Foot Rot of Prickly Pear Cactus Caused by *Phytophthora nicotianae*

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ABSTRACT

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A foot rot of prickly pear cactus (*Opuntia ficus-indica*) was observed in commercial orchards of a major producing area in Sicily. Symptoms were exudates oozing from the basal stem, soft rot and discoloration of internal tissues, and eventual wilting of the plant. The disease occurred in clay soils after prolonged periods of rain. *Phytophthora nicotianae* was consistently isolated from infected tissues. Koch's postulates were completed with plants of prickly pear cactus grown in containers. This is the first record of *P. nicotianae* as a pathogen of prickly pear cactus.

Plantations of prickly pear cactus (*Opuntia ficus-indica* Mill.) in Sicily extend over 4,000 ha. In recent years, plants in some orchards of a major producing area near the village of San Cono have wilted rather than resuming growth after dormancy. Diseased plants showed a water-soaked area with brown irregular margins on the stem surface at the soil line and a softening of internal tissues that turned brown or reddish. Dark brown mucoid and profuse gummy, amber-colored exudates oozed from the lesion surface (Fig. 1).

In most of the plants, the rotting of the stem never extended more than 15–20 cm above the ground. Affected plants were girdled and collapsed. The disease affected random groups of plants in clay or clay loam soils after prolonged periods of rain.

A species of *Phytophthora* de Bary with papillate sporangia was consistently isolated from rotted tissues. The objective of this study was to identify the fungus and ascertain whether it was the causal agent of foot rot of prickly pear cactus.

MATERIALS AND METHODS

Isolation and culture. Tissue pieces, cut with a sterile scalpel from the advancing margins of the infected portion of the stem, were plated onto potato-dextrose agar (PDA) or Difco cornmeal agar (CMA). Plates were then incubated at 25 C in the dark. Subcultures were grown on CMA, V-8 juice agar (V8A), and carrot broth (CB) (1,16).

Sporangia were produced in 250-ml Erlenmeyer flasks containing 25 ml of CB inoculated with mycelial plugs taken from the edge of cultures actively growing on V8A. After 6 days of incubation at 25 C in the dark, mycelial mats were aseptically filtered, washed several times with distilled water in a Büchner funnel, and transferred onto 9-cm-diameter petri plates containing 10 ml of sterile Petri's salt solution (16). The plates were incubated for 3–6 days under 40W daylight-type fluorescent lamps at room temperature (19–22 C).

Isolates of *Phytophthora*. The following isolates of *Phytophthora* spp. with papillate sporangia were used in the present study: a single zoospore isolate ($C_{91\text{-}2}$) from prickly pear cactus; an isolate (C_{55}) of *P. cactorum* (Leb. & Cohn) Schroet. from feijoa (*Feijoa sellowiana* Berg); an isolate (C_{102}) of *P. capsici* Leon. (A_1 mating type) from pepper (*Capsicum annuum* L.); three isolates (C_{25}, C_{42}, and C_5) of *P. citrophthora* (Smith & Smith) Leon. from citrus soils; two single zoospore isolates ($C_{18\text{-}22}$ and $C_{19\text{-}40}$) of *P. nicotianae* van Breda de Haan var. *nicotianae* Waterh. from citrus soils (A_1 and A_2 mating types, respectively); two mass isolates (C_{92} and C_{93}) of *P. nicotianae* from kentia (*Howeia forsterana* (Moore & Muell.) Becc.) (A_1 mating type) and hibiscus (*Hibiscus rosa-sinensis* L.) (A_1 mating type), respectively; a single zoospore isolate ($C_{88\text{-}1}$) of *P. n.* var. *parasitica* Water. from jojoba (*Simmondsia chinensis* (Link) Schneider) (A_2 mating type); and an isolate (C_{38}) of *P. palmivora* (Butler) Butler from chamaedorea palm (*Chamaedorea elegans* Mart.) (A_1 mating type). The identification numbers of the isolates are those of the culture collection at the Institute of Plant Pathology, University of Catania, Italy.

