Role of Antibiotics Produced by *Chaetomium globosum* in Biocontrol of *Pythium ultimum*, a Causal Agent of Damping-Off

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We gratefully acknowledge H. Fritz, Ciba-Geigy AG, Basel, for performing NMR.

Accepted for publication 23 July 1991 (submitted for electronic processing).

ABSTRACT

Di Pietro, A., Gut-Rella, M., Pachlatko, J. P., and Schwinn, F. J. 1992. Role of antibiotics produced by *Chaetomium globosum* in biocontrol of *Pythium ultimum*, a causal agent of damping-off. Phytopathology 82:131-135.

Production of antifungal compounds by *Chaetomium globosum* and the role in suppression of Pythium damping-off of sugarbeet were evaluated. Two metabolites with antifungal activity against *Pythium ultimum*, 2-(buta-1,3-dienyl)-3-hydroxy-4-(penta-1,3-dienyl)-tetrahydrofuran (BHT) and the epidithiadiketopiperazine, chaetomin, were isolated from liquid cultures of *C. globosum*. BHT was produced in 1% malt extract medium by six of eight tested *C. globosum* strains, whereas chaetomin was produced in 1% corn steep medium by five of nine tested strains. The ability of the nine *C. globosum* strains to produce chaetomin in liquid culture was correlated with the efficacy to suppress Pythium damping-off of sugarbeet in heat-pasteurized soil. Moreover, chaetomin was extracted from pasteurized soil inoculated with *C. globosum* strain Cg-13, an effective biocontrol strain, but not from pasteurized soil inoculated with ADP-13, a spontaneous variant of Cg-13 unable to suppress Pythium damping-off. Chaetomin was a hundred times as inhibitory to mycelial growth of *P. ultimum* as BHT in a bioassay in microtiter plates. Activity of chaetomin was comparable to the fungicide metalaxyl and five to 10 times higher than that of the epidithiadiketopiperazine, gliotoxin. Results suggest that chaetomin production in soil plays an important role in antagonism of *C. globosum* against *P. ultimum*.

Additional keywords: antibiosis, mode of action, soilborne diseases.

Considerable losses are due to plant diseases caused by soilborne pathogenic fungi. In recent years, biological control of soilborne pathogens has received increasing attention as a promising supplement or alternative to chemical control. To improve efficacy of biological control, however, improved understanding of mechanisms of action, nutrition, and ecology of biocontrol agents is needed (7). Such knowledge will lead to substantial progress in selection of superior strains, mass production, and appropriate formulation of biocontrol organisms. The mode of action of fungal antagonism to soilborne plant pathogens has been elucidated conclusively only in a few cases. A major problem lies in the difficulty of proving that results obtained in vitro are relevant in the soil system (15).

Pythium ultimum Trow is involved in seed rots and preemergence damping-off of many plant species (14). The pathogen survives in soil as oospores and sporangia during intersubstrate periods (1,26). Propagules germinate rapidly in response to nutrient stimuli such as soluble or volatile exudates from germinating seeds (21). *P. ultimum* can colonize sugarbeet seeds within 12 h under favorable conditions (22). Biological control of seed rot and damping-off by *P. ultimum* in the greenhouse (11,23,30) and under field conditions (12,13) with different fungal antagonists has been described.

The saprophytic ascomycete, *Chaetomium globosum* Kunze:Fr., is a potential antagonist of several soilborne and seedborne plant pathogens (10,17,19,27,28,30). Several antagonistic mechanisms may play a role in disease suppression by *C. globosum*. In vitro hyphal coiling was observed in dual culture with *Rhizoctonia solani* (30) and *Alternaria brassicicola* (28), indicating potential mycoparasitism of *C. globosum* against these pathogens. No coiling was observed around hyphae of *P. ultimum* (17,28) although *C. globosum* effectively suppressed Pythium damping-off in the greenhouse (11,30). A nondiffusable antibiotic was recovered from pea seeds (*Pisum sativum*) treated with *C. globosum* ascospores (17). Control of *P. ultimum* therefore was attributed to antibiosis, but definitive evidence still is lacking. Previous studies on the relevance of the three potential modes of action (competition, mycoparasitism, and antibiosis) in the antagonism of *C. globosum* against *P. ultimum* showed no evidence of competition and mycoparasitism as mechanisms of antagonism (10). Therefore, the present study was conducted to determine the role of antibiotic production by *C. globosum* in suppression of damping-off of sugarbeet caused by *P. ultimum*.

