the four cultivars in which seedling lengths and weights were significantly reduced (Barhee and Empress) were infected and destroyed after inoculations of callus and meristem cultures with *B. circulans* B02-3.

B. circulans, a proven pathogen of date palm tissue cultures (4), is also a pathogen of date palm seedlings under the conditions used in this study. The potential of *B. circulans* as a pathogen of date palm trees is similar to the results of Feather et al (2) concerning *Fusarium oxysporum* Schlecht. as a potential danger to date palms in California. Although *F. oxysporum* has never been reported as a pathogen of date palms in California, Feather et al (2) showed that *F. oxysporum* isolated from diseased Canary Island palm (*P. canariensis* Hort. ex Chab.) was pathogenic to date palm seedlings under greenhouse conditions.

Table 2. Disease incidence on seedlings of different cultivars of date palm (*Phoenix dactylifera*) inoculated with *Bacillus circulans* isolate B02-3 and recovery of *B. circulans* from diseased tissues

Cultivar ^a	No. of treated plants		<i>B. circulans</i> recovered from:			
	Germinated	Diseased	Root	Crown	Cotyledon	Nongerminated seed
Amir Hajj	29	0	— ^b	—	—	—
Barhee	55	18	+++	+++	+++	+
Dayri	48	9	++	++	++	+
Deglet Beida	29	8	+++	+++	+	+
Deglet Noor	53	0	—	—	—	—
Empress	51	11	++	+++	++	+
Khadrawy	38	0	—	—	—	NT
Medjool	15	0	—	—	—	NT

^aData for Amir Hajj and Medjool are from one experiment; data for other cultivars are combined from three experiments.

^b— = None, + = less than 10³ cfu/g of tissue, ++ = 10³–10⁴ cfu, +++ = 10⁵–10⁶ cfu, NT = not tested.

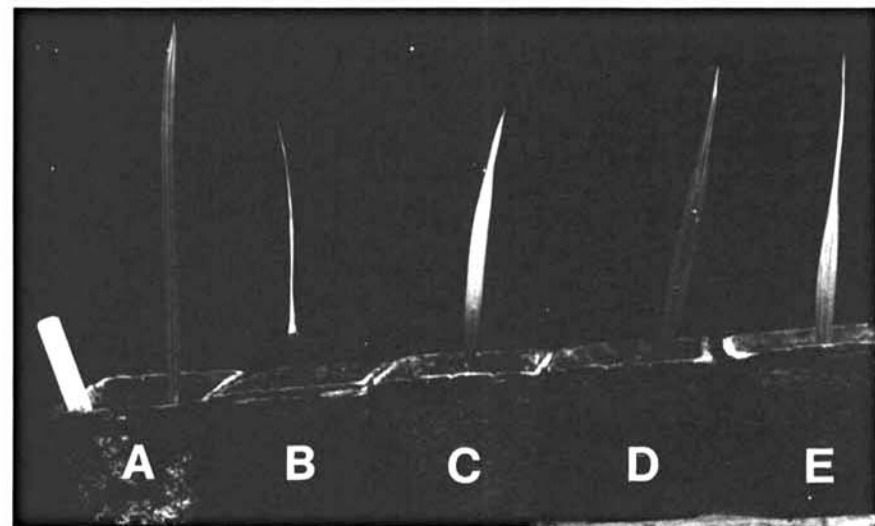


Fig. 1. Severity of symptoms on seedlings of the susceptible date palm cultivar Barhee inoculated with *Bacillus circulans*. (A) Noninoculated control. (B–E) Inoculated seedlings: (D) initial tip necrosis, (C) progressive downward necrosis, (E) total necrosis and wilt of the cotyledon, and (B) death of the seedling. All seedlings are shown approximately 5 mo after being planted.

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LITERATURE CITED

- Broadbent, P., Baker, K. F., Franks, N., and Holland, J. 1977. Effect of *Bacillus* spp. on increased growth of seedlings in steamed and in nontreated soil. *Phytopathology* 67:1027-1034.
- Feather, T. V., Ohr, H. D., Munnecke, D. E., and Carpenter, J. B. 1989. The occurrence of *Fusarium oxysporum* on *Phoenix canariensis*, a potential danger to date production in California. *Plant Dis.* 73:78-80.
- Hosford, R. M., Jr. 1982. White blotch incited in wheat by *Bacillus megaterium* pv. *cerealis*. *Phytopathology* 72:1453-1459.
- Leary, J. V., Nelson, N., Tisserat, B., and Allingham, E. A. 1986. Isolation of pathogenic *Bacillus circulans* from callus cultures and healthy offshoots of date palm (*Phoenix dactylifera* L.). *Appl. Environ. Microbiol.* 52:1173-1176.
- Matkin, O. A., and Chandler, P. A. 1972. The U.C.-type soil mixes. Pages 68-85 in: *The U.C. system for producing healthy container-grown plants*. K. F. Baker, ed. Calif. Agric. Exp. Stn. Ext. Serv. Man. 23.
- Mundt, J. O., and Hinkle, N. F. 1976. Bacteria within ovules and seeds. *Appl. Environ. Microbiol.* 32:694-698.
- Wolf, J., and Barker, A. N. 1968. The genus *Bacillus*: Aids to the identification of its species. Pages 93-109 in: *Identification Methods for Microbiologists*. Part B. B. M. Gibbs and D. A. Shapton, eds. Academic Press, New York.