Effect of *Chaetomium cupreum* on Seed Germination and Antagonism to Other Seedborne Fungi of Soybean

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ABSTRACT

YEH, C. C., and J. B. SINCLAIR. 1980. Effect of *Chaetomium cupreum* on seed germination and antagonism to other seedborne fungi of soybean. Plant Disease 64:468-470.

Chaetomium cupreum was isolated from three soybean seed lots of two cultivars grown at three locations in Illinois in 1979. This is the first report of *C. cupreum* in soybean seeds. In dual cultures, zones of inhibition developed between *C. cupreum* and *Fusarium* sp., *Macrophomina phaseolina, Phomopsis* sp., and *Rhizoctonia solani.* The growth of *Cercospora kikuchii, Colletotrichum dematium* var. *truncata, Fusarium* sp., *M. phaseolina, Phomopsis* sp., and *R. solani* but not of *Cercospora sojina* or *Gliocladium roseum* was inhibited on water agar mixed with the culture filtrate of *C. cupreum* and autoclaved. Ethyl ether soluble fractions of the culture filtrate of *C. cupreum* inhibited the growth of all fungi mentioned and of *Alternaria* sp. and delayed germination of soybean seeds. The fraction had absorption maxima at 230, 250, 280, 290, and 505 nm in water.

Fungi associated with soybean (*Glycine max* (L.) Merr.) seeds can reduce seed viability and seedling vigor (5) as well as the quality of flour and oil derived from infected seeds (2). Several *Chaetomium* spp., but not *C. cupreum*, have been associated with soybean seeds (4). *C. cupreum* Ames was isolated in 1979 from seeds of Clark 63 (Belleville, Illinois) and Essex (Brownstown and Carbondale, Illinois). *C. cupreum* has been isolated from soil (1), but this is the first report of its isolation from soybean seeds. When *C. cupreum* occurred in culture plates of surface-sterilized soybean seeds, inhibition of other fungi was observed.

We studied the interaction in culture between *C. cupreum* and nine fungi from soybean seeds and roots, the effect of a culture filtrate of *C. cupreum* and an extract from the culture filtrate on the nine fungi for indications of biologic control of other fungi, and the effect of the extract on soybean seed germination for phytotoxicity.

Supported in part by the Illinois Agricultural Experiment Station and the U.S. Agency for International Development (USAID). The views and interpretations are those of the authors and should not be attributed to USAID or to any individual acting on their behalf.

00191-2917/80/05046803/$03.00/0
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MATERIALS AND METHODS

Fungi. *C. cupreum* (ILLS 39316, ATTC 42000) was isolated from a seed of Clark 63 soybean. The fungus first was distinguished through its abundant production on potato-dextrose agar (PDA, Difco) of a copper-colored pigment that diffused into the medium. At first, the pigment may be confused with that produced by *Cercospora kikuchii* under similar conditions. The fungus was identified by its perithecium and appendages and its ascus and ascospores (Fig. 1A–C). Because of its

resemblance in growth characteristics to *Cercospora kikuchii* on PDA, *C. cupreum* on soybean seeds may be overlooked.

The other fungi isolated from soybean seeds and studied were: *Alternaria* sp., *Cercospora kikuchii* (T. Matsumoto & Tomoyasu) Gardner (ATTC 36864), *Cercospora sojina* Hara., *Colletotrichum dematium* (Pers ex Fr.) Grove var. *truncata* (Schw.) Arx., *Fusarium* sp., *Macrophomina phaseolina* (Tassi) Goid., *Phomopsis* sp., and *Rhizoctonia solani* Keuhn. The isolate of *Gliocladium roseum* Bainier was obtained from soybean roots. All cultures were maintained on PDA at 25 C.

Dual cultures. Agar disks (5-mm diam) from 7-day-old cultures of *C. cupreum* were placed on one side of each of four 9-cm PDA culture plates. After the disks were incubated for 5 days at 25 C, another disk from a 7-day-old PDA culture of one of the other nine fungi was placed 5 cm from the *C. cupreum* disk. The zone of inhibition between the *C. cupreum* culture and the test fungus was measured after 7 days. The percentage of inhibition of radial growth (PIRG) was calculated as $(r_1 - r_2)/r_1 \times 100$, where r_1 and r_2 are the longest and shortest colony radium, respectively, from the edge of the test fungus (3).