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MATERIALS AND METHODS

Microorganisms. *C. globosum* strains Cg-1, Cg-3, Cg-13, Cg-14, Cg-20, Cg-29, Cg-40, and Cg-43 were obtained from D. Gindrat (Swiss Federal Agricultural Station, Changins, Switzerland). A spontaneous variant of strain Cg-13 was isolated during the early phases of the studies. This variant, showing enhanced production of aerial mycelium and reduced number of perithecia compared to the parental strain, was designated ADP-13. *P. ultimum* strain P-71 was obtained from Ciba-Geigy AG, Switzerland. All microorganisms were stored at −196 C (9).

Biological control of *P. ultimum*. Biocontrol activity of the *C. globosum* strains against damping-off of sugarbeet caused by *P. ultimum* was assayed in a soil from Stein, Switzerland. Soil characteristics were: pH 7.8, 55.6% sand, 20.1% silt, 24.2% clay, 2.4% organic matter. Heat-pasteurized Stein soil was used for some experiments. *P. ultimum* was grown in 500-ml Roux culture bottles for 14 days in 150 ml of liquid carrot medium (9). The contents of one bottle was added to an equal amount of tap water and blended in a blender (Turmix AG, Switzerland) for 60 s. Three-hundred milliliters of this liquid, containing mainly sporangia of *P. ultimum*, were mixed with 5 L of soil. Control soil was mixed with a corresponding amount of tap water. The soil was incubated in plastic bags for 3 days under the growth chamber conditions described below. Inoculum density of *P. ultimum* at the time of planting was 1,000–1,500 propagules per gram of soil as determined by the soil dilution plating method (25). *C. globosum* was applied either as ascospores coated onto seeds or as wheat bran inoculum. Ascospores of *C. globosum* were produced as described previously (30). Thirty milligrams of asco-

spore powder was suspended in 400 µl of 20% Vinamul (Vinyl Products, U.K.) and transferred into a small plastic bag. Five grams of sugar beet seed (*Beta vulgaris* L., 'KW,' monogerm) was added, and the closed bag was shaken vigorously to assure an even distribution of the ascospores on the seed surface, providing 1.5×10^5 spores per seed. Seeds were subsequently dried overnight under a continous air flow. Ascospore density on seed coats was determined by separately vortexing five seeds in 5 ml of sterile water for 10 min and plating serial dilutions on 1% malt extract agar.

Wheat bran inoculum of *C. globosum* was prepared as follows: 50 g of wheat bran and 130 ml of distilled water were mixed in autoclavable plastic bags and autoclaved for 30 min. The bran was inoculated with an ascospore suspension of *C. globosum* (10^6 spores per bag). The bran was mixed, and the closed bag was incubated at 25 C for 7 days. Wheat bran inoculum was added to the soil at a ratio of 2%, w/v (10^4–10^5 cfu/g fresh weight of soil) immediately before planting. Noninoculated bran (2%, w/v) was added to soil as a control.

As a chemical control standard, metalaxyl (Ciba-Geigy AG, Switzerland) was applied to soil mixed with noninoculated bran, as a soil drench at the time of planting, providing a final concentration of 10 µg a.i. per gram of soil. Rectangular plastic pots (100 × 120 × 45 mm) were filled with soil, and 15 sugar beet seeds were planted in each pot (five replicate pots per treatment). Pots were incubated in a growth chamber at 14 h light, 23 C, 65% RH; 10 h dark, 18 C, 75% RH. Pots were watered as needed to maintain a moist soil surface. After 20 days, the number of healthy plants in each pot was recorded.

