Pathogenicity of *Cylindrocladium clavatum* Causing Potato Tuber Rot

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ABSTRACT

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Peanut, eucalyptus, soybean, pepper, tomato, tobacco, papaya, and eggplant were tested for response to artificial inoculation with *Cylindrocladium clavatum* isolated from a potato tuber. Tobacco, tomato, papaya, and eggplant were not affected by *C. clavatum*, but severe root rot developed on peanut, and eucalyptus, soybean, and pepper showed slight to moderate symptoms of root infection. An in vitro soil temperature of 25 C and moisture level of −3 bars were more favorable for tuber surface rot development than lower soil temperatures or a soil moisture level of −86 bars. Tuber rot was less at 15 and 20 C, and at 10 C no measurable disease resulted. The optimum temperature for vegetative growth and conidial germination of *C. clavatum* correlates well with the effect of soil temperature on disease development on potato tubers.

A disease of potato (*Solanum tuberosum* L.) tubers, first noticed in June 1977 in Brazil, was recently described (1). The causal organism was a species of *Cylindrocladium* (1), which we identified (confirmed by C. Booth and S. A. Alfieri, Jr.) as *C. clavatum* Hodges and May. This is the fungus associated with a root disease of *Araucaria angustifolia* (Bertol) Kuntze, *Eucalyptus saligna* Sm., and several species of *Pinus* in Brazil (4).

We have isolated *C. clavatum* from roots of *Albizia, Inga,* and *Acacia* spp. and of *Glycine max* (L.) Merrill in central Brazil. The fungus was also isolated from eucalyptus roots in Almerim district in the state of Para (F. C. Albuquerque, *personal communication*) and from diseased leaves of *Vigna unguiculata* (L.) Walp. (2). These records indicate that *C. clavatum* has a wider host range and distribution than previously reported (4).

Field observations indicate that soil temperature and moisture may influence potato tuber infection and disease development. The current investigation was done to determine the reaction of some plant species to inoculation with *C. clavatum* from a potato tuber and to determine the effect of soil temperature and moisture on the development of Cylindrocladium tuber rot under controlled conditions.

MATERIALS AND METHODS

Inoculum preparation and infestation of soil. A single-spore isolate of *C. clavatum*, designated UnB 295 (ATCC 42088), was used. The fungus was isolated from surface rot lesions on a potato tuber of cultivar Bintje and maintained on

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potato-dextrose agar (PDA) at 10 C. Soil (red latosol, pH 5.2) was sifted through a 2-mm mesh sieve and steamed for 1 hr at 120 C.

Inoculum for infestation of soil was prepared by comminuting a 10-day-old culture in 100 ml of sterile distilled water for 3 min with a Waring Blendor. Fifteen milliliters of this suspension of agar, mycelium, conidia, and microsclerotia was thoroughly mixed with 400 g of soil (dry wt equivalent) in plastic pots (13 × 11 × 11 cm). The infested soil was kept moist for 3 days before planting.

Pathogenicity tests. Pathogenicity tests were done in a greenhouse with temperatures varying from 18 C at night to 30 C during the day. Seeds of the following plant species were used: peanut (*Arachis hypogaea* L. 'Tatui'), soybean (cv. UFV-1), eucalyptus (*Eucalyptus saligna* Sm., cultivar unknown), papaya (*Carica paypaya* L., cultivar unknown), tobacco (*Nicotiana tabacum* L. 'TNN'), eggplant (*Solanum melangena* L. 'Roxa Comprida'), tomato (*Lycopersicon esculentum* Mill. 'Santa Cruz'), and pepper (*Capsicum annuum* L. 'Agronomo 10').

Nine seeds were planted in each of four replicate pots of infested soil and uninfested soil. Uninfested soil was amended with autoclaved inoculum. Thirty days after planting, seedlings from each pot were uprooted and washed under running tap water, and the severity of root rot was evaluated on a 0–4 scale. All data were analyzed by Duncan's multiple range test.

Effect of soil temperature and moisture. Tubers of the cultivar Bintje were treated with 1% sodium hypochlorite for 20 min and rinsed twice in sterile water. The tubers were then wounded (1) and buried in 400 g of infested steamed soil in plastic pots (13 × 11 × 11 cm), three tubers per pot. Tubers similarly prepared but buried in uninfested steamed soil served as controls.

Soil moisture was adjusted to 10 and 25% (w/w, dry wt equivalent) by adding sterile water; soil was mixed thoroughly for 30 min in plastic bags before burial of the tubers. Containers were covered with paper to reduce evaporation. The soil moisture contents approximated soil matric potentials of −86 and −3 bars, as determined by the method described by Fawcett and Collis-George (3).