Culture filtrate of *C. cupreum*. Agar disks (5-mm diam) cut from a 7-day-old PDA culture of *C. cupreum* were used to inoculate 50 ml of sterile potato-dextrose broth in 250-ml Erlenmeyer flasks. After 2 wk at 25 C, the culture was filtered through a Buchner funnel containing three layers of filter paper (Whatman No. 1). The culture filtrate was incorporated into 2% water agar to concentrations of 5, 10, 15, and 20% by volume and autoclaved for 15 min at 121 C. Water agar without the culture filtrate served as a control. After 20 ml of each treatment was poured into 9-cm diameter culture plates, 5-mm agar disks from PDA or water agar cultures of each of the nine test fungi were placed in the center of each plate. Each treatment was replicated four times. Colony diameters were recorded after 7 days at 25 C. The experiment was done three times.

Extract from culture filtrate of *C. cupreum*. A culture filtrate of *C. cupreum* was prepared as described, and 300 ml of methanol was added to 700 ml of the culture filtrate and stored at 5 C overnight for deproteination. The filtrate-methanol mixture was filtered through a Buchner funnel with three layers of filter paper as described, then concentrated to 150 ml in a flash evaporator at 90 C. The mixture was extracted four times with 100 ml of ether. The ether fraction was dried in a flash evaporator and the residue resuspended in 200 ml of boiling distilled water. The maximum absorbancy was recorded with a GCA/McPherson spectrophotometer.

The ether extract in water was mixed with 2% water agar to give concentrations of 125, 250, 500, and 1,000 μg/ml and autoclaved. After 20 ml per plate was poured into 9-cm culture plates, the agar was inoculated with the test fungi. Water agar without the extract was a control. Each treatment/fungus combination was

Fig. 1. (A) Perithecium, **(B)** immature ascus, and **(C)** mature asci with ascospores of *Chaetomium cupreum*. Potato-dextrose agar culture plates showing inhibition of growth of four fungi by *C. cupreum* in dual cultures: **(D)** *Macrophomina phaseolina*, **(E)** *Fusarium* sp., **(F)** *Phomopsis* sp., and **(G)** *Rhizoctonia solani*.

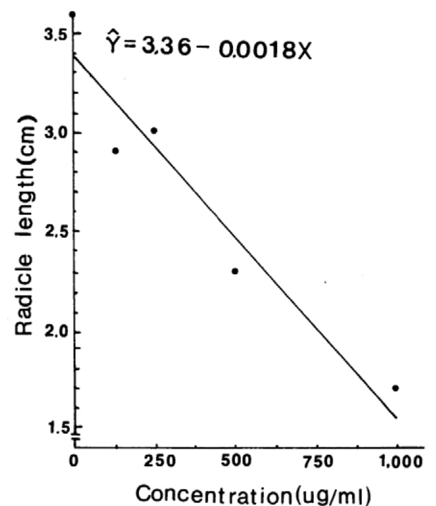

Fig. 2. Relationship between radicle length of soybean (cv. Amsoy 71) and concentration of extract from culture filtrate of *Chaetomium cupreum* after 30 hr in the dark at room temperature (25 ± 2 C).

Table 1. Colony diameters (cm) of fungi isolated from soybean and grown on 2% water agar containing different concentrations of a culture filtrate from *Chaetomium cupreum* after 7 days at 25 C

Fungus	Culture filtrate concentration (%)[x]					FLSD at 5%[y]
	0	5	10	15	20	
Alternaria sp.	4.3 bc[z]	4.2 bc	4.0 c	4.8 ab	5.0 a	0.54
Cercospora kikuchii	1.7 ab	2.0 a	1.7 a	1.5 b	1.7 b	0.28
C. sojina	1.7	1.6	1.7	1.7	1.6	ns
Colletotrichum dematium var. *truncata*	4.8 bc	5.2 a	4.9 b	4.6 c	4.7 c	0.32
Fusarium sp.	8.5 a	3.2 b	2.3 c	2.6 c	2.4 c	0.39
Gliocladium roseum	4.4	4.4	4.3	4.3	4.3	ns
Macrophomina phaseolina	8.5 a	7.5 b	7.0 b	7.1 b	2.1 c	0.66
Phomopsis sp.	6.3 a	6.6 b	5.8 c	4.3 d	3.0 e	0.29
Rhizoctonia solani	8.4 a	7.4 b	6.2 c	4.8 d	3.0 e	0.45

[x]Percent based on combined means of three experiments with four replicates per treatment.
[y]FLSD = Fisher least significant difference.
[z]Means followed by the same letter are not significantly different ($P = 0.05$).