Assay for production of inhibitory metabolites by *C. globosum*. One hundred milliliters of 1% (w/v) malt extract broth (Oxoid Ltd., Basingstoke, U.K.) was autoclaved in 500-ml Erlenmeyer flasks, and three agar plugs from a colony of *C. globosum* on 1% malt extract agar were transferred to each flask. No fungus was added to control flasks. The flasks were incubated on a rotary shaker at 175 rpm for 12 days at 10, 15, 20, 25, or 30 C. After incubation, cultures were centrifuged at 15,000 *g* and 4 C for 10 min. The supernatant was sterilized through a syringe filter (X-60, 0.45 µm; Gelman Sciences, Ann Arbor, MI) and added at a ratio of 1:4 (v/v) to a malt extract agar medium that had been autoclaved and cooled to 60 C, providing a final concentration of 0.5% malt extract and 1.5% agar. The medium was poured into petri dishes, and an agar plug from a 4-day-old culture of *P. ultimum* on 1% malt extract agar was placed in the center of each petri dish (three plates per treatment and test organism). Dishes were incubated at 20 C , and the colony radius was measured daily. Growth rates between 1 and 2 days after inoculation were calculated. Growth inhibition was expressed as percentage compared to control (0%).

Extraction of fungitoxic metabolites from liquid cultures. *C. globosum* was grown in 500-ml Erlenmeyer flasks containing 100 ml of the following growth media: 1% malt extract broth; 1% corn steep powder broth (Ciba-Geigy AG, Switzerland); or 0.5% malt extract + 1% corn steep powder broth. Three agar plugs from a colony of *C. globosum* on 1% malt extract agar plates were transferred to each flask. Controls consisted of medium without the fungus. The flasks were incubated at 25 C and 175 rpm for 12 days. Cultures were filtered through two layers of filter paper (MN 713, Macherey-Nagel, FRG), and the filtrates were extracted twice with equal volumes of ethyl acetate. The solvent was evaporated on a rotary evaporator, and the solid extracts were dissolved in 1 ml of ethyl acetate.

Extraction of fungitoxic metabolites from soil. Wheat bran inoculum of *C. globosum* strains Cg13 and ADP-13 was prepared as described above and added to 500 ml of pasteurized Stein soil at a ratio of 5% (w/v). Control soil was mixed with 5% noninoculated wheat bran. The soil samples were incubated in sealed sterile bottles for 6 days at 20 C. After the incubation period, the soil samples were extracted twice with equal volumes of ethyl acetate. The extracts were filtered through two layers of filter paper (MN 713; Macherey-Nagel, FRG), and the solvent was removed on a rotary evaporator. The solid extracts were dissolved in 1.5 ml of ethyl acetate.

Thin-layer chromatography (TLC) bioassay with *P. ultimum*. Different quantities of the extracts from *C. globosum* liquid cultures or from soil were applied to silica gel plates for TLC (60 F_{254}; Merck AG, FRG). As a reference, 100 µg of pure chaetomin dissolved in ethyl acetate were also applied to the plates. The plates were developed in methylene chloride/methanol (95:5, v/v). For additional separation of the metabolites, the plates were subsequently developed in hexane/ethyl acetate (3:2, v/v). For the antibiosis-bioassay, *P. ultimum* was grown in carrot medium. The mycelial mat was transferred to a sterile blender (Sorvall Inc., Newtown, CT). Sterile V8 broth (200 ml of V8 juice, 3 g of $CaCO_3$, 5 g of glucose, and 800 ml of distilled water) was added, providing 100 ml of solution per 15 g of mycelium. To prevent bacterial growth, ampicillin (Sigma Chemicals, St. Louis, MO) was added at 500 mg a.i. per liter of medium. The mixture was blended for 2 min at maximum speed and transferred to a sterile spraying bottle. The developed TLC-plates were sprayed with the *P. ultimum* suspension and incubated in a plastic box with moist filter paper for 48 h at 20 C. To visualize the zones of growth inhibition, the plates were dipped in an aqueous 4% carbon powder suspension. Carbon adsorbed to the mycelium, whereas the inhibition zones remained clear.

