Role of *Pythium* Species in the Seedling Disease Complex of Cotton in California

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ABSTRACT

DeVay, J. E., Garber, R. H., and Matheron, D. 1982. Role of *Pythium* species in the seedling disease complex of cotton in California. Plant Disease 66:151-154.

Survival of cotton seedlings (*Gossypium hirsutum*, cv. Acala SJ-2) grown from acid-delinted seed in six field tests during 1976 in the San Joaquin Valley of California was directly related to the concentration of soilborne propagules of *Pythium ultimum*. The viable propagules of *P. ultimum* recovered from soil were mainly oospores, but sporangia were occasionally found. Plant residues were the second most frequent source of viable propagules. Seedling survival ranged from 90 to 22% at 0 and 217 propagules of *P. ultimum* per gram of soil, respectively, with the death of 50% of the seedlings at approximately 100 propagules per gram of soil. *Pythium* species other than *P. ultimum* were present but at relatively low concentrations in soil samples. Pathogenicity tests indicated that *P. ultimum* and *P. aphanidermatum* were the most virulent species on cotton seedlings. Assays of *Pythium* species in samples of naturally infested field soils that were air-dried and stored at 23 C or kept moist and stored in sealed polyethylene bags at 4 or 23 C indicated that neither the period of storage (up to 5 mo) nor the conditions of storage caused any significant changes in populations of *Pythium* species.

Seed rot and preemergence damping-off of cotton seedlings in California have been attributed mainly to *Pythium* Pringsh. species, particularly to *P. ultimum* Trow., whereas *Rhizoctonia solani* Kuehn has been considered the main cause of postemergence damping-off (3,4,8). In years characterized by low soil and air temperatures during early growth of cotton seedlings, black root rot caused by *Thielaviopsis basicola* (Berk. & Br.) Ferr. also has been an important cause of seedling disease. Other soilborne organisms may contribute to seed rot and damping-off of seedlings; however, the effect of interaction of known pathogens and other organisms in natural soil populations on the development of seedling diseases is not well understood.

In a recent study (7), the relative importance of several seed and seedling pathogens in commercial fields in the San Joaquin Valley was determined by the use of selective fungicides, assays of soil for fungi, and isolations of pathogenic fungi from diseased seedlings. *Pythium* species were the major cause of seedling disease and reduced stands during the period of the study.

The present study was made to examine further the relationship between soil populations of several *Pythium* species and the survival of cotton seedlings. Data are also presented on survival of propagules of *Pythium* species in soil samples stored under different conditions. Brief reports of these results have been made (3,4).

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Accepted for publication 10 June 1981.

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0191-2917/82/02015104/$03.00/0

MATERIALS AND METHODS

Field tests. In conjunction with chemical seed treatment trials in the San Joaquin Valley for selective control of seedling disease of cotton (7), we conducted a study on the death of seedlings grown from untreated seed in control plots in 1976 in relation to soil populations of *Pythium* species. Tests were made in six different fields with eight single-row replicates, 80 m long, randomly placed among the chemical seed treatments. Seeds were planted for the tests in conjunction with the general seeding of the fields. The planting dates were from 25 March to 20 April 1976. The fields had been preirrigated, and the seeds were planted at depths where the soil moisture was near field capacity. Soil temperatures at planting depths were variable but ranged at night from 5 to 15 C and during the day from 15 to 27 C. The percentage of survival of cotton seedlings (*Gossypium hirsutum* L., cv. Acala SJ-2) was determined by comparing the number of seeds planted with the number of surviving seedlings. Counts were made within 6 wk after planting.

To estimate the populations of *Pythium* species in the field plots at planting time, we collected moist soil samples (15–27 C) by bulking and mixing eight cores measuring 15 × 2.5 cm from each replicate in each field. Soil samples were stored at 4 C. Soil types in the six field plots were Traver clay loam (sand, 72%; silt, 19%; clay, 9%; moisture equivalent [ME], 46%; pH 8.0) at Mahoney ranch; Foster fine sandy loam (sand, 75%; silt, 10%; clay, 15%; ME, 19%; pH 7.7) at Vosler ranch; Tulare clay (sand, 15%; silt, 20%; clay, 65%; ME, 48%; pH 8.0) at Boswell I; Wasco fine sandy loam (sand, 60%; silt, 25%; clay, 15%; ME, 13%; pH 6.4) at Shafter I and II; and Chino fine sandy loam (sand, 51%; silt, 29%; clay, 20%; ME, 18%; pH 7.8) at Clay ranch.

