Root Rot of Soybean Caused by *Cylindrocladium clavatum* in Central Brazil

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ABSTRACT

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A new root disease of soybeans caused by *Cylindrocladium clavatum* in central Brazil is reported. The fungus causes black rot of the taproot and base of the hypocotyl. Under field conditions, patches of stunted plants are found that may wilt and die. Isolates of the fungus from soybeans also infected pea (*Pisum sativum*). *Rhizoctonia solani, Neocosmospora* spp., *Fusarium oxysporum, F. solani, F. roseum, Macrophomina* sp., *Meloidogyne incognita*, and *M. javanica* were also isolated from diseased soybean roots. Pathogenicity of *R. solani* and *Neocosmospora* sp. was established in single inoculations and in combinations with *C. clavatum*. The role of other fungi and nematodes is being investigated.

Additional key words: *Glycine max*, soybean root rot

Cylindrocladium clavatum was originally described in Brazil by Hodges and May (12) and was shown to cause root diseases of *Eucalyptus saligna, Arucaria angustifolia*, and *Pinus* spp. It was reported to cause brown eye of potato tubers (4,5,14), root rot of peas (15), root and pod rot of peanuts (7), and leaf spot of cowpeas (6). It also infects native species such as *Vochysia thyrsoidea* and *Inga* sp. (J.C. Dianese, *unpublished*). The fungus is commonly found in the latosols of central Brazil (1), where it occurs associated with other soybean pathogens (8) such as *Rhizoctonia solani* (13) and *Neocosmospora* spp. (10,17).

Greenhouse inoculation of soybean seedlings with potato and soil isolates of *C. clavatum* has been reported to result in discrete root lesions (2,5).

Following the increase in soybean acreage in the Cerrado plateau of Brazil, a black root rot of soybeans was recognized in 1982. The purpose of this paper is to show that the disease is due to infection by *C. clavatum*, which may be associated with other fungi. Because *Neocosmospora vasinfecta* is a well-known pathogen of soybean in the southern United States (10,17) and *R. solani* causes soybean "dead patch" in southern Brazil (9,13), local isolates of these fungi were used for inoculations in soybean seedlings to study their effects and possible interactions with *C. clavatum*.

MATERIALS AND METHODS

In 1982, a dark root rot affecting mainly the taproot of soybean cultivar Cristalina was first observed in two fields in the Distrito Federal. This cultivar is responsible for 30% of the soybean production in the Cerrado region of Brazil. One of the fields had plants naturally infected with *Meloidogyne incognita* and *M. javanica*, whereas the

Table 1. Fungi isolated from soil around the root system of soybean cultivar Cristalina in two fields in Distrito Federal, Brazil

	Propagules per gram of dry soil[a]			
	Dry season[b]		Rainy season	
Fungi	Field 1[c]	Field 2	Field 1	Field 2
Aspergillus	2,567	759	3,680	870
Aspergillus niger	90	30	388	32
Cladosporium	1,178	195	198	133
Colletotrichum dematium	0	10	0	0
C. gloeosporioides	36	0	0	0
Conyothyrium	0	10	0	0
Curvularia	0	0	11	0
Cunninghamella	173	50	0	0
Chaetomium	432	30	1,599	1,876
Cylindrocladium clavatum	1	20	0	0
Fusarium oxysporum	760	399	718	999
F. roseum	230	43	42	5
F. solani	322	93	182	56
Geotrichum candidum	22	0	0	0
Gilmaniella	10	0	93	11
Gliocladium	4,968	991	1,040	350
Gonatobotrys	0	0	21	0
Gonytrichum	0	20	0	0
Macrophomina	0	0	11	0
Metarryzium	0	1	11	119
Menisporella	45	71	10	11
Monocillium	0	10	0	11
Monacrosporium	1	0	11	43
Myrothecium	259	41	115	103
Mucor	317	161	575	462
Neocosmospora	637	159	509	586
Paecilomyces	0	10	0	0
Penicillium	4,192	2,901	5,771	2,115
Phomopsis	24	71	0	0
Phoma	54	1	0	11
Pyrenochaeta	0	0	21	11
Rhizopus stolonifer	86	5	189	0
Sporotrix	1	10	0	0
Syncephalastrum	0	0	179	0
Torulomyces	0	0	21	20
Trichoderma	1,308	331	1,040	350
Volutella	11	0	0	0
Not identified	4,089	4,799	5,726	3,214

[a] Each figure expresses the average number of propagules per gram of dry soil isolated from 10 soil samples. Each sample was a mixture of five subsamples from the rhizosphere of five different plants at a depth of 5–10 cm from the surface.

