

Pathogenicity of *Cylindrocladium clavatum* to Pea, a New Host, and Preliminary Evaluation of Its Virulence

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ABSTRACT

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Cylindrocladium clavatum causes a foot rot of field-grown peas in central Brazil, a new host record. Field symptoms—yellowing, wilting, and death—are similar to those caused by *Fusarium solani*. The pathogenicity of two isolates of *C. clavatum* obtained from peas was compared with that of three isolates from potatoes in a greenhouse. All five isolates were pathogenic to both peas and potatoes, two isolates from potato being the most pathogenic to peas. This apparently is a new disease of peas in Brazil.

Additional key words: *Pisum sativum*, resistance, root rot

Peas (*Pisum sativum* L.) are becoming an important seed crop in the Cerrado region of the Federal District, Brazil. In June 1981, sprinkler-irrigated, flowering pea plants in a field previously cultivated to apparently healthy potatoes at the experimental field of the Centro Nacional de Pesquisa de Hortaliças, EMBRAPA, showed symptoms resembling those

caused by *Fusarium solani* (Mart.) Appel & Wr. Dark brown lesions developed from the soil line down to the roots, girdling the epicotyl. Decay of the cortical tissue caused the shoot to wilt, turn yellow, and die.

In the same month, during a survey of diseased pea plants for *F. solani* using pentachloronitrobenzene (PCNB) and water agar media for isolations (11), three of the isolates obtained from plants resistant to *F. solani* (PI 140165) yielded a species of *Cylindrocladium* (4) that had apparently not been reported previously on peas (10,12,13).

C. clavatum Hodges & May was first described in Brazil in 1972, attacking *Pinus*, *Araucaria*, and *Eucalyptus* (8). Since then, it has been found on potato

(9), cowpea (6), peanut (7), and soybean (2). It has been detected in several states of Brazil, including Amazonas, Distrito Federal, Goiás, Mato Grosso do Sul, Minas Gerais, and São Paulo (6-9). *C. clavatum* has also been isolated from virgin Cerrado soils (1), thus indicating that this species is native to Brazil.

Laboratory and greenhouse studies were undertaken to identify the pathogen and to characterize the disease and the pathogenicity of isolates from potatoes (9) and peas on three pea cultivars.

MATERIALS AND METHODS

Cultures of *Cylindrocladium* sp., originally isolated on PCNB and water-agar media, were maintained on potato-dextrose agar and identified based on colony morphology and conidial, conidiophore, and vesicle characters.

For pathogenicity tests, *Cylindrocladium* isolates A and B from peas and isolates C, D, and E (ATCC 44649) of *C. clavatum* from potatoes were used. Conidial suspensions for all tests were prepared by scraping 14- to 20-day-old cultures in sterile distilled water with a rubber spatula, followed by filtration through a double-layered cheesecloth. Suspensions were adjusted to $1.0-2.0 \times 10^5$ conidia per milliliter. The viability of conidia, determined on potato-dextrose

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agar after 2 hr at 22–26 C, was approximately 85%.

Preliminary pathogenicity tests were conducted by inoculating pea cv. Mikado and potato tubers cv. Achat with pea isolates. Pea plants 7–10 days old that were grown in either vermiculite or sterile soil were inoculated by dipping unwounded or wounded roots into the conidia suspensions for 30 sec. Plants were wounded by severing the lower third portion of the roots. After inoculation, plants were repotted in vermiculite and moved to a greenhouse at 18–26 C. Disease progress was recorded over a 5- to 20-day period. Potato tubers were inoculated by being dipped into the conidial suspensions for 30 sec. Tubers were then placed on a rack in a moist chamber (24–26 C). As soon as symptoms developed, tubers were removed from the moist chamber. Reisolations were made 7 days after inoculation, thus fulfilling Koch's postulates.

The pathogenicity of isolates to three pea cultivars (Mikado, Rag 1020, and

Triofin), as well as possible interactions among isolates and cultivars, were studied. A split-plot design was used, with cultivars as main plots and isolates as subplots and with five replicates. Subplots consisted of four plants in a vermiculite-filled, 18-cm-diameter plastic pot. The inoculation procedures were similar to those of the preliminary trial, with wounded roots. Plants were rated 17 days after inoculation by two observers using a 0–2 scale, where 0 = healthy, 1 = wilting, and 2 = dead plants. The rating of each subplot was equal to the average of the four individually rated plants. Appropriate checks were included in both trials.

RESULTS AND DISCUSSION

Isolates of *Cylindrocladium* sp. from peas were all identified as *C. clavatum* (8–10), with no cultural or morphological differences when compared with isolate E (ATCC 44649) from potato (9).

Isolates A and B were pathogenic to both pea and potato. The first symptoms

on both unwounded and wounded peas were seen 4 days after inoculation. Reddish brown streaks on the stem at the soil line progressed downward, causing a general necrosis of the epicotyl and the root system. On potato tubers, round, superficial, dark brown lesions developed with well-defined margins (Fig. 1), the site of entry being the lenticels (9). It was impossible to distinguish these symptoms from those of brown eye, a potato tuber disease recently described in Brazil and caused by *C. clavatum* (9).

Table 1 summarizes the results of the inoculation experiments. Highly significant differences in pathogenicity were found only among isolates ($P = 0.01$), isolates from potato being more virulent than those from peas when inoculated on pea. Studies are under way surveying a larger number of *C. clavatum* isolates and pea cultivars.

