Identity and Relative Virulence of Some Heterothallic Phytophthora Species Associated with Root and Stem Rot of Safflower

J. M. Klisiewicz

Research Plant Pathologist, Agricultural Research Service, U.S. Department of Agriculture, Department of Plant Pathology, University of California, Davis, CA 95616.

Mention of trade mark or proprietory product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that also may be suitable.

Accepted for publication 19 April 1977.

ABSTRACT

KLISIEWICZ, J. M. 1977. Identity and relative pathogenicity of some heterothallic Phytophthora species associated with root and stem rot of safflower. Phytopathology 67:1174-1177.

Phytophthora species isolated from safflower plants affected with root and/or stem rot in production areas in Arizona and California were identified as *P. cryptogea*, *P. drechsleri*, and *P. parasitica*. Only *P. drechsleri* previously had been implicated as a causal organism of root rot in nature. Isolates of each species induced root and stem rot of artificially inoculated plants of susceptible cultivar Nebraska 10 and moderately resistant cultivar Gila. Most isolates did not induce root or stem rot in resistant cultivars VFR 1 and US Biggs. Highly virulent isolates of *P. cryptogea* and *P. drechsleri* induced root rot of root-rot-resistant VFR 1 and stem rot of stem-rot-resistant US Biggs.

Phytophthora root and stem rot of safflower (*Carthamus tinctorius* L.) was first reported in the United States from Nebraska in 1949 (4). The causal agent was identified as *Phytophthora drechsleri* Tucker (6, 7). The same disease found later in fields and experimental plantings of safflower in California and states of the Great Plains also was attributed to *P. drechsleri* (13). Other *Phytophthora* spp. also have been reported to be pathogenic to safflower. *Phytophthora cactorum* (Leb. & Cohn) Schroet., isolated from diseased plants in safflower plantings at Beltsville, Maryland, was less pathogenic to safflower in greenhouse tests than were *P. drechsleri* or a *P. parasitica* Dast. isolate from tomato (13). *Phytophthora cactorum* from an apple orchard soil was pathogenic to safflower seedlings (2). *Phytophthora megasperma* Drechs. (2) and *P. capsici* Leonian (12) were only slightly pathogenic to artificially inoculated safflower. A pathogenic siolate throught to be *P. drechsleri* was reclassified (8) as *P. cryptogea* Pethyb. and Laff., suggesting that the latter species is also an incitant of root and stem rot of safflower.

Phytophthora root and stem rot remains an important disease of safflower, especially in surface-irrigated fields. The disease usually occurs in irrigated fields of susceptible and moderately resistant cultivars. Root and stem rot occurs also in breeding nurseries and in field tests for resistance of genetically diverse cultivars and breeding lines. Dying plants of certain cultivars with high resistance suggested that some strains of *P. drechsleri* are more virulent than realized (15) or that other *Phytophthora* spp. may be inciting the disease. Identity of pathogenic *Phytophthora* spp. associated with root and stem rot of safflower and knowledge of variability in virulence of strains within species is important to understanding differences in resistance to root and stem rot expressed by the same safflower cultivars in different production areas.

This paper presents information on identification and variability in virulence of isolates of three heterothallic *Phytophthora* spp. repeatedly isolated in this study from rotted roots and stems of irrigated safflower.

MATERIALS AND METHODS

Isolation.—*Phytophthora* isolates used in this study were obtained from safflower plants with symptoms of root and stem rot collected from irrigated breeding nurseries and fields in California and Arizona. Roots and stems were washed in tap water, surface-sterilized for 2 min in 1.0% sodium hypochlorite, and thoroughly rinsed in sterile distilled water. Small pieces of tissue from roots and stems were placed on a PVP medium (11) containing pimaricin, vancomycin, and pentachloronitrobenzene plus Difco cornmeal agar (CMA), and distilled water. Single hyphal tips from mycelia originating from tissue on PVP medium were transferred to Difco lima bean agar (LBA).

Growth and morphological characteristics.—Isolates were cultured on Difco CMA and incubated for 96 hr at 3-C intervals from 6 to 39 C to determine growth-temperature relationships. Average growth of mycelia at each temperature was determined by measurements of replicate single colonies.

To induce formation of sporangia, plastic dishes containing disks (4 mm in diameter) with mycelia from margins of 4- to 7-day-old cultures on LBA were flooded with 5 to 10 ml of autoclaved soil extract and kept at 21 to 24 C in light for 24 to 48 hr. Soil extract was prepared from 100 g of loam soil suspended in 1 liter of distilled water for 12 hr, then filtered in a Büchner funnel

(Whatman paper No. 1) until clear and autoclaved at 121 C for 15 min.