Purification and identification of fungitoxic metabolites. Extracts of liquid cultures or of soil colonized by *C. globosum* were applied to silica gel plates, and the plates were developed as described above. The inhibitory metabolites were visible as quenched spots under UV-light (254 nm), so this method of localization was used for the further purification steps. Zones containing the relevant metabolites were scraped from the plates with a spatula and eluted three times with ethyl acetate. The solvent was evaporated on a rotary evaporator. Metabolites were further purified by recrystallization in hexane. The identification of the fungitoxic metabolites was carried out with nuclear magnetic resonance analysis and mass spectroscopy.

Effect of *C. globosum* metabolites on mycelial growth of *P. ultimum*. A bioassay in microtiter plates (20) was used to test the inhibition of hyphal growth of *P. ultimum* by the purified metabolites from *C. globosum*. Sporangia of *P. ultimum* strain P-71 were prepared as described previously (24). The sporangia were suspended in cleared V8 medium at 2.5×10^3 sporangia per milliliter. Aliquots (90 µl) of the suspension were pipetted into wells of sterile microtiter plates (96-well-plates, A/S Nunc, Denmark), and the plates were incubated at 20 C for 7 h. After incubation, at least 95% of the sporangia had germinated, as determined by microscopical observation. The metabolites of *C. globosum* were not readily soluble in water, so 2 mg of the determined metabolite was dissolved in a minimal volume of ethyl acetate, and 150 µl of PEG 200 (Fluka AG, Switzerland) was added. Then the ethyl acetate was evaporated on a rotary evaporator. Sterile distilled water (2 ml) was added, and the sample was sonicated for 5 min to provide an aqueous dispersion containing 1 mg a.i. of metabolite per milliliter and 7.5% PEG 200. The dispersion was diluted to different concentrations, and 10-µl aliquots were added to the sporangial suspension in the wells to provide the desired end concentration of metabolite. Metabolites were tested at concentrations of 0.1, 0.25, 0.5, 0.75, 1, 2.5, 5, 7.5, 10, and 50 mg a.i./L. As a blank control, a comparable quantity of PEG was added to the sporangial suspension.

Gliotoxin from *Gliocladium fimbriatum* (Sigma) and metalaxyl WP 25 (Ciba-Geigy AG, Switzerland) were included as toxicity standards. Five wells with *P. ultimum* and two wells with cleared V8 medium (blanks) were used for each treatment. Microtiter plates were incubated at 20 C in a plastic box with moist filter paper. Mycelial growth of *P. ultimum* was determined quantitatively by measuring the optical density in the wells after 0, 12, 18, and 36 h of incubation (20). Optical density was measured in an ELISA-plate reader (Titertrek Multiskan plus MK 11, Skan Laboratories, Switzerland) with a 578-nm filter. Values of EC_{50} for mycelial growth were determined by calculating the growth

rates between the measurements at 18 and 36 h after the start of the incubation. The minimal concentration with which no measurable growth occurred was designated as minimum inhibitory concentration (MIC) value.

Analysis of data. All experiments were conducted at least twice. Growth chamber experiments were arranged in a randomized complete block design. Data were subjected to analysis of variance, and standard errors were calculated. Data from the experiments on the effect of different compounds on mycelial growth of *P. ultimum* were subjected to regression analysis to determine EC_{50} values.

RESULTS

Efficacy of *C. globosum* strains in reducing Pythium damping-off. The efficacy of three *C. globosum* strains in suppressing damping-off of sugar beet caused by *P. ultimum* was determined. Experiments 1 and 2 were conducted in pasteurized soil, whereas experiments 3 and 4 were conducted in natural soil. In both experiments Cg-13 was the only effective strain in reducing Pythium damping-off (Table 1). However, whereas Cg-13 applied as wheat bran inoculum was comparable to metalaxyl in pasteurized soil, the efficacy of Cg-13 was considerably less than that of metalaxyl in the natural soil. Applied as wheat bran inoculum, Cg-43 was considerably less effective than Cg-13, and the variant ADP-13 was completely ineffective. Ascospore seed coating was less effective than wheat bran inoculum. Only strain Cg-13 gave some protection against Pythium damping-off. Cg-43 and ADP-13 had no beneficial effect.