Compatibility type. The isolate from prickly pear cactus was paired in culture with isolates of *P. nicotianae* of the compatibility type A_1 (isolate $C_{18\text{-}22}$) or A_2 (isolate $C_{88\text{-}1}$). Paired cultures were grown in petri dishes containing V8A in the dark at 25 C.

Polyacrylamide gel electrophoresis. Mycelial extracts were prepared according to the procedure of Gill and Zentmyer (7) with a slight modification. Mycelium from 8- to 9-day-old cultures, grown on CB and incubated in the dark at 25 C, was harvested by filtration onto muslin and washed three times with sterile distilled water. The buffer-soluble proteins were extracted by grinding blotted dry mycelium with a pestle in a mortar containing quartz sand and 0.1 M phosphate buffer, pH 7.0, with 0.001 M EDTA (1 ml of buffer per gram of mycelium). The mycelial fragments were removed by centrifugation at 10,000 *g* for 15 min, and the resulting supernatant was centrifuged at 96,000 *g* for 80 min. The soluble proteins of the supernatant were precipitated with 70% ammonium sulfate and then recovered by centrifugation at 9,800 *g* for 25 min. The resulting pellet was dissolved in the sample buffer (0.5 M Tris-HCl, pH 6.8, 10% glycerol and 0.002% bromophenol blue). All the above steps were carried out at 4 C.

The proteins extracted were analyzed either on linear gradient slab gels (5–20%

Fig. 1. The most characteristic symptom of foot rot caused by *Phytophthora nicotianae* on prickly pear cactus is gum exudate oozing from the basal stem.

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polyacrylamide) or on 7.5% polyacrylamide gels in a nondissociating buffer system (3,14). Each sample containing approximately 400 μg of proteins, as determined by the method of Lowry et al (10), was pipetted onto the stacking gel (5%). A Protean-Dual apparatus (Bio-Rad Laboratories, Richmond, CA) was used. Electrophoresis was done either at 4 C or at room temperature (19–22 C). The gels were stained with Coomassie Blue (4). The protein patterns were compared by means of a similitude index (SI) (8). SI values were calculated by multiplying the reciprocals of the percentage similarities by 100 (6).

Pathogenicity tests. The pathogenicity of the isolate under study was compared to the pathogenicity of two isolates of *P. nicotianae* from citrus soil (A_1 mating type) and jojoba, respectively, on 3-yr-old plants of prickly pear cactus. The plants were grown in containers filled with a sterilized soil mix of two parts clay loam and one part sand. The plants were inoculated on the stem, 2–3 cm below the soil line, by removing a single bark disk 5 mm in diameter. A plug from the edge of cultures actively growing on CMA was inserted into the hole. The bark disk was replaced and covered with waterproof tape. Six plants were inoculated with each fungal isolate. Control plants were inoculated with CMA only. The plants were kept in a greenhouse from March to September. During this period the temperature in the greenhouse ranged from 20 to 37 C.

Quantitative determination of *Phytophthora* population in soil. In June 1986, soil samples were collected from eight commercial prickly pear cactus orchards in the San Cono area. The soil texture, according to USDA soil texture classes (2), was sandy loam in four orchards and clay in four. Four orchards—two of each soil type—were irrigated in summer. In each orchard, four 0.5-kg samples of rhizosphere soil were collected from four different plants (one composite sample per plant) at a depth of 5–15 cm. In the orchards with clay soils, samples were taken from around four diseased and four apparently healthy plants.

The population of *Phytophthora* was estimated by the soil-dilution plating technique using the selective medium BNPRA (12) and expressed as the number of viable propagules per gram of dry soil (ppg). At least 15 replicate plates were used for each sample. Before plating, the soil was diluted either 1:10 or 1:50 (w/v) with deionized water. For identification, colonies of *Phytophthora* on the selective medium were subcultured on CMA.