[b] Dry season: April to September with an average rainfall of 200 mm. Rainy season: October to March with an average rainfall of 1,200 mm.

[c] Field 1: nematode-infested area where 20% of the plants showed root-knot caused by *M. incognita* and/or *M. javanica*. Field 2: area where plants did not show root-knot.

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other field had no plants showing root-knot symptoms. Soil samples from the rhizosphere of diseased plants from both areas were collected during the dry season in May 1984 and during the rainy season in January 1985 to determine and quantify the fungal population present using direct dilution plating in Martin's rose bengal medium (19). To detect the fungi associated with rotten roots, plants with apparently healthy roots as well as those with root rot (with and without nematode galls) were sampled in May 1985. Root pieces 1 cm long were surface-sterilized with 1% sodium hypochlorite for 30 sec before plating in potato-dextrose agar containing 300 ppm chloramphenicol. Ten plates, each containing 10 root pieces, were used to

Table 2. Frequency of fungal colonies isolated from 1-cm-long segments of secondary roots of soybean cultivar Cristalina[a]

Fungi	Frequency per 100 root segments		
	Normal	Dry-rotted	Galled
Cylindrocladium clavatum	6.3	9.7	14.0
Rhizoctonia solani	2.3	9.7	5.3
Neocosmospora	4.3	7.0	6.0
Fusarium oxysporum	20.7	13.0	17.7
F. solani	3.0	4.0	9.0
F. roseum	7.3	4.3	3.0
Gliocladium	29.0	19.7	24.7
Penicillium	0.0	0.0	0.6
Aspergillus	0.0	0.0	0.3
Macrophomina	0.3	0.0	0.0
Not identified	5.0	6.3	7.2

[a] Root pieces were plated in potato-dextrose agar with 300 ppm chloramphenicol and incubated at 24 ± 2 C for 6 days. Root samples were collected at the end of the crop cycle in May 1985 from a nematode-infested field (field 1 in Table 1). Data represent the average frequency per 100 pieces.