Eight replicates of infested and uninfested soil per moisture level were maintained at 10, 15, 20, and 25 C in laboratory incubators. After 6-day incubation, tubers from each treatment were recovered, washed under running water, and rated on a 0–4 scale for Cylindrocladium tuber rot. The experiment was repeated four times. Although disease index values differed somewhat among experiments, the trends remained the same.

Temperatures above 25 C were not used in this study, since preliminary work showed that at temperatures above 25 C the tubers rapidly rot due to bacterial infection, which hinders accurate Cylindrocladium rot indexing.

Vegetative growth and germination of conidia. Vegetative growth of *C. clavatum* and germination of conidia was observed at 10, 15, 20, 25, 28, 30, 33, and 35 C. Vegetative growth was studied in petri plates containing 15 ml of PDA inoculated with mycelium plus agar (4-mm diameter) cut from a 10-day-old culture of *C. clavatum* on PDA. Mycelial growth in six replicate cultures at each temperature was measured after 7 days.

A conidial suspension (10^6 spores per milliliter) in sterile distilled water was prepared from a 10-day-old culture of *C. clavatum* for spore germination tests. About 0.2 ml of conidial suspension was deposited with a pipette and spread over the surface of 2.5% water agar with the aid of a sterile glass rod with U-shaped tip.

Germination percentages were determined by microscopic observation of 200 conidia per plate after 3, 6, 9, and 24 hr of incubation at 10, 15, 20, 25, 28, 30, 33, and 35 C. Conidia were considered to be germinated when they produced germ tubes discernibly longer than the length of the conidium. Two replicate plates were counted for each temperature, and the experiment was repeated twice.

RESULTS

Pathogenicity tests. *C. clavatum* showed different degrees of susceptibility among the eight species (Table 1). Tobacco, tomato, papaya, and eggplant remained symptomless throughout the experiment. Peanut developed the most disease and eucalyptus the next greatest amount of disease. Both species showed preemergence and postemergence damping-off. Soybean and pepper had less severe disease and looked normal aboveground, except for slightly reduced growth.

None of the control plants develped symptoms. *C. clavatum* was reisolated from all infected plants but not from roots of tobacco, tomato, papaya, or eggplant.

Effect of soil temperature and moisture. Cylindrocladium tuber rot was most severe when potato tubers were kept in moist soil (−3 bars) at 25 C (Table 2). No surface rot developed on tubers maintained at 10 C at either moisture level. At 15 C, no surface rot was observed on tubers in dry soil (−86 bars), but at that temperature a low degree of tuber rot was evident in moist soil (Table 2). At 20 C, more tuber rot occurred in moist than in dry soil, but this difference was not statistically significant. At 25 C, rot severity was significantly ($P = 0.01$) greater than that at 20 C at both soil moisture levels. Rot severity in moist soil was significantly ($P = 0.01$) greater than that in dry soil at 25 C.

Potato tubers in uninfested soil (control) remained without surface rot. The fungus was readily reisolated from all tubers with symptoms of Cylindrocladium rot.

Radial growth and spore germination. The optimum temperature for radial growth and conidial germination of *C. clavatum* was between 25 and 28 C (Fig.

Table 1. Pathogenicity of *Cylindrocladium clavatum* isolated from surface rot lesions on a potato (*Solanum tuberosum* L.) tuber

Test plant	Disease reaction	Seedlings infected[x]	Disease index[y]
Peanut	Damping-off before and after emergence	36/36	3.40 c[z]
Eucalyptus	Damping-off before and after emergence	36/36	1.51 b
Soybean	Reduced growth	36/36	0.95 b
Pepper	Reduced growth	16/36	0.45 b
Tomato	None	0/36	0.00 a
Tobacco	None	0/36	0.00 a
Papaya	None	0/36	0.00 a
Eggplant	None	0/36	0.00 a

[x] Number of infected seedlings/total number inoculated.
[y] Severity of Cylindrocladium root rot was rated on a scale of 0 (no visible damage) to 4 (root system completely decayed).
[z] Mean values in each column followed by the same letter do not differ significantly ($P = 0.05$) from each other by Duncan's multiple range test.