Inhibition of *P. ultimum* by culture filtrates of *C. globosum*. The inhibition of mycelial growth of *P. ultimum* by culture filtrates of *C. globosum* varied with the strain and growth temperature of *C. globosum* (Fig. 1). Culture filtrates of strain Cg-13 from all growth temperatures were inhibitory to *P. ultimum*. Filtrates of the variant ADP-13 caused only very low inhibition. With Cg-43, maximum inhibitory activity was detected in filtrates from 30 C. Production of inhibitory compounds decreased at lower growth temperatures, and no inhibitory activity was found in the filtrate from 10 C.

Isolation of fungitoxic metabolites by TLC. A TLC-bioassay of extracts of *C. globosum* cultures grown in 1% malt extract broth revealed the presence of a metabolite toxic to *P. ultimum*. The metabolite was purified and identified by nuclear magnetic resonance and mass spectroscopy as 2-(buta-1,3-dienyl)-3-hydroxy-4-(penta-1,3-dienyl)-tetrahydrofuran (BHT; Fig. 2). BHT was produced at detectable quantities by six of eight tested strains of *C. globosum* in 1% malt extract broth (Table 2). The variant ADP-13 and strain Cg-43 did not produce BHT. However, a different, yet unidentified metabolite inhibitory to *P. ultimum* was extracted from the 1% malt extract culture of Cg-43.

Another fungitoxic metabolite was isolated from the *C. globosum* cultures in 1% corn steep powder broth. This metabolite was produced in addition to BHT when Cg-13 was grown in a medium containing 0.5% malt extract + 1% corn steep powder broth. The metabolite was identified as chaetomin (Fig. 2) by nuclear magnetic resonance analysis and mass spectroscopy. Five of nine tested strains of *C. globosum* produced chaetomin in 1% corn steep powder broth (Table 2).

Cg-13, but not the spontaneous variant ADP-13, produced a fungitoxic metabolite in heat-pasteurized soil when added as wheat bran inoculum at a ratio of 5% (w/v) and incubated in the soil for 6 days at 20 C. The metabolite was purified from the soil extract and identified as chaetomin by nuclear magnetic resonance analysis and mass spectroscopy. BHT was not detected in the soil extracts.

Efficacy of chaetomin-producing and nonproducing *C. globosum* strains against Pythium damping-off of sugarbeet. To test the hypothesis that the metabolite chaetomin plays a role in reduction of Pythium damping-off, nine *C. globosum* strains were evaluated for their efficacy in reducing damping-off of sugarbeet in pasteurized soil inoculated with *P. ultimum*. Four of the nine strains did not produce chaetomin at detectable quantities in liquid culture (see Table 2). Results are shown in Figure 3. The most effective *C. globosum* strain Cg-1 gave 50% reduction of Pythium damping-off. The four strains that did not produce chaetomin (i.e., ADP-13, Cg-40, Cg-43, and Cg-3 [marked with asterisks]) were less effective in reducing damping-off than the chaetomin-producing strains. However, ADP-13 was the only completely ineffective strain.

Effect of *C. globosum* metabolites on mycelial growth of *P. ultimum*. A bioassay in microtiter plates was used to determine inhibition of mycelial growth of *P. ultimum* by different compounds. Results are shown in Table 3. BHT had a low inhibitory activity against *P. ultimum*. The EC_{50} and MIC values for inhibition of mycelial growth were higher than 50 mg a.i./L. Conversely, the inhibitory activity of chaetomin against *P. ultimum* on a weight basis was in the same range as that of the fungicide metalaxyl, and five to 10 times higher than that of gliotoxin (Table 3).

DISCUSSION

A role of antibiosis in the antagonistic activity of *C. globosum* against various plant pathogens has been suggested (8,10,17,27,30). The present work provides further evidence for production of antibiotics in soil as an important mechanism in the antagonism of *C. globosum* against *P. ultimum*. *C. globosum* strain Cg-13, which effectively suppressed Pythium damping-off of sugarbeet in the growth chamber, produced at least two metabolites inhibitory to *P. ultimum* in liquid culture, BHT, and chaetomin. The metabolites also were produced by several other *C. globosum* strains. Chaetomin was extracted from soil to which Cg-13 had been added previously. Moreover, ADP-13, a spontaneous variant of strain Cg-13 that failed to produce the fungitoxic metabolites