RESULTS

Morphological and cultural characteristics. The fungal isolate from prickly pear cactus grown on agar media (Fig. 2) formed uniform colonies producing sporangia (Fig. 2C) that were subspherical, ovoid, obpyriform, or ellipsoid, papillate, occasionally with two apexes, having a mean length:breadth ratio <1.6. Chlamydospores (Fig. 2B) were differentiated both in agar and in liquid media; diameters on V8A ranged from 19 to 45 μm (mean and modal values, 29 and 28 μm, respectively). Hyphal swellings were observed in neither agar nor liquid media. Cardinal temperatures for radial growth of colonies on agar media (PDA, CMA, and V8A) were 9–10 (minimum), 26–27 (optimum), and 35–37 C (maximum). In paired cultures with the A_2 mating type of *P. nicotianae*, the isolate differentiated amphiginous antheridia and oogonia (Fig. 2A). The diameter of the isolate ranged from 21 to 31 μm (mean and modal values, 26 and 28 μm, respectively), and the diameter of oospores ranged from 17 to 28 μm (mean and modal values, 23 and 24 μm, respectively).

Polyacrylamide gel electrophoresis. The isolate from prickly pear cactus showed protein patterns identical or very similar to those of the isolates of *P. nicotianae* from other hosts (Fig. 3). The

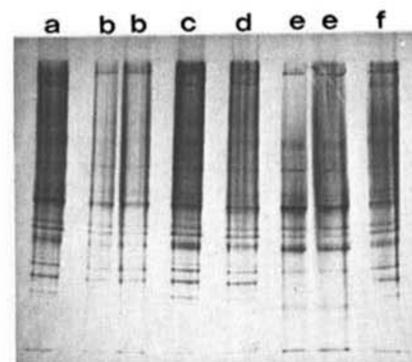

Fig. 3. Electrophoretic protein patterns shown by isolates of *Phytophthora nicotianae* in 5–20% polyacrylamide gradient gel: a = isolate $C_{19\text{-}40}$ from citrus soil, b = isolate C_{93} from kentia, c = isolate $C_{91\text{-}2}$ from prickly pear cactus, d = isolate $C_{18\text{-}22}$ from citrus soil, e = isolate C_{92} from hibiscus, and f = isolate $C_{88\text{-}3}$ from jojoba. Isolates $C_{18\text{-}22}$, C_{92}, and C_{93} are of A_1 mating type, and isolates $C_{19\text{-}40}$, $C_{88\text{-}3}$, and $C_{91\text{-}2}$ are of A_2 mating type.