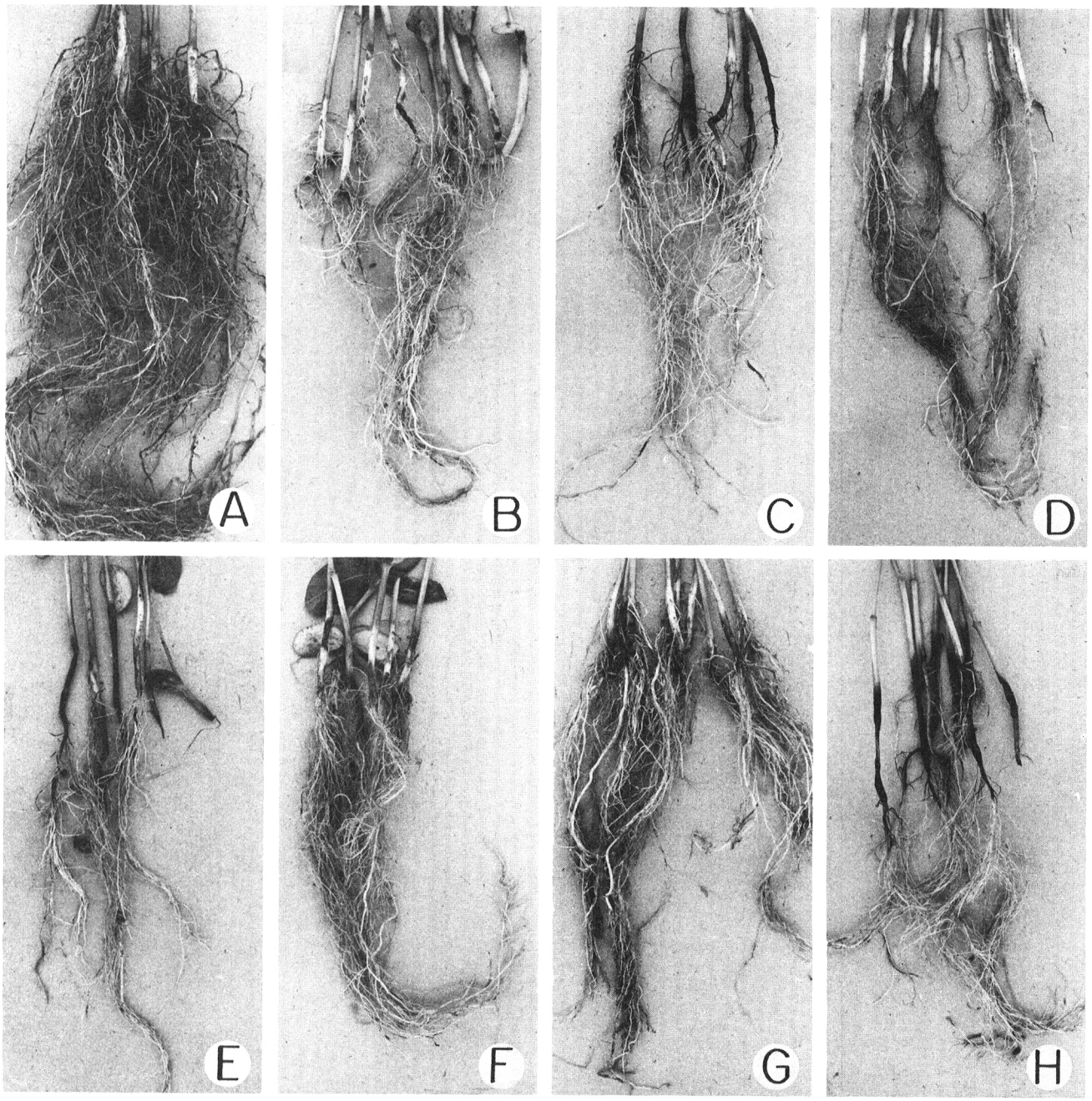

Fig. 1. Symptoms resulting from inoculating soybean cultivar Cristalina with different combinations of *Rhizoctonia solani, Cylindrocladium clavatum*, and *Neocosmospora* sp. **(A)** Control plants, **(B)** *R. solani*, **(C)** *C. clavatum*, **(D)** *Neocosmospora* sp., **(E)** *R. solani* and *Neocosmospora* sp., **(F)** *R. solani* and *C. clavatum*, **(G)** *C. clavatum* and *Neocosmospora* sp., and **(H)** *C. clavatum, R. solani*, and *Neocosmospora* sp.