Populations of *Pythium* species in soil samples were measured by using a modified Anderson Sampler (2) through which soil was distributed by sieve plates onto Mircetich's pimaricin-vancomycin agar medium in petri plates (14). The modified Anderson Sampler method was used because of ease of use, reproducibility of results, and capability of yielding approximately 12% more propagules of *Pythium* species than the dilution or wet plating method (4,13). For each analysis, five subsamples of soil were used that had been thinly spread, air-dried (relative humidity, 16–24%) for about 18 hr, and mixed in a roller mill (size 11 jar containing eight large burundum cylinders, 21 × 21 mm). Based on tests with two field soils, milling up to 10 min had no statistically significant effect on the number of viable propagules of *Pythium* species. However, increasing the milling time decreased the number of viable propagules: few, if any, viable propagules remained after 60 min.

Twenty-five or 100 mg of soil, depending on the concentrations of *Pythium* propagules, were plated. After the soil was impacted on the agar medium, the plates were incubated in the dark at 23 C, and *Pythium* colonies were counted after 72 hr. Transfers from developing colonies of *Pythium* species were then made to an agar medium containing V-8 juice and β-sitosterol to stimulate sporangial and oospore formation for identification of the isolates (3).

Longevity of *Pythium* species. The longevity of *Pythium* species in soil stored under different conditions was determined as part of a study on propagule density of these fungi in field soils and survival of cotton seedlings. Moist soil was collected as previously described from three fields in different counties of the San Joaquin Valley

during July 1976 and stored as follows: a) approximately 2 kg of soil was placed in open paper bags and air-dried at 23 C at 16–45% relative humidity; b) similar amounts of moist soil were placed in sealed polyethylene bags and kept at 23 C; c) same as b), except that the soil was stored at 4 C. The water potential (Ψ) of the soil stored in plastic bags was approximately −5 bars, whereas that of the air-dry soil was less than −800 bars. Soil Ψ was measured by isopiestic determinations by using a thermocouple psychrometer according to the method of Duniway (5).

Assays of soil for *Pythium* spp. were made with the modified Anderson Sampler at 0 time (within 24 hr after collection of soil) and subsequently at various intervals up to 5 mo. Before milling, soil samples stored in plastic bags were air-dried for 18 hr. The individual samples stored in plastic bags were thoroughly mixed on a roller mill as previously described under field tests.

Pathogenicity tests. Representative isolates of *Pythium* species from field soils used in the soil storage experiments were compared for pathogenicity on cotton seedlings, cv. Acala SJ-4 (seed germination ≥90%). Each isolate was grown on potato-dextrose agar in petri plates for 10 days, and each petri plate culture was then blended for 1 min in 100 ml of distilled water and mixed manually with uninfested soil per 15-cm plastic pot (350 cm^3). The pots were placed in a greenhouse (20–26 C); after 2 days, six seeds were planted per pot. Five replicates (pots) were prepared for each isolate. An isolate of *P. ultimum* (F-367) of known pathogenicity (seedling death for 50% of seedlings [SD_{50}] required 62 propagules per gram of soil) was used as one control. Another control consisted of planting seeds in uninfested soil. The uninfested soil was a 1:1 mixture of Reiff fine sandy loam (sand, 65%; silt, 18%; clay, 17%; ME, 20%; pH 7.0) and sand. The soil was autoclaved 3 hr at 121 C. Surviving seedlings were counted 16 days after planting.

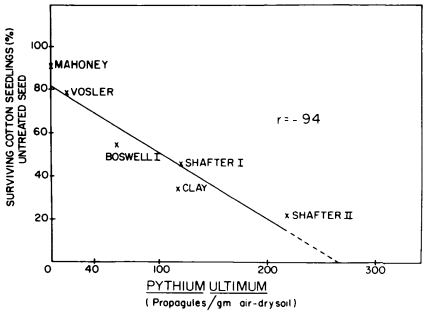

Fig. 1. Survival of cotton seedlings grown from untreated seed in relation to the concentration of propagules of *Pythium ultimum* in six field soils in the San Joaquin Valley, CA.

RESULTS

Field tests. Survival of cotton seedlings from untreated seed planted in six field tests was negatively correlated ($r = -0.94$) with the concentrations of propagules of *P. ultimum* in the field soils (Fig. 1). The correlation was highly significant ($P \leq 0.01$).