Mating structures and compatibility types were studied on CV8A medium (11) consisting of clear V-8 juice, β-sitosterol, $CaCl_2$, agar, and distilled water modified by substituting for 50 ml of water an equal volume of safflower plant extract (obtained by boiling 200 g of fresh plant stems in 1 liter of distilled water for 15 min).

Safflower isolates were identified from descriptions of *Phytophthora* spp. (5, 18, 19, 20).

Pathogenicity and cultivar response.—The pathogenicity of the *Phytophthora* isolates was evaluated from symptoms on stems and roots of inoculated plants of four safflower cultivars. Plants of root-rot-susceptible Nebraska 10, moderately root-rot-resistant Gila (16), root-rot-resistant VFR 1 (14), and stem- and root-rot-resistant US Biggs (16, 17) were grown in glazed crocks, six plants per crock, containing UC mix (1). Six- to 7-wk-old plants in a combination of two crocks/cultivar/isolate were inoculated as described below. To avoid the effects of drought on susceptibility to root rot injury, the plants were not water-stressed before inoculation (9).

Inoculum consisted of 2- to 3-wk-old cultures growing on sterile vermiculite medium (10) containing V-8 juice, distilled water, and $CaCO_3$. Three g of the inoculum mixed with 22 g of autoclaved soil were spread on the surface of soil in each crock. Soil in the crock was saturated and drained before and after inoculum was added. After the crocks were kept at 21 C for 24 hr to enhance the formation of sporangia, crock drain holes were plugged and the crocks were placed in water-bath tanks at 27 C. Then water was added and maintained 1 cm above the soil surface for 24 hr to enhance infection of the lower stem. Crocks were drained and retained in the waterbath for an additional 24 hr and then were placed on greenhouse benches. The soil was flushed with water and allowed to drain. Plants were irrigated once daily thereafter and observed for symptoms. Noninoculated plants in control crocks were treated similarly. Final counts of dead plants were made 3 wk after inoculation.

To obtain only root infection, inoculum was placed in the root zone. A glass tube 25 cm in diameter that was placed in the center of each crock at planting extended from the soil surface to the crock bottom. Three g of inoculum mixed with 22 g of autoclaved soil were placed in the opening left in the soil in each crock after removal of the tube. The crocks were cared for as above except that during the flooding period crocks with drain holes open were placed into plastic containers set in water baths. Water was added to the containers and maintained at a level that allowed flooding of the root-zone in the crocks. Crocks containing noninoculated soil were similarly flooded.

RESULTS

Isolation from diseased plants.—*Phytophthora* spp. were isolated from the rotted basal tips of tap roots of wilted safflower plants up to 6-wk-old in late spring. Wilted plants later died in the field under moderate to high temperatures. *Phytophthora* spp. also were isolated from tissues of rotted roots and lower stems of plants in early bud stage in June or flowering stage in July. Leaves of the older plants showed a change in color from dark to light green, without obvious wilting, and died shortly thereafter. Partially dry roots and stems of different aged dead plants also yielded *Phytophthora* spp. on selective media. Plants with root or stem rot collected in the same field usually yielded isolates of only one *Phytophthora* spp.

Isolates designated as P. parasitica.—Safflower isolates 169 from Arizona and 166, 273, and 375 from California were studied. The minimum and maximum temperatures for growth on CMA were 9 and 36 C, respectively, and the optimum was between 27 and 33 C. Growth of cultures on CMA at 24 C was submerged and of an irregular rosette pattern with sparse to moderate amounts of aerial mycelia. Primary hyphae were of uneven width (5 to 10 μm), with irregular branching giving a gnarled appearance. Sporangia formed in CMA cultures and on mycelia growing from LBA disks in soil extract at 21 and 24 C. Terminal sporangia were papillate, broadly ovate to obpyriform (Fig. 1-A), measuring 37-45 μm × 47.55 μm (mean 41-51 μm). Terminal and intercalary chlamydospores formed in 2-wk-old CMA cultures. Oogonia and oospores were not observed in single cultures but developed with amphigynous antheridia in paired cultures with A^2 compatibility types of *P. cryptogea* on CV8A containing safflower extract. These four isolates from safflower were designated as *P.*