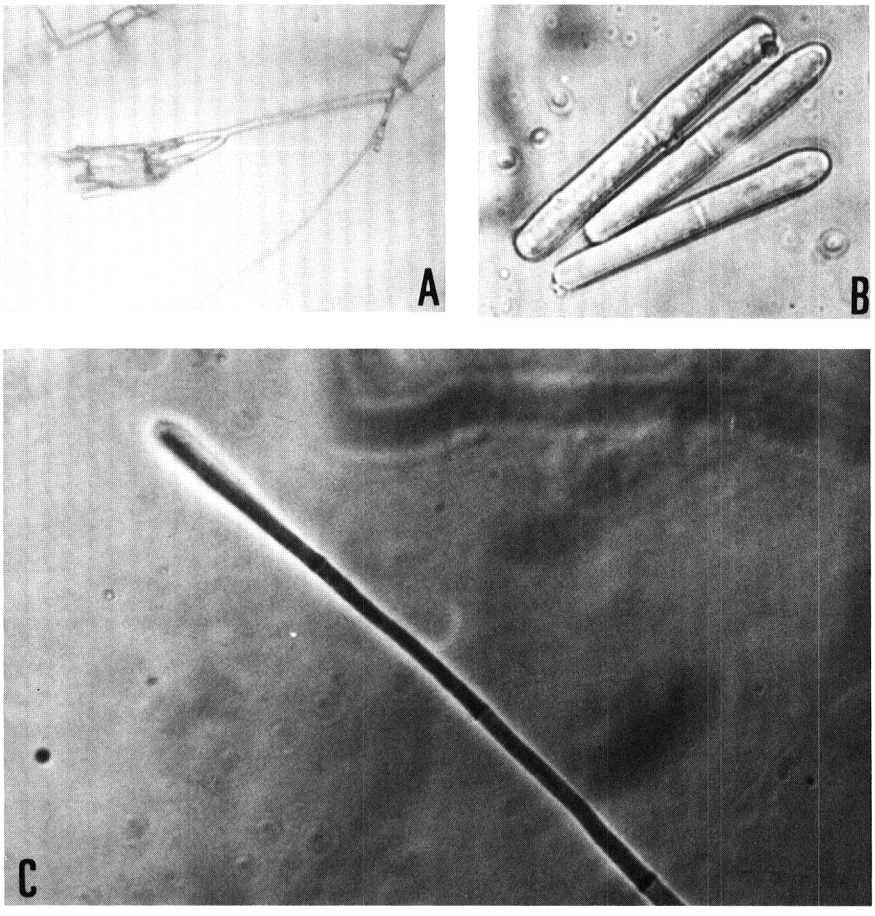

Fig. 2. Soybean isolate of *Cylindrocladium clavatum*. **(A)** Conidial head on branched conidiophore (×300), **(B)** uniseptate conidia (×1,600), and **(C)** clavate vesicle (×850).

isolate the fungi associated with each of the three types of roots.

Pathogenicity of *C. clavatum* (ATCC 60587), *Neocosmospora* sp., and *R. solani* was tested by growing soybean plants in 1.5-L pots filled with autoclaved soil inoculated with spore or mycelial suspensions. Inoculum suspensions contained 6×10^7 conidia of *C. clavatum*, 2×10^7 ascospores of *Neocosmospora* sp., and washed mycelium of *R. solani*. Spore suspensions were applied at 10 ml per pot. Mycelial suspension was produced by growing *R. solani* for 6 days at 25 C in 250-ml flasks with potato-dextrose broth. Mycelium mass was separated from the medium by filtration and suspended in 100 ml of sterile distilled water. Inoculated and control plants were grown in a greenhouse with temperatures ranging from 18 C at night to 29 C during the day. Three weeks later, plants were observed for symptoms and biopsied for pathogenic fungi.

RESULTS AND DISCUSSION

Fungal propagules present in the rhizospheres of soybeans with and without a history of nematode infection are given in Table 1. Most fungi detected were saprophytes and were present in both fields. Among the fungi isolated, those with a potential for root pathogenicity were *C. clavatum, Fusarium oxysporum, F. solani, Macrophomina* sp., and *Neocosmospora* sp. Because the method used (19) was selective for sporulating fungi, *R. solani* was not isolated. In Brasília, soybeans are seeded in October or November and grow through a rainy season with 1,200 mm of rainfall until harvest in March or April. Isolates of *C. clavatum* were always obtained from plants showing black root rot as early as 1 mo after planting. Isolation of fungi from the roots of plants just after harvest showed a population different from the one isolated directly from the rhizosphere soil (Table 2). Species with a history of involvement in root rots were present, including *R. solani*. Disinfecting the root surface eliminated most of the typical saprophytes. A total of seven species were selectively and preferentially associated with roots. Except for *R. solani*, the remaining six species were also detected by direct isolation from the soil. *C. clavatum* was detected in low frequency in Martin's medium but was isolated with high frequency from soybean roots (Table 2).