Survival of seedlings grown from untreated seed varied from 90 to 22% at 0 and 217 propagules of *P. ultimum* per gram of soil, respectively, with an SD_{50} of approximately 100 propagules per gram of soil under field conditions. The propagules of *P. ultimum* recovered from soil were determined by microscopically examining the plated soil (25–100 mg) that was distributed by the Anderson Sampler at 400 locations on each plate. Propagules that germinated were transferred to V-8 juice agar for identification of *Pythium* species. The propagules were mainly oospores with oogonial walls clearly visible; however, sporangia were occasionally found. *P. ultimum* was the most frequently isolated species; however, *P. aphanidermatum* (Edson) Fitz. and *P. irregulare* Buis. were also found.

Longevity of *Pythium* species. The longevity of *Pythium* species in soils stored under different conditions was determined in conjunction with the study of their populations in field soils and the survival of cotton seedlings in field tests (7). Neither the period nor the conditions of soil storage caused any statistically significant changes in populations of *Pythium* species during the storage period (Fig. 2). Among the soils that were air-dried and assayed within 24 hr after collection, the Shafter I soil contained 187 propagules of *Pythium* species per gram of soil, consisting of 146 of *P. ultimum*, 9 of *P. aphanidermatum*, 32 of *P. acanthicum* Dres., and several of unidentified *Pythium* species. Soil samples from the Clay ranch contained 162 propagules of *Pythium* species per gram of soil, consisting of 19 of *P. ultimum*, 29 of *P. irregulare*, 19 of *P. acanthicum*, and some of other unidentified *Pythium* species. A third soil sample, from the Mahoney ranch, contained 19 propagules of *Pythium* species per gram of soil, all of which were *P. ultimum*. The concentrations of *P. ultimum* and *P. aphanidermatum* in the Shafter I soil remained relatively constant for at least 5 mo, although the counts of viable propagules fluctuated during the storage period (Fig. 3).

Colony counts for *Pythium* species were greatly affected by the incubation temperature of the soil assay plates (Fig. 4); incubation at 23 C was optimal.

Microscopic examination of soil particles on plates indicated that thick-walled spores were the most frequent origin of colonies on the soil assay plates. Propagules within plant residues, however, were also origins of colonies. The kinds of propagules of *Pythium* species detected in the analysis of two Shafter I soil samples (air-dried and assayed within 24 hr after collection) consisted of thick-walled spores (72 and 60%); sporangia (8 and 10%); plant residues (17 and 30%); and mycelial fragments (3 and 0%), respectively, for the two soil samples.

Pathogenicity tests. Representative isolates of *Pythium* species from among those recovered from the soils used in the longevity tests were compared in pathogenicity tests by inoculating cotton, cv. Acala SJ-4 (Table 1). Greenhouse temperatures ranged from 20 to 26 C. Isolates of *P. ultimum* from the various soils ranged from highly virulent to nonpathogenic; *P. aphanidermatum*, only detected in the Shafter soil, was highly virulent; and *P. irregulare*, *P.*

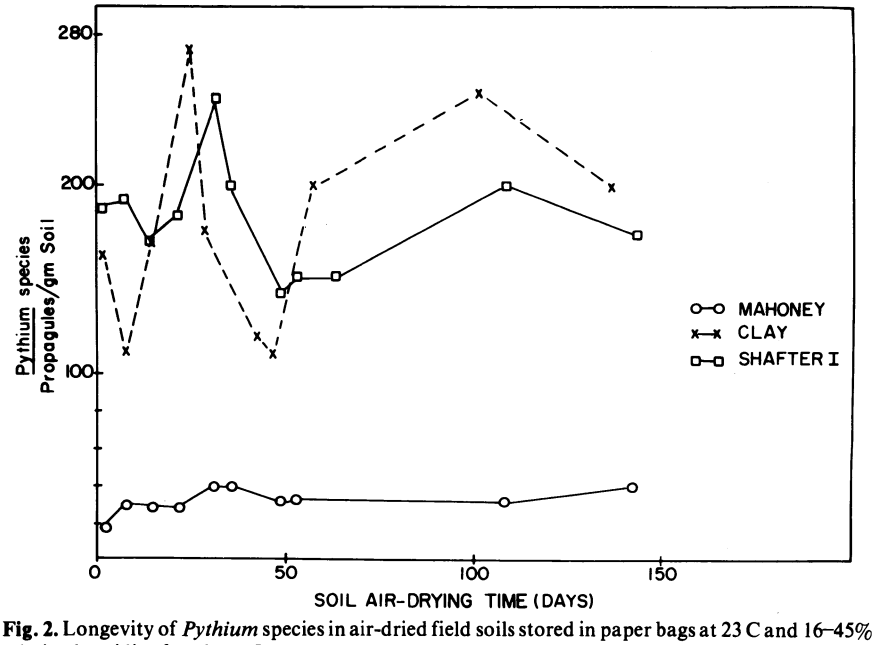

Fig. 2. Longevity of *Pythium* species in air-dried field soils stored in paper bags at 23 C and 16–45% relative humidity for about 5 mo.

acanthicum, and several unidentified species of *Pythium* were nonpathogenic. Reisolations of *Pythium* species isolates were made from selected seedlings.