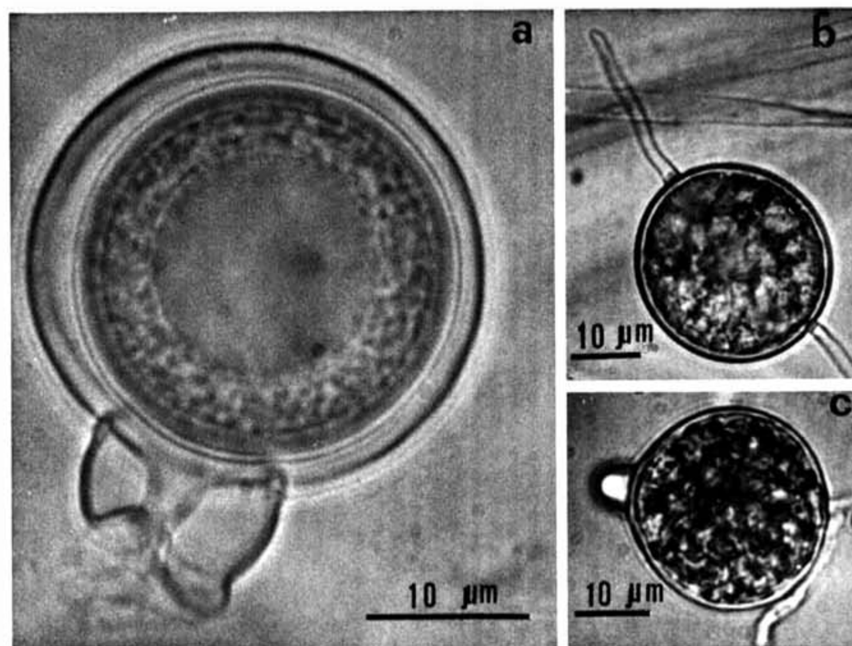

Fig. 2. (A) Gametangia and oospore, **(B)** chlamydospore, and **(C)** sporangium produced on agar media by the isolate of *Phytophthora nicotianae* from prickly pear cactus.

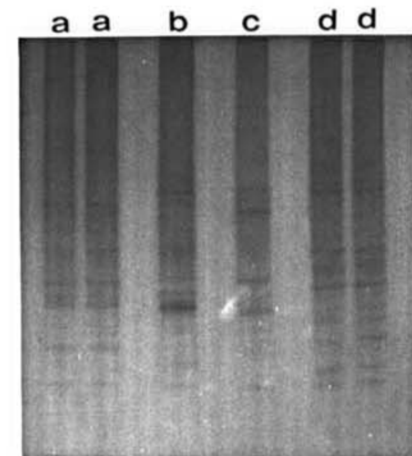

Fig. 4. Electrophoretic protein patterns shown by isolates of *Phytophthora* spp. in 7.5% polyacrylamide slab gel: a = isolate C_{38} of *P. palmivora*, b = isolate $C_{91\text{-}2}$ of *P. nicotianae* from prickly pear cactus, c = isolate C_{24} of *P. citrophthora*, and d = isolate C_{55} of *P. cactorum*.

corresponding SI values ranged from 1.0 to 1.3 (Table 1).

When the protein patterns of the isolate from prickly pear cactus and isolates of other species of *Phytophthora* were compared (Fig. 4), the corresponding SI values ranged from 2.2 (SI between the prickly pear cactus isolate and *P. palmivora*) to 3.8 (SI between the prickly pear cactus isolate and *P. cactorum*) (Table 2). SI values between the prickly pear cactus isolate and isolates of *P. citricola* and *P. capsici* were 6.0 and 5.0, respectively, indicating that the isolates were taxonomically distinct (*data not shown*).

Pathogenicity tests. All the plants inoculated with the isolate from prickly pear cactus, as well as those inoculated with the isolates from citrus soil and jojoba, showed symptoms within 30 days of inoculation. The symptoms were identical to those observed in natural infections in commercial orchards. The control plants showed no symptoms.

Fungal isolates obtained from rotted tissues of inoculated plants were identical to the fungus initially isolated from naturally infected stem tissues.

***Phytophthora* populations in the soil.** Population density of *P. nicotianae* in soil samples collected from nonirrigated orchards never exceeded 8 ppg, whereas samples from the irrigated orchards contained 12–48 ppg.

Independently of the irrigation regime, very similar population density values were obtained in rhizosphere soil of both symptomatic and asymptomatic plants. Furthermore, the incidence of foot rot caused by *P. nicotianae* among plants in clay soils was not correlated with the values of soil population density of this fungus (Table 3).

Similar levels of propagules of *P. nicotianae* were found in sandy loam and clay soils. Foot rot was found only in plants grown on clay soils, however.

DISCUSSION

The *Phytophthora* isolate from prickly pear cactus was classified as *P. nicotianae* according to the taxonomic key of Newhook et al (13). The results of the polyacrylamide gel electrophoresis confirmed the validity of this identification. On the basis of morphological criteria, Waterhouse (17) differentiated two varieties of this species, *nicotianae* and *parasitica*. The isolate from prickly pear cactus showed morphological characteristics that fit both varieties, confirming that the grouping of *P. nicotianae* into two varieties is questionable (5).