C. clavatum is known in Brasília as a pathogen of potato (5,14) and pea (15), whereas *R. solani* causes "dead patch," a major disease of soybeans in southern Brazil (13). Both fungi were selected for inoculation of Cristalina soybean to compare their effects with the root rot observed in the field. Because *Neocosmospora* sp. appeared in high frequencies both in soil and root samples (Tables 1 and 2) and *N. vasinfecta* can infect soybeans systemically (10,17), an isolate of this fungus was also selected for pathogenicity tests. Figure 1 shows the extent of root damage whenever the three

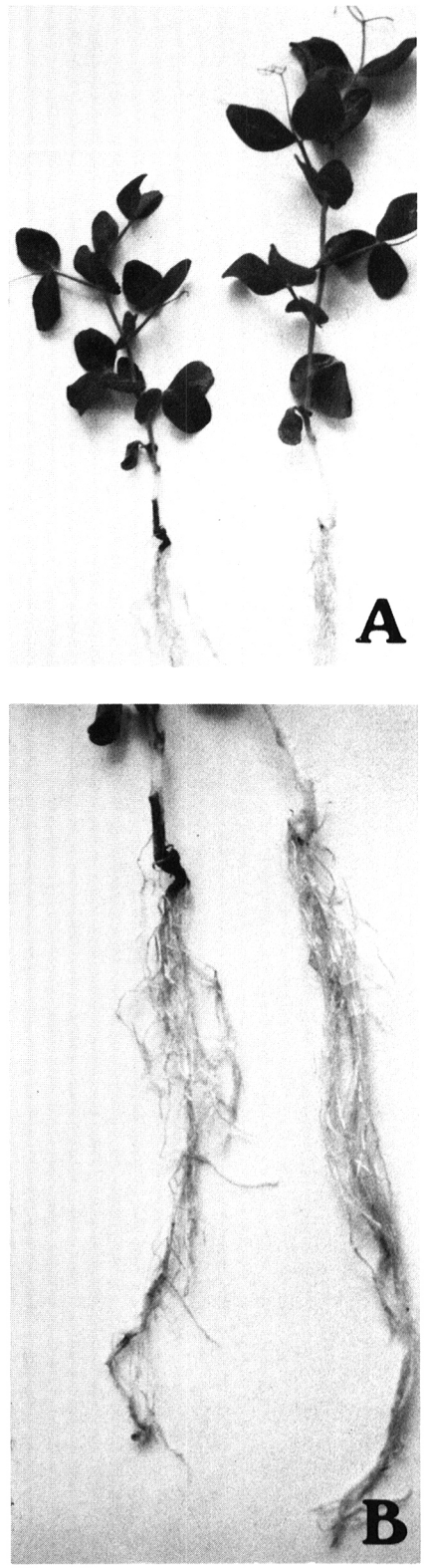

Fig. 3. Inoculation of pea seedlings (cultivar Triofin) with a soybean isolate of *Cylindrocladium clavatum* showing (left) black rot of the taproot and lower hypocotyl compared with (right) control (B is an enlargement of the lower part of A).

fungi were inoculated alone or in combination with each other. The highest level of damage occurred when all three pathogens were inoculated simultaneously (Fig. 1H). This suggests a possible synergistic effect that in the field may lead to a disease complex involving nematodes, *F. oxysporum, F. solani*, and *Macrophomina* sp., which will be the objective of future research. A characteristic black root rot was present only in those plants inoculated with *C. clavatum* (Fig. 1C,E,G,H), but a major reduction in the quantity of roots was caused by all three fungi individually or in combinations (Fig. 1A–H). These characteristic black rot symptoms were not observed by Almeida and Bolkan (2) when they inoculated soybean with soil isolates of *C. clavatum*, although some root rot was reported. *Neocosmospora* sp. did not induce the expected systemic symptoms described by Phillips (17) but caused a reduction in the quantity of roots (Fig. 1D). *R. solani* produced typical depressed lesions on the main root and lower hypocotyl and it also reduced the root systems of the inoculated plants (Fig. 1B,F).

A disease of soybeans with identical symptoms caused by *C. crotalariae* has been reported in the United States (11) and in Korea (18). The morphological features of *C. clavatum* (12,16) (Fig. 2) can be used to distinguish it from *C. crotalariae* (3).

Our soybean isolate of *C. clavatum* induced the same symptoms Lopes and Reifschneider (15) described after they inoculated pea seedlings with the isolate (Fig. 3).

The role of *F. oxysporum, F. solani*, and *Macrophomina* sp. in soybean root rot is under investigation.

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