Table 2. Response of potato (*Solanum tuberosum* L. 'Bintje') tubers to *Cylindrocladium clavatum* in artificially infested soil

Temperature (C)	Disease index[x] and moisture[y] 10%	25%
10	0.0 a[z]	0.0 a
15	0.0 a	0.3 b
20	0.2 b	0.6 b
25	0.9 c	2.1 c

[x] Severity of Cylindrocladium tuber rot was rated on a 0–4 scale: 0 = tubers free of surface rot, 1 = ≤ 15% of tuber surface rotted, 2 = 16–30% of tuber surface rotted, 3 = 31–45% of tuber surface rotted, 4 = > 45% of tuber surface rotted.
[y] Soil moisture contents of 10 and 25% approximate soil matric potentials of −86 and −3 bars, respectively, as determined by the method of Fawcett and Collis-George (3).
[z] Means (average of four replicates of three tubers each) in each column followed by the same letter do not differ significantly ($P = 0.01$) from each other by Duncan's multiple range test.

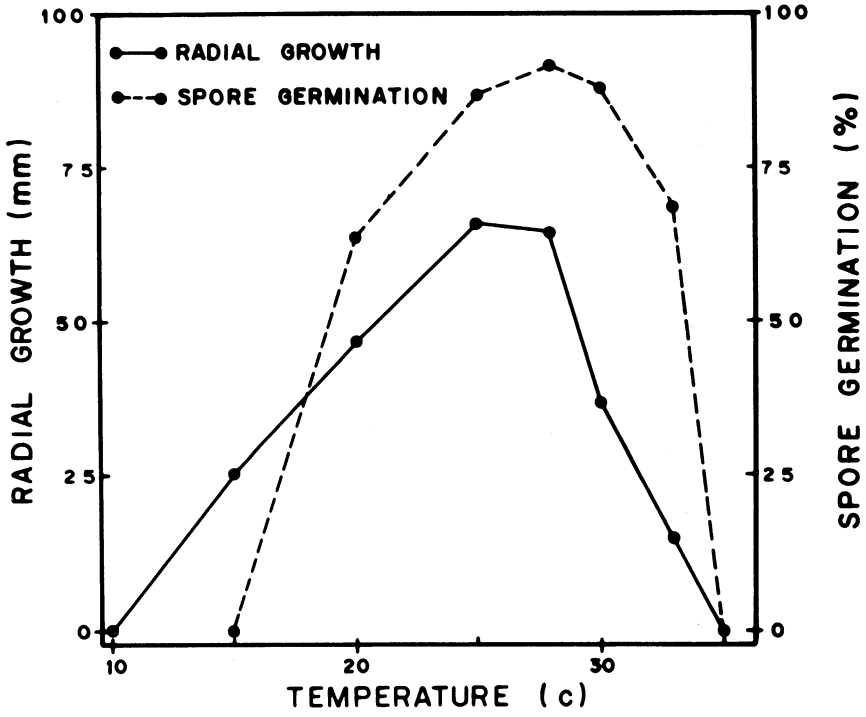

Fig. 1. Effect of temperature on radial growth of *Cylindrocladium clavatum* on potato-dextrose agar and spore germination on water agar. Radial growth and spore germination were assessed 7 days and 24 hr after incubation, respectively.

1). Mycelial growth occurred between 10 and 35 C, whereas conidia germinated between 15 and 35 C.

DISCUSSION

C. clavatum was previously reported to cause a leaf spot disease on *V. unguiculata* (2) and to damage plantations of perennial crops (4). Our results indicate that *C. clavatum* is also pathogenic to peanut, soybean, eucalyptus, and pepper. These findings confirm the suggestion that the pathogen has a greater host range than reported (2,4).

Because of its pathogenicity to such a wide range of species, *C. clavatum* must be regarded as a potential problem to rapidly growing agriculture in the cerrado region of central Brazil. It is significant that, experimentally, peanut was the most susceptible and soybean the third most susceptible to *C. clavatum*. Both are currently being considered for extensive cultivation in the cerrado region.

C. clavatum is most aggressive in soil at 25 C. Soil temperatures of 10, 15, and 20 C were less favorable or not favorable for development of surface rot by *C. clavatum*. The results also show that moist soil conditions are more favorable for disease development than dry soil conditions. At temperatures above 10 C, *C. clavatum* was more aggressive in moist soil, a characteristic reported for *C. crotalariae* on peanut (5).

The effect of temperature on vegetative growth and spore germination of *C. clavatum* correlates well with the effect of soil temperature on disease development. At 15 and 20 C, the growth rate of *C. clavatum* on PDA was approximately 62 and 30% less than that at 25 C. No vegetative growth of the fungus occurred at 10 C during the 7-day inoculation period.

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