TABLE 1. Effect of different *Chaetomium globosum* strains on damping-off of sugar beef caused by *Pythium ultimum*

Treatment	Number of healthy plants[a,b]			
	Experiments 1 + 2[c]		Experiments 3 + 4[d]	
Noninfested control	12.4 ± 0.8	12.8 ± 1.0	9.8 ± 0.7	10.6 ± 0.8
Pythium-infested control	0.2 ± 0.2	0.4 ± 0.2	0.2 ± 0.2	0
Metalaxyl 10 mg a.i./L soil	9.8 ± 0.9	12.0 ± 0.8	10.2 ± 1.1	11.4 ± 0.8
Cg-13 WBI[e]	8.1 ± 1.1	10.4 ± 0.9	2.8 ± 1.2	4.1 ± 1.0
Cg-13 seed coating[f]	3.0 ± 1.1	4.1 ± 1.0	2.0 ± 0.9	2.8 ± 1.1
ADP-13 WBI	0.4 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2
ADP-13 seed coating	0.6 ± 0.2	0.4 ± 0.2	0	0.2 ± 0.2
Cg-43 WBI	1.8 ± 0.9	2.8 ± 0.8	1.8 ± 0.8	2.2 ± 1.1
Cg-43 seed coating	0.4 ± 0.2	0.6 ± 0.2	0.2 ± 0.2	0.4 ± 0.2

[a] Values are the means of five replicates, 15 seeds per replicate. Healthy plant stand was determined two weeks after planting.
[b] Values followed by standard error.
[c] Experiments conducted in heat-pasteurized soil.
[d] Experiments conducted in natural soil.
[e] Wheat bran inoculum of *C. globosum* strains was added to the soil at a ratio of 2% (w/v).
[f] Seeds were coated with ascospores of *C. globosum* (1.5×10^5 spores per seed)

in liquid culture and in soil, was ineffective in suppressing Pythium damping-off of sugarbeet. Previous studies had shown no significant differences between ADP-13 and Cg-13 in growth rates, spore germination rates, or root colonization ability (10). Finally, the *C. globosum* strains that produced chaetomin in liquid culture were more effective in reducing Pythium damping-off than the nonproducing strains.

Production of antibiotics by *C. globosum* is well documented. Isolates of different *Chaetomium* spp. from soil produced several antibiotic compounds, including chaetomin, in liquid culture (5). In the present study chaetomin and BHT were isolated from liquid culture of *C. globosum*. BHT first was isolated from culture broth of *Chaetomium coarctatum* (6). No antibiotic properties of this compound have yet been described. A different unidentified fungitoxic metabolite was produced by strain Cg-43 in malt extract medium. This metabolite apparently accounts for the inhibitory effect of the Cg-43 culture filtrates. The epidithiadiketopiperazine chaetomin was described earlier (29). In the present study, production of the two metabolites in liquid culture was strongly dependent on the nutritional composition of the growth medium. BHT was produced in malt extract medium, whereas production of chaetomin was dependent on corn steep powder medium. Corn steep liquor has been described previously as particularly effective in enhancing chaetomin production (4).

Our results suggest that chaetomin is the only one of the two metabolites that is important in biocontrol of *P. ultimum*. First, chaetomin was approximately 100 times as inhibitory to *P. ultimum* as BHT in a microtiter plate bioassay. Second, chaetomin, but not BHT, was extracted in detectable quantities from pasteurized soil colonized by *C. globosum*. The present study provides the first report on the production of chaetomin by *C. globosum* in soil. The chemically related antibiotic gliotoxin has been iso-

TABLE 2. Production of chaetomin and 2-(buta-1,3-dienyl)-3-hydroxy-4-(penta-1,3-dienyl)-tetrahydrofuran (BHT) by different strains of *Chaetomium globosum*

C. globosum strain	Chaetomin[a]	BHT[b]
Cg-1	+[c]	+
Cg-3	−	+
Cg-13	+	+
APD-13	−	−
Cg-14	+	+
Cg-20	+	+
Cg-29	+	+
Cg-40	−	ND[d]
Cg-43	−	−

[a] *C. globosum* strains were grown in liquid culture medium containing 1% corn steep powder.
[b] *C. globosum* strains were grown in liquid culture medium containing 1% malt extract.
[c] Presence of fungitoxic metabolites was determined by a thin-layer chromatography bioassay with *Pythium ultimum*. Lowest detectable quantities were 5 μg for chaetomin and 20 μg for BHT.
[d] Not determined.