The recent expansion of the acreage planted to peas and potatoes in the Cerrado region of Brazil, the possibility of using the same land for peas in the dry

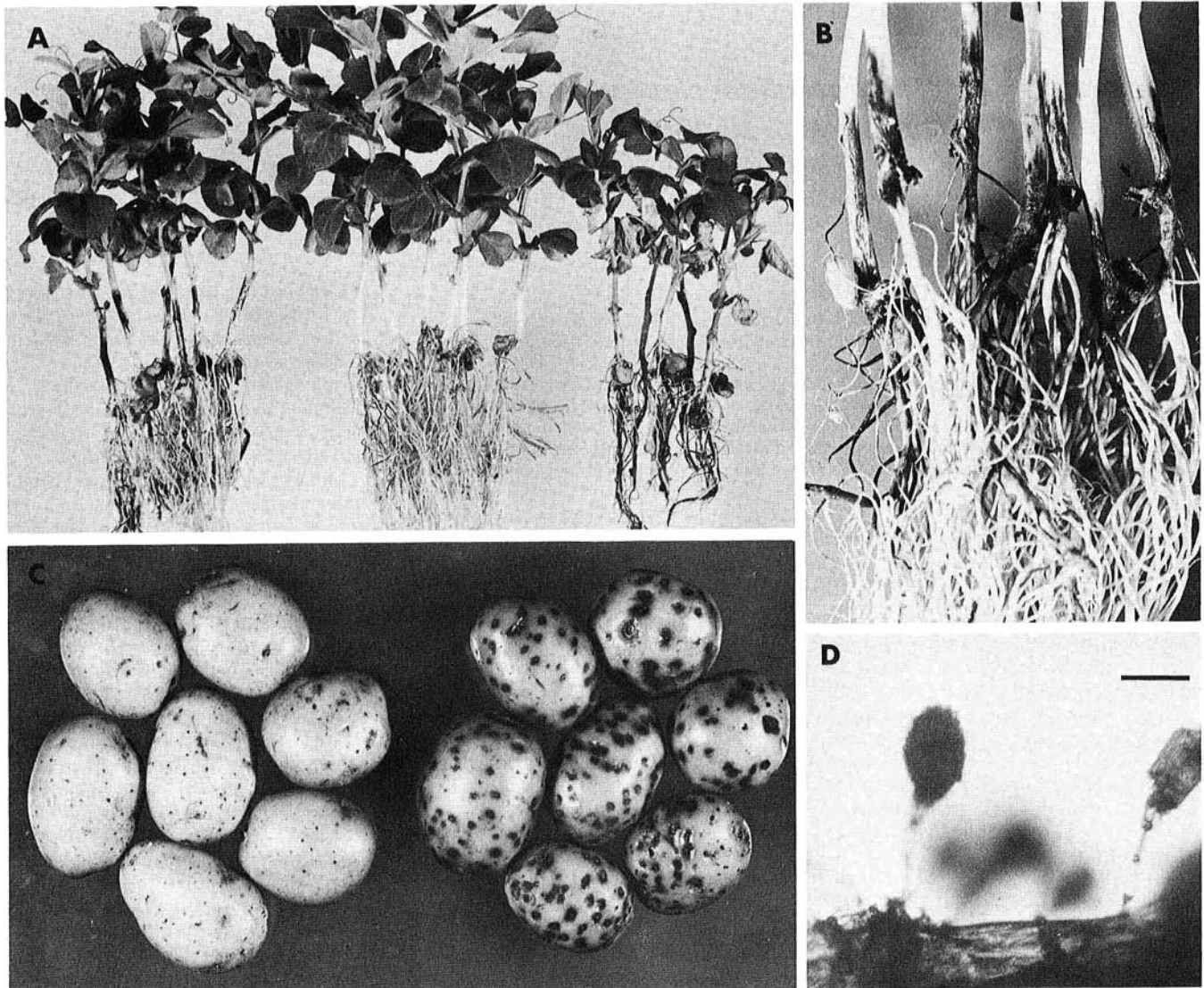


Fig. 1. Peas (cv. Mikado) and potato (cv. Achat) tubers inoculated with *Cylindrocladium clavatum*: (A) Symptoms produced by isolate A (left), check (center), and isolate E (right). (B) Detail of foot rot symptoms. (C) Symptoms on tubers produced by isolate A (right) and check (left). (D) Fruiting bodies of the fungus on pea roots (scale bar = 50 μ m).

Table 1. Mean disease rating of three pea cultivars inoculated with five isolates of *Cylindrocladium clavatum*

Cultivar	Isolates					Uninoculated	Cultivar mean
	E	C	D	B	A		
Mikado	1.7 ^x	0.7	0.5	0.2	0.2	0.0	0.5 ^y
Rag 1020	1.7	1.0	0.5	0.5	0.5	0.0	0.7
Triofin	1.7	1.5	0.7	0.7	0.2	0.0	0.8
Isolate mean	1.7 a ^z	1.1 b	0.6 c	0.5 c	0.3 d	0.0 d	

^x Mean of five replicates, where 0 = healthy, 1 = wilting, and 2 = dead plants. Ratings, done by two observers 17 days after inoculation, are the mean for each subplot.

^y Cultivar means are not significantly different at $P = 0.01$.

^z Means followed by the same letter do not differ according to Duncan's multiple range test at $P = 0.01$.

(April–September) and potatoes in the wet (October–March) seasons, and the tremendous destructive potential of *Cylindrocladium* black rot of peanuts in Georgia, USA (3), indicate that *C. clavatum* should be considered a potentially serious pathogen of both crops in central Brazil. Long crop rotations, which are usually recommended as a control measure for soilborne diseases, should be studied in great detail because of the wide host range and endemic occurrence of *C. clavatum* in Brazilian soils (1,5–9).

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