Table 2. Colony diameters (cm) of fungi isolated from soybean and grown on 2% water agar containing various concentrations of an ether extract from *Chaetomium cupreum* after 7 days at 25 C

Fungus	Culture filtrate concentration (μg/ml)[x]					FLSD at 5%[y]
	0	125	250	500	1,000	
Alternaria sp.	4.8 a[z]	3.7 b	2.8 c	1.5 d	0.7 e	0.36
Cercospora kikuchii	2.3 ab	2.0 a	2.3 ab	1.8 b	1.1 c	0.58
C. sojina	2.6 a	2.0 b	1.5 c	1.0 c	0.7 d	0.51
Colletotrichum dematium var. *truncata*	4.8 a	4.5 b	3.8 c	3.6 d	3.0 e	0.13
Gliocladium roseum	4.0 a	3.4 b	2.6 c	2.7 c	2.3 c	0.42
Macrophomina phaseolina	8.2 a	7.4 b	6.6 c	3.8 d	1.5 d	0.30
Phomopsis sp.	7.1 a	6.5 b	5.3 c	3.6 d	1.5 d	0.48
Rhizoctonia solani	8.5 a	2.9 b	1.1 c	0.5 d	0.5 d	0.15

[x]Mean percent based on combined means of three experiments with three relicates per treatment per experiment.
[y]FLSD = Fisher least significant difference.
[z]Means followed by the same letter are not significantly different ($P = 0.05$).

replicated three times, and the study was done three times.

The extract also was mixed with distilled water to give the four concentrations and autoclaved. Then, 10 ml of the solution was poured into three sterile 5-cm diameter culture plates for each treatment. Distilled water was a control. Five germinating soybean seeds (cv. Amsoy 71) were placed in each plate. All plates were incubated in the dark. Radial growth was recorded after 24–36 hr at 25 ± 2 C. The study was done three times.

RESULTS AND DISCUSSION

In dual cultures, zones of inhibition developed only between *C. cupreum* and *Fusarium* sp., *M. phaseolina*, *Phomopsis* sp., and *R. solani* (Fig. 1D-G); the zones measured 0.9, 0.7, 1.5, and 1.4 cm, respectively. No zones of inhibition developed between *C. cupreum* and the other five fungi. The PIRG for each combination was 31.4, 48.7, 66.6, and 64.7%, respectively, suggesting that *C. cupreum* produced a compound toxic to these four seedborne fungi. *C. cupreum* may be useful for biologic control of these soybean pathogens.

The nine pathogens varied in sensitivity to culture filtrates of *C. cupreum* (Table 1). Inhibition of growth increased with increased concentration of the culture filtrate for all fungi except *Alternaria* sp., *Cercospora sojina*, and *G. roseum*. *Alternaria* sp. increased significantly in growth at the 15 and 20% concentrations of the culture filtrate; this growth stimulation could not be explained. When the culture filtrate was partially purified, concentrating the active ingredient, all nine test fungi were inhibited in growth, the inhibition increasing with increased concentration of the filtrate (Table 2). *Alternaria* sp., *Cercospora sojina*, and *G. roseum*, which were not sensitive to the crude culture filtrate, were sensitive to the concentrated, partially purified culture filtrate.

The toxic component in the culture filtrate of *C. cupreum* can be autoclaved. It may be composed of more than one compound because the maximum absorbance of the partially purified extract in water was detected at 230, 250, 280, and 290 nm in ultraviolet light and at 505 nm in visible light. The partially purified culture filtrate was toxic to soybean seedlings (Fig. 2). Inhibition of radicle growth increased with increased concentration of the partially purified culture filtrate, with almost complete inhibition at 1,000 μg/ml.

C. cupreum produces a compound that is toxic not only to certain seedborne fungi in soybeans but also to germinating seeds. If the fungitoxic compound is used in biologic control studies, a concentration must be selected that will inhibit the fungi but allow the seed to germinate and emerge, even though the process may be slowed.

ACKNOWLEDGMENTS

We thank E. Muller, Inst. Botansk, Switzerland, and J. L. Crane, Illinois State Natural History Survey, Urbana, for confirming the identification of *C. cupreum*, and E. G. Jordan, D. D. Mueller, and J. T. Yorinori for providing soybean seeds from which *C. cupreum* was isolated and/or cultures of the test fungi.

LITERATURE CITED

1. AMES, L. M. 1949. New cellulose destroying fungi isolated from military material and equipment. Mycologia 41:637-648.
2. HEPPERLY, P. R., and J. B. SINCLAIR. 1978. Quality losses in *Phomopsis*-infected soybean seeds. Phytopathology 68:1684-1687.
3. ROYSE, D. J., and S. M. RIES. 1978. The influence of fungi isolated from peach twigs on the pathogenicity of *Cytospora cincta*. Phytopathology 68:603-607.
4. SINCLAIR, J. B., and O. D. DHINGRA. 1975. An annotated bibliography of soybean diseases. Univ. Illinois Intern. Agric. Publ. Series No. 7, Urbana. 280 pp.
5. TERVET, I. W. 1945. The influence of fungi on storage, on seed viability and seedling vigor of soybeans. Phytopathology 35:3-15.