DISCUSSION

The primary involvement of *P. ultimum* as a cause of seed rot and damping-off of cotton seedlings during 1976 was indicated by the selective action of field applications of the fungicide ETMT[5ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole] against *Pythium* species, by the direct relationship of seedling disease to the concentration of propagules of *Pythium* species in field soils, and by the frequency of isolation and pathogenicity of *P. ultimum* isolates from soil samples in cotton seedlings compared with the other isolates of *Pythium* species. This view was further strengthened by a continuing study in 1977 by Garber et al (7) of the selective action of chemical seed treatments. Under field conditions, *P. ultimum* showed an SD_{50} for cotton seedlings at 100 propagules per gram of soil. In contrast, under greenhouse conditions with autoclaved soil, a single, highly virulent isolate of *P. ultimum* at 60 propagules per gram of soil caused an SD_{50} of cotton seedlings. Among the other *Pythium* species isolated, only *P. aphanidermatum* from the Shafter soil was highly virulent in cotton seedlings. Ebbels (6) has reported that damping-off of cotton seedlings caused by *P. aphanidermatum* can be important in parts of West Africa; it is the species of *Pythium* principally involved. However, in the San Joaquin Valley, where soil temperatures are low in early season plantings, *P. ultimum* is the species of *Pythium* most important as a seedling pathogen.

The survival of propagules of *Pythium* species in stored soils was analyzed because it was uncertain what changes would occur in natural populations of these fungi under the variable conditions of soil storage. Previously, Hoppe (10) reported that *Pythium* species survived for at least 12 yr in air-dried muck soil, whereas Stanghellini and Hancock (15) found that sporangia of *P. ultimum* survived for at least 11 mo in both air-dried and moist soils without a decrease in rate or percentage germination. More recently, Hancock (9) found that populations of *P. ultimum* were not substantially affected when stored in air-dried soil in plastic bags at 10 C for 1–3 mo. In the study by Stanghellini and Hancock (15), sporangia of *P. ultimum* were the major survival structure in cultivated soils. Stanghellini and Nigh (16) reported that oospores were the main survival structure for *P. aphanidermatum* and that the oospores remained viable in both water-saturated and air-dried field soil at 4 C for at least 13 mo.

In the present study, microscopic examinations of plated soils indicated that thick-walled oogonia or oospores of several species of *Pythium*—including *P. ultimum*, *P. acanthicum*, and *P. aphanidermatum*—appeared to be the main survival structures. Sporangia of *P. aphanidermatum* and thin-walled globose sporangia of other species were also the foci of colonies of *Pythium* species. Second to free oospores, fungal propagules in plant debris were an important source of colonies in plated soils.

These results on germinability of oospores of *P. ultimum* appear to be at variance with the definitive work of Lumsden and Ayers (11), who reported that the thick-walled oospores do not germinate readily. Their findings were based on oospores prepared from cultures grown on a V-8 juice-cholesterol medium; however, in the present work, naturally occurring propagules were studied. The oospores observed in this study were easily distinguished from sporangia based on their thick oogonial walls. Dormancy of the oospores apparently had been broken during the aging of oospores under field conditions.

These results also seem to be at variance with those of Stanghellini and Hancock (15), who did not observe oospores for *P. ultimum* in the naturally infested soil they studied. The reason for this difference is unclear, but all of the soils used in the present study were from cotton fields, and the concentrations of *Pythium* propagules seldom exceeded 250 propagules per gram of air-dried soil.