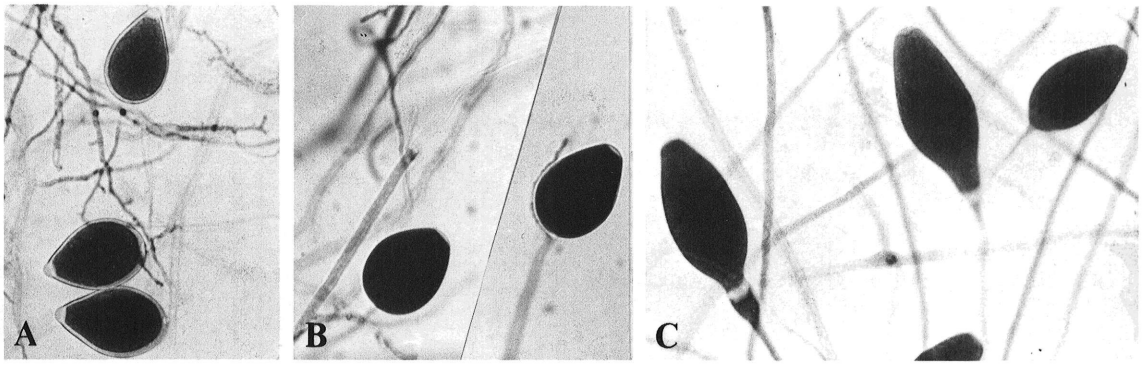

Fig. 1-(A to C). Typical sporangia of *Phytophthora* spp. causing root and stem rot of safflower. **A)** *P. parasitica* (×720); **B)** *P. cryptogea* (×720); and **C)** *P. drechsleri* (×720).

parasitica based on the similarities of their general morphological characteristics to those described for the species (5).

Isolates designated as P. cryptogea.—Isolates from safflower in Arizona (165, 261, 265) and California (162, 174) were studied. Isolates grew slightly at 6 C, and the maximum temperature for good growth was 36 C. Isolates from Arizona grew slightly at 39 C. Optimum growth of isolate 162 occurred between 24 and 30 C. Isolates 165, 174, 261, and 265 had an optimum temperature range of 27 to 33 C. Colony growth on CMA at 24 C was uniform, with sparse to moderate amounts of aerial mycelia. Primary hyphae were of uniform width (6.2 mm), with frequent branches of uneven width giving the mycelium a gnarled appearance. Sporangia formed sparsely in CMA cultures, but were abundant on mycelia growing from LBA disks placed in soil extract at 21 C for 6 to 12 hr. Typical sporangia were ovate, nonpapillate, uniformly broadly rounded at the base (Fig. 1-B), and 34 to 37 μm $\times$ 39-52 μm (mean 36 $\times$ 44 μm). Proliferation of sporangiophores was by growth outside and at the base of a nondischarged sporangium or within an evacuated sporangium. Hyphal swellings formed on mycelium in soil extract. Oogonia with single amphigynous antheridia formed readily in paired cultures with known A^1 and A^2 mating types. This group of safflower isolates from Arizona and California is comprised of A^1 and A^2 mating types.

The morphology of hyphae and sporangia of isolates 165, 261, 265, 162, and 174 as well as the sexual organs were similar to *P. cryptogea* as described by Waterhouse (19, 20).

Isolates designated as P. drechsleri.—Isolates 266 and 473 from safflower in California were studied. The minimum and maximum temperatures for growth were 6 and 36 C, and the optimum temperature was between 24 and 30 C. Growth on CMA at 24 C was submerged, slightly plumelike and slightly rosette, and radiating with a sparse amount of aerial mycelia. Primary hyphae were uniform in width (4.5 μm), with slightly acute branching of uniform width. Nonpapillate sporangia formed rarely on CMA but formed in soil extract in 12 to 24 hr at 21 C. Occasionally sporangia were elongated and ellipsoidal in form, but the majority of sporangia were elongated and tapered at the base (Fig. 1-C). Sporangia ranged from 25 to 35 μm $\times$ 56 to 79 μm. Sporangiophores often were broadened below the sporangium and proliferated within a previous evacuated sporangium or by continuous growth outside and at the base of a nondischarged sporangium. Hyphal swellings formed on mycelia in soil extract. Numerous oogonia containing clearly differentiated oospores formed with amphigynous antheridia in paired cultures when isolates 266 and 473 were paired with *P. cryptogea* A^1 mating types. A few thick-walled dark-brown mating structures formed in paired cultures with A^2 types of *P. drechsleri*, but oospores were not clearly differentiated within the oogonia. Pairing with an A^2-type *P. cinnamomi* stimulated the production of *P. cinnamomi* mating structures. The mating type of the isolates 266 and 473 was uncertain, requiring further study.

