took almost a year to show symptoms. Only two indicators inoculated with symptomless carrier material developed symptoms after transfer between the temperature regimes. Test seedlings inoculated with tissue from symptomless carrier sources developed symptoms more rapidly when cut back at 6 mo if they had been maintained continuously at the higher temperature. However, the 3-mo cutback delayed symptom development in indicators inoculated with tissue from the source with symptoms.

DISCUSSION
Because sunblotch symptoms may take as long as 2 yr to develop under uncontrolled temperatures, there is obviously a need to accelerate the indexing procedure. One way is to try to develop a rapid laboratory technique, and research in this direction is being done.

A faster reacting indicator plant would be a help, but so far no avocado cultivars more sensitive than Hass and Collinson are known, and the only other host so far recorded, cinnamon, is no faster (4). Our experiment showed a clear difference in the rate of symptom development between plants kept continuously at 30/28 C and those maintained continuously at 20/18 C. Within 8 mo of inoculation, slightly more than 90% of the plants at the higher temperature range had symptoms, although only two of the plants kept at the lower temperature had symptoms and expression required an average of 350 days. The effect of the higher temperature was further demonstrated with plants that were inoculated at 18/20 C and transferred to 30/28 C after 3 mo. Symptoms developed faster in them than in plants maintained continuously at 18/20 C and in plants transferred to the lower temperature after 3 mo.

Indexing of symptomless carriers was also helped by cutting the plants back 3 mo after inoculation, instead of 6 mo as recommended by Wallace (9), if plants were maintained at the higher temperature. This treatment impeded symptom appearance, however, in the indicators receiving tissue from sources with symptoms. Normally, indexing takes longer in symptomless carriers than in those with symptoms (10), so presumably the 3-mo cutback of the latter’s indicators removes the growth that is on the point of developing symptoms. In the symptomless carrier source indicators, cutting back forces new growth in which the symptoms appear more rapidly.

The reason for the time difference for the different carriers to induce symptoms in indicators is unknown. Wallace and Drake (10) suggested that plants with symptoms might have a higher concentration of the agent.

LITERATURE CITED

Pathogenicity of Cylindrocladium clavatum Causing Potato Tuber Rot

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ABSTRACT


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 (C. A. 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-sporic 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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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 papaya 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 × 1 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.

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

<table>
<thead>
<tr>
<th>Test plant</th>
<th>Disease reaction</th>
<th>Seedlings infected</th>
<th>Disease index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut</td>
<td>Damping-off before and after emergence</td>
<td>36/36</td>
<td>3.40 c</td>
</tr>
<tr>
<td>Eucalyptus</td>
<td>Damping-off before and after emergence</td>
<td>36/36</td>
<td>1.51 b</td>
</tr>
<tr>
<td>Soybean</td>
<td>Reduced growth</td>
<td>36/36</td>
<td>0.95 b</td>
</tr>
<tr>
<td>Pepper</td>
<td>Reduced growth</td>
<td>16/36</td>
<td>0.45 b</td>
</tr>
<tr>
<td>Tomato</td>
<td>None</td>
<td>0/36</td>
<td>0.00 a</td>
</tr>
<tr>
<td>Papaya</td>
<td>None</td>
<td>0/36</td>
<td>0.00 a</td>
</tr>
<tr>
<td>Eggplant</td>
<td>None</td>
<td>0/36</td>
<td>0.00 a</td>
</tr>
</tbody>
</table>

a Severity of Cylindrocladium root rot was rated on a scale of 1 (no visible damage) to 4 (root system completely decayed).

b 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.

c Severity of Cylindrocladium root rot was rated on a scale of 0 (no visible damage) to 4 (root system completely decayed).

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 pre-emergence and post-emergence damping-off. Soybean and pepper had less severe disease and looked normal above ground, except for slightly reduced growth.

None of the control plants developed 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. 1).

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

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Disease index 10%</th>
<th>Disease index 25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.0 a</td>
<td>0.0 a</td>
</tr>
<tr>
<td>15</td>
<td>0.0 a</td>
<td>0.3 b</td>
</tr>
<tr>
<td>20</td>
<td>0.2 b</td>
<td>0.6 b</td>
</tr>
<tr>
<td>25</td>
<td>0.9 c</td>
<td>2.1 c</td>
</tr>
</tbody>
</table>

1 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.

2 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).

Mean values (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.
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.

ACKNOWLEDGMENTS
We thank C. Booth, Commonwealth Mycological Institute, Kew, Surrey, England, and S. A. Alfieri, Jr., and Doyle Conner, Division of Plant Industry, Gainesville, FL, for confirming the identification of Cylindrocladium clavatum. We thank João Vítor M. Agresta for technical assistance.

LITERATURE CITED

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.

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.