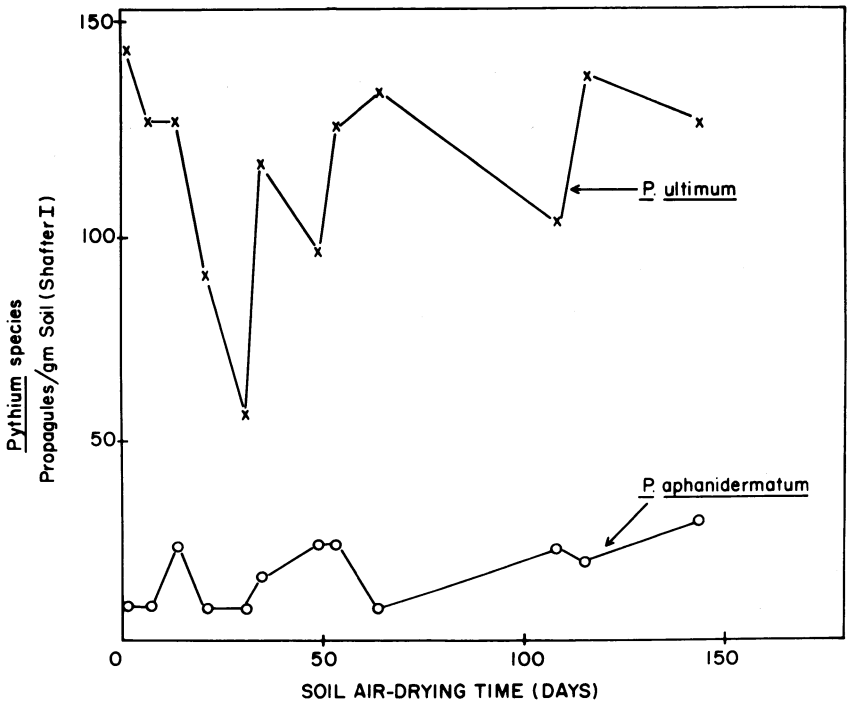

Fig. 3. Populations of *Pythium ultimum* and *P. aphanidermatum* in air-dried Shafter I soil samples stored at 23 C and 16–45% relative humidity for about 5 mo.

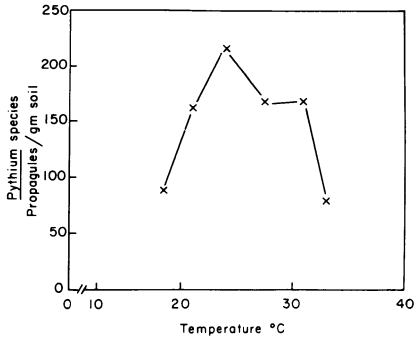

Fig. 4. Effect of incubation temperature of soil assay plates on germination of propagules of *Pythium* species per gram of soil.

Table 1. Pathogenicity on cotton seedlings (cv. Acala SJ-4) of representative isolates of *Pythium* species isolated from soils of three cotton fields in the San Joaquin Valley of California

Pythium isolate and source	Propagules per gram of soil (no.)	Seedling survival (%)[y]
P. ultimum (F-367), Shafter I	380	0 a
P. aphanidermatum, Shafter I	608	10 a
P. ultimum, Shafter I	440	13 a
P. ultimum, Clay ranch	358	60 b
P. irregulare, Clay ranch	236	70 b
P. ultimum, Mahoney ranch	350	80 b
Uninfested soil[z]	0	77 b

[y]Each value is the mean of five replicates (six seeds per pot per isolate) grown for 16 days in a greenhouse at 20–26 C. Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[z]Mixture (1:1) of sand and Reiff fine sandy loam soil autoclaved 3 hr at 121 C.

The naturally infested soil used by Stanghellini and Hancock (15), however, contained 3,814 propagules per gram of soil. The conditions in the naturally infested soil that favored the development of such a high concentration of sporangia but not viable oospores of *P. ultimum,* in contrast to that found in the soils used in the present study, are unkown.

An extensive study was made by Hancock (9) of seasonal fluctuations of *P. ultimum* populations in cotton field soils from 10 sites in the San Joaquin Valley. He found that soil conditions affected by organic amendments, moisture levels, and temperature definitely influenced populations of *P. ultimum.* He also reported that the propagules of *P. ultimum* in these soils consisted mainly of sporangia. Again, the reasons for the high concentrations of viable oospores of *P. ultimum* found here, in contrast to the low oospore-high sporangial populations reported by Hancock (9), are unknown.

Dormancy of oospores of *P. ultimum* as described by Ayers and Lumsden (1) was not apparent in the germination of oospores or in the population estimates of oospores in field soils used here or in the study by Lumsden et al (12) on the ecology of *P. ultimum* in field soil. Seasonal fluctuations in the relative concentrations of oospores and sporangia of *P. ultimum* and perhaps differences in methods of soil plating and observations of propagules may have contributed to the differences in the nature of propagules observed by us and by Hancock (9).

ACKNOWLEDGMENTS

We are grateful for the financial support of the California Planting Cotton Seed Distributors, Bakersfield 93302. We also appreciate the cooperation of the following farm advisors of the University of California Cooperative Extension: B. L. Weir, Merced County; A. George, Tulare County; O. D. McCutcheon, Kings County; D. L. West, Kern County; R. Vargas, Madera County; and L. K. Stromberg, Fresno County. We thank Diane Zumwalt for assistance with soil water potential measurements.

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