The general morphology of cultures, hyphae, and sporangia of isolates 266 and 473 was judged as similar, more so to that described for *P. drechsleri* (19, 20) than to *P. cryptogea* (19, 20).

Pathogenicity and cultivar response.—Symptom development from root and stem rot was similar in susceptible plants regardless of cultivar or *Phytophthora* species. Stem-infected plants in the greenhouse wilted, with the leaves changing from dark- to light-green or yellow, and died within 5 to 10 days of inoculation. Constriction and discoloration of the stems extended upward from the soil line. Symptom development on leaves was similar but slower in root-infected plants, which died 10 to 21 days after inoculation. Tap and lateral roots of dead plants were totally rotted.

Growth of lateral roots near the soil surface and daily irrigation seemed to prolong the life of diseased plants. Small necrotic lesions developed on the lower stem of resistant plants.

Phytophthora parasitica, *P. drechsleri*, and *P. cryptogea* isolates from safflower were pathogenic to

TABLE 1. Relative virulence of *Phytophthora parasitica*, *P. drechsleri*, and *P. cryptogea* to four safflower cultivars at 27 C

Phytophthora spp. and safflower isolate	Percentage of plants/cultivar dead from root or stem rot							
	Nebraska 10[a]		Gila		VFR 1		US Biggs	
	Root (%)	Stem (%)	Root (%)	Stem (%)	Root (%)	Stem (%)	Root (%)	Stem (%)
P. parasitica								
169	100	100	75	100	0	0	0	0
166	100	100	100	100	0	0	0	0
P. cryptogea								
265	16	100	0	75	0	0	0	0
162	58	100	8	100	0	0	0	0
165	100	100	75	100	0	0	0	0
261	100	100	83	100	0	75	0	0
174	100	100	100	100	100	100	0	83
P. drechsleri								
266	65	100	0	100	0	83	0	0
473	100	100	100	100	83	100	0	16

[a]Known reactions of cultivars to root rot caused by *P. drechsleri*: Nebraska 10, susceptible; Gila, moderately resistant; VFR 1, resistant; Biggs, resistant.

stems and roots of Nebraska 10 safflower plants, and were recovered from infected parts of the plants.

The relative virulence of *P. parasitica* isolates 166 and 169 as shown in Table 1 is representative of the virulence of four *P. parasitica* isolates from safflower to susceptible Nebraska 10 and moderately resistant Gila cultivars. VFR 1 and US Biggs showed no root or stem rot symptoms, but small necrotic flecks were observed on the lower stem.

Phytophthora drechsleri isolates attacked stems of Gila and VFR 1 killing plants, but differed in virulence to roots of the same cultivars (Table 1). Isolate 473 from roots of safflower growing on heavy rice-land soil incited root rot in resistant VFR 1 plants. Isolate 473 was avirulent to roots of US Biggs plants, but incited stem rot on a small percentage of the plants.

Isolates of *P. cryptogea* were highly virulent to the stems of Nebraska 10 and Gila plants, and weakly to highly virulent to their roots (Table 1). Most isolates failed to incite root or stem rot of VFR 1 and US Biggs plants, but isolate 174 (from safflower growing on heavy rice-land soil) was highly virulent, and incited stem and root rot on VFR 1 and stem rot on a high percentage of US Biggs.

DISCUSSION

Root and stem rot of safflower has been attributed mostly to *P.* drechsleri (the originally identified causal organism) and *Pythium* spp. (21). This study shows that *P. cryptogea* and *P. parasitica* also incite the disease. The association of other possible pathogenic heterothallic *Phytophthora* spp. with the disease of safflower is not excluded, though none was isolated in this study.

The maximum temperatures for growth of *P. cryptogea* isolates from safflower exceeded the maximum (31-33 C) temperatures for growth reported for the species (20). Growth of *P. cryptogea* isolates at 36 C and survival of some at 39 C adds to speculation on the role of climatic conditions in the region of origin in determining temperature tolerance of various isolates (3). Similar temperatures for growth of *P. drechsleri* and *P. cryptogea* isolates in the study casts doubt on the value of temperatures for growth in separation of the two species. In this study, after close examination and comparison of culture characteristics, hyphae and sporangia of safflower isolates 266, 473, 265, 162, 165, 261, and 174, it was apparent that all the isolates were not of the same species. Thus, the isolates were designated as the species (*P. drechsleri* or *P. cryptogea*) to which their morphology most closely conformed.