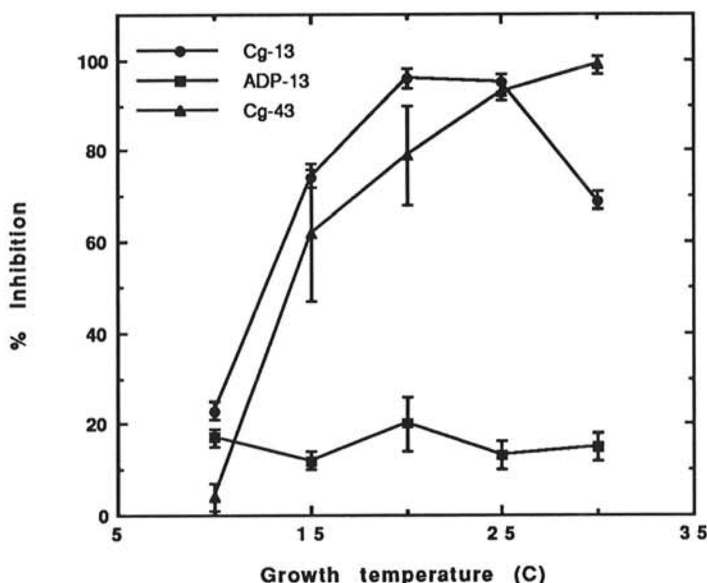

Fig. 1. Inhibition of mycelial growth of *Pythium ultimum* by culture filtrates of *Chaetomium globosum* strains Cg-13, ADP-13, and Cg-43 grown in 1% malt extract broth at different temperatures. Culture filtrates were incorporated in malt extract agar. Each point represents the means of three replicate plates from each of three experiments. Error bars represent standard errors.

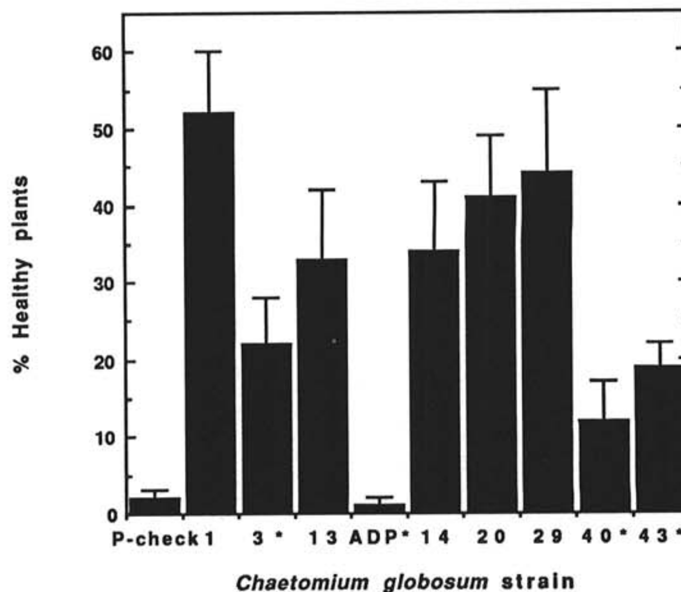

Fig. 3. Effect of chaetomin-producing and nonproducing *Chaetomium globosum* strains on Pythium damping-off of sugarbeet. *C. globosum* strains marked with an asterisk did not produce chaetomin in 1% corn steep powder broth. Wheat bran inoculum of the *C. globosum* was added to heat-pasteurized soil at a ratio of 2% (w/v). Values are the means of five replicates, 15 seeds per replicate, from each of three growth chamber experiments. Plant stands were determined 2 wk after planting. Data were expressed as a percentage of the noninfested control. Error bars represent standard errors.