Foot rot of prickly pear cactus is caused by *P. nicotianae* in Sicily. *P. nicotianae* is a polyphagous pathogen that parasitizes over 72 genera of plants (16). Although it has previously been recognized as a pathogen on plants of the Cactaceae family (9), this is the first report of *P. nicotianae* on prickly pear cactus. Pathogenicity tests done with isolates from prickly pear cactus, jojoba, and citrus soil revealed they are not host-specific.

Symptoms of foot rot caused by *P. nicotianae* differ from those of crown rot of prickly pear cactus incited by *Armillaria mellea* Kummer by the presence of abundant gum exudates and the absence of white mycelial mats in rotted tissues (11,15). Foot rot and crown rot have so far been detected in two distinct areas in Sicily (11).

Populations of *P. nicotianae* appeared to be affected by irrigation. The incidence and severity of foot rot, however, appeared to be independent of pathogen population levels. It seems likely that the reduction of oxygen as a consequence of the saturation of clay soil during rainy seasons (autumn and winter) makes prickly pear plants extremely susceptible to the disease.

At present, foot rot caused by *P. nicotianae* does not pose a serious threat to the cultivation of prickly pear cactus in Sicily because most orchards are planted in sandy or sandy loam soils and are not irrigated in summer. The price of prickly pear cactus fruits has risen in recent years, however, and its cultivation is being extended to marginal clay or clay loam soils, with irrigation becoming a common practice in commercial orchards. The incidence of foot rot in prickly pear cactus orchards could thus be expected to increase in the future.

Table 1. Values of similitude index[a] obtained from comparison of electrophoretic protein patterns[b] of six isolates of *Phytophthora nicotianae*

	Isolates				
Isolates	$C_{88\text{-}3}$	$C_{19\text{-}40}$	C_{93}	$C_{91\text{-}2}$	$C_{18\text{-}22}$
C_{92}	1.3	1.3	1.2	1.3	1.3
$C_{18\text{-}22}$	1.0	1.0	1.1	1.0	...
$C_{91\text{-}2}$	1.0	1.0	1.1	...	...
C_{93}	1.1	1.1	...	...	...
$C_{19\text{-}40}$	1.0	...	...	...	...

[a] Calculated by multiplying reciprocal of percentage similarities (*PS*) by 100, where *PS* = (no. pairs similar bands × 100)/(no. different bands + no. pairs similar bands).
[b] See Figure 3.

Table 2. Values of similitude index[a] obtained from comparison of electrophoretic protein patterns[b] of isolates of different species of *Phytophthora*

		Isolates		
Isolates	**Species**	C_{24}	$C_{91\text{-}2}$	C_{38}
C_{38}	*P. palmivora*	4.3	2.2	...
$C_{91\text{-}2}$	*P. nicotianae*	2.6	...	2.2
C_{24}	*P. citrophthora*	...	2.6	4.3
C_{55}	*P. cactorum*	9.5	3.8	7.1

[a] Calculated by multiplying reciprocal of percentage similarities (*PS*) by 100, where *PS* = (no. pairs similar bands × 100)/(no. different bands + no. pairs similar bands).
[b] See Figure 4.

Table 3. Populations of *Phytophthora nicotianae* in rhizosphere soil of prickly pear cactus plants at eight commercial orchards in San Cono area, Sicily

		Propagules per gram of dry soil[z]	
Soil type Orchard no.	**Irrigation**	**Plants with symptoms of foot rot**	**Plants without symptoms of foot rot**
Sandy loam			
1	Yes	...	21.5 a
2	Yes	...	17.3 ab
3	No	...	0.5 b
4	No	...	1.0 b
Clay			
5	Yes	31.8 a	13.5 ab
6	Yes	27.1 a	25.8 a
7	No	0.3 b	0.5 b
8	No	1.1 b	0.8 b

[z] Each value is the mean of four composite soil samples. Values followed by the same letters are not significantly different according to Duncan's multiple range test ($P = 0.05$).

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