Phytophthora drechsleri and *P. cryptogea* isolates showed variation in virulence and certain isolates appeared to be more virulent on stems than on roots. Highly virulent strains (Table 1) of these two *Phytophthora* spp., revealed in this study, undoubtedly contribute to the death of resistant safflower plants in the field. Variation in virulence among *Phytophthora* spp. that incite root and stem rot of safflower may influence the development of resistant cultivars, particularly if highly virulent strains prevail in soils of certain production areas.

The most virulent strains of *P. drechsleri* and *P. cryptogea* that have been isolated to this time from safflower are avirulent to roots of US Biggs but virulent to the stems. Continued improvement of resistance to Phytophthora root and stem rot will require screening of cultivars and breeding lines to identify other safflowers with resistance to these strains and any new strains that might occur on safflower in nature.

LITERATURE CITED

1. BAKER, K. F. 1957. The U. C. system for producing healthy container-grown plants. Calif. Agric. Exp. Stn. Man. 23:68-86.
2. BANIHASHEMI, Z., and J. E. MITCHELL. 1975. Use of safflower seedlings for the detection and isolation of Phytophthora cactorum from soil and its application to population studies. Phytopathology 65:1424-1430.
3. BUMBIERIS, M. 1974. Characteristics of two Phytophthora species. Aust. J. Bot. 22:655-660.
4. CLAASSEN, C. E., M. L. SCHUSTER, and W. W. RAY. 1949. New diseases observed in Nebraska on safflower. Plant Dis. Rep. 33:73-74.
5. DASTUR, J. F. 1913. Phytophthora parasitica n. sp., a new disease of the castor oil plant. Mem. Dept. Agric. India. Bot. Ser. 5:177-231.
6. ERWIN, D. C. 1950. Phytophthora root rot of safflower in Nebraska caused by Phytophthora drechsleri. Plant Dis. Rep. 34:306.
7. ERWIN, D. C. 1952. Phytophthora root rot of safflower. Phytopathology 42:32-35.
8. DUNIWAY, J. M. 1976. Movement of zoospores of Phytophthora cryptogea in soils of various textures and matric potentials. Phytopathology 66:877-882.
9. KNOWLES, P. F., and M. D. MILLER. 1965. Safflower. Calif. Agric. Exp. Stn. Ext. Serv. Circ. 532. 51 p.
10. MIRCETICH, S. M., and H. W. FOGLE. 1969. Role of Pythium in damping-off of peach. Phytopathology 59:356-358.
11. MIRCETICH, S. M., and M. E. MATHERON. 1976. Phytopthora root and crown rot of cherry trees. Phytopathology 66:549-558.
12. SATOUR, M. M., and E. E. BUTLER. 1967. A root and crown rot of tomato caused by Phytophthora capsici and P. parasitica. Phytopathology 57:510-515.
13. THOMAS, C. A. 1951. The occurrence and pathogenicity of Phytophthora species which cause root rot of safflower. Plant Dis. Rep. 34:454-455.
14. THOMAS, C. A. 1976. Resistance of VFR 1 safflower to Phytophthora root rot and its inheritance. Plant Dis. Rep. 60:123-125.
15. THOMAS, C. A., and J. M. KLISIEWICZ. 1963. Selective pathogenesis within Phytophthora drechsleri. Phytopathology 53:368.
16. THOMAS, C. A., and D. E. ZIMMER. 1970. Resistance of Biggs safflower to Phytophthora root rot and its inheritance. Phytopathology 60:63-64.
17. THOMAS, C. A., and D. E. ZIMMER. 1971. Registration of USB safflower germplasm. Crop Sci. 11:606.
18. TUCKER, C. M. 1931. Taxonomy of the genus Phytophthora deBary. Missouri Res. Bull. 153. 208 p.
19. WATERHOUSE, G. M. 1956. The genus Phytophthora. Commonwealth Mycol. Inst., Mycol. Pap. 12. 120 p.
20. WATERHOUSE, G. M. 1963. Key to the species of Phytophthora deBary. Commonwealth Mycol. Inst., Mycol. Pap. 92. 22p.
21. ZIMMER, D. E., and C. A. THOMAS. 1969. Pythium root rot of safflower. Plant Dis. Rep. 53:473.