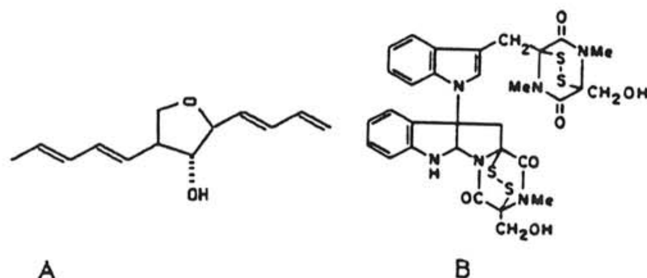

Fig. 2. Structure of **A**, 2-(buta-1,3-dienyl)-3-hydroxy-4-(penta-1,3-dienyl)-tetrahydrofuran (BHT) and **B**, chaetomin.

TABLE 3. Inhibition of mycelial growth of *Pythium ultimum* by different compounds

Compound	EC_{50}	MIC[a]
Chaetomin	0.5[b]	2.5
BHT[c]	50.0	>50.0
Gliotoxin	7.5	10.0
Metalaxyl	0.25	1.0

[a] Minimum inhibitory concentration.
[b] Values are expressed in mg a.i./L
[c] 2-(Buta-1,3-dienyl)-3-hydroxy-4-(penta-1,3-dienyl)-tetrahydrofuran.

lated from the coats of pea seeds in soil colonized by a strain of *Trichoderma viride* (31). Furthermore, the extraction of a nondiffusable antibiotic from squash seeds (*Cucurbita pepo*) coated with *C. globosum* ascospores has been described (17). The nondiffusable antibiotic inhibited growth of many soil-inhabiting fungi. Our results suggest that the compound isolated by these authors (17) was probably chaetomin.

The toxicity of chaetomin to fungi, bacteria, and mammals (3,4) has been described. A possible role of this metabolite in the antagonism of *C. globosum* to the apple scab pathogen *Venturia inaequalis* has been proposed (2,8). Great sensitivity of *P. ultimum* to gliotoxin has been reported as well (18,24). A rapid uptake of the toxin by *P. ultimum* was observed (18). Sporangial germination and mycelial growth of *P. ultimum* were strongly inhibited by the gliotoxin-containing culture supernatant from *Gliocladium virens* (24). In a similarly designed assay described here, chaetomin was five to 10 times as inhibitory to mycelial growth of *P. ultimum* as gliotoxin. Inhibitory activity of chaetomin was comparable to that of metalaxyl. Thus, even low levels of chaetomin in the soil may cause considerable inhibition or at least a delay of sporangial germination and hyphal growth of *P. ultimum*, leading to suppression of damping-off. Substantial colonization of the seed pericarps of sugarbeet by *P. ultimum* in soil occurs within 8–12 h under favorable conditions, and the severity of damping-off is related directly to the incidence of pericarp colonization (22). Therefore, a temporary inhibition of *P. ultimum* growth may be sufficient for disease suppression.

The positive correlation between production of chaetomin by *C. globosum* strains in liquid culture or soil, and the efficacy in suppressing Pythium damping-off in the growth chamber, provides further evidence for the importance of this metabolite in antagonism of *C. globosum* against *P. ultimum*. In a similar case, a mutant of *G. virens* that did not produce the fungitoxic metabolite gliovirin was obtained by UV-mutagenesis (16). The mutant also failed to protect cotton (*Gossypium hirsutum*) from Pythium damping-off. In the present study, the chaetomin-producing *C. globosum* strains were superior to the nonproducing strains in biocontrol efficacy. However, except for the variant strain ADP-13, all the nonproducing strains still provided some degree of protection against Pythium damping-off. This may be due to the production of other fungitoxic compounds, such as the unidentified metabolite isolated from strain Cg-43. Furthermore, additional mechanisms such as competition and mycoparasitism may also play a role in suppression of Pythium damping-off by *C. globosum*, although previous studies gave no evidence for such mechanisms (10,17,30). Finally, resistance of the host plant induced by the antagonist may be another potential mechanism, but has not been investigated in this system.

LITERATURE CITED

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