Effect of a Water-Soluble Guar Root Extract on Spore Germination and Mycelial Growth of Various Fungi

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ABSTRACT

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A water extract from the bark of guar roots inhibited germination of conidia and growth of mycelia of several plant pathogenic fungi. Germination of conidia and growth of mycelia of other test fungi were either stimulated or not affected by the extract. Fewer sclerotia were produced by *Phymatotrichum omnivorum* in soil with the extract than in soil without the extract.

Additional key words: antifungal substance, *Cyamopsis tetragonoloba,* inhibition

Field observations indicated that wheat (*Triticum aestivum* L.) double cropped with guar (*Cyamopsis tetragonoloba* (L.) Taub) is less severely infected by common root rot than is wheat double cropped with other crops or wheat not rotated with another crop (2). In addition, water extracts from the bark of guar roots inhibit germination of conidia of *Bipolaris sorokiniana* (Sacc. in Sorokin) Shoem. and decrease the severity of infection by *B. sorokiniana* in crowns and subcrown internodes of wheat grown in environmental chambers (2).

This study was undertaken to determine the effect of the extract from the bark of guar roots on the germination of conidia and growth of mycelia of several other fungi.

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MATERIALS AND METHODS

The following fungi were used in this study: *B. sorokiniana* isolated from the subcrown internode of wheat; *Cladosporium* sp. Link ex Fr. isolated in the laboratory; *Colletotrichum graminicola* (Ces.) G. W. Wils isolated from sorghum, *Sorghum bicolor* (L.) Moench; a second culture of *C. graminicola* isolated from oats, *Avena sativa* L.; *C. trifolii* Bain isolated from alfalfa, *Medicago sativa* L.; *Drechslera avenacea* (Curt. ex Cke.) Shoem., isolated from oat; *Drechslera turcica* (Pass.) Subram & Jain isolated from sorghum; *Fusarium moniliforme* Sheldon and *F. semitectum* Berk & Rav. both isolated from sorghum; *F. oxysporum* Schlect. f. sp. *vasinfectum* (Atk.) Snyd. & Hans isolated from cotton, *Gossypium hirsutum* L.; *Neocosmospora vasinfecta* E. F. Sm. isolated from peanut, *Arachis hypogaea* L.; *Phymatotrichum omnivorum* (Shear) Dug. isolated from cotton; *Puccinia recondita* Rob. ex Desm. f. sp. *tritici* collected from wheat; *Rhizoctonia solani* Kuehn isolated from peanut; *Sclerotium rolfsii* Sacc. isolated from guar; *Septoria tritici* Rob. ex Desm. isolated from wheat; *Ustilago tritici* (Pers.) Rostr. collected from wheat; and *Verticillium dahliae* Kleb. isolated from cotton.

Guar plants were collected from field plots, and the bark was stripped from the roots. Bark was air dried and then ground in a Wiley mill using a 20-mesh screen. Extracts of the bark were prepared from 35 g of ground bark stirred in 200 ml of methanol and then in 200 ml of acetone for 30 min. The mixture was passed through four layers of cheesecloth, and the methanol and acetone insoluble bark material was spread on a sheet of aluminum foil to air dry. This dried bark material was blended in 420 ml of water at low speed for 5 sec and then stirred for 1 hr. The mixture was drained and then squeezed through four layers of cheesecloth. The liquid portion was transferred to a beaker and placed in the refrigerator at 5 C for about 16 hr to allow sedimentation to occur. The upper solution was then pipetted from the beaker and flash evaporated to near dryness at 45 C; it was further dried and

Table 1. Spore germination and germ tube growth of fungi exposed to two concentrations of guar root extract[a]

	Spore germination (%)			Germ tube length (μm)		
	mg extract/ml H_2O					
Fungus	**0**	**7.5**	**15**	**0**	**7.5**	**15**
Bipolaris sorokiniana	42–60	0.5–1	0	306–510	50–61	···
Colletotrichum graminicola[b]	44–56	0.1–0.5	0	50–300	35–40	···
Colletotrichum graminicola[c]	49–61	0	0	50–153	···	···
Colletotrichum trifolii	71–85	2–5	0	20–55	16–30	···
Drechslera avenacea	75–89	0.5–1	0	102–490	41–51	···
Fusarium oxysporum f. sp. *vasinfectum*	6–10	80–90	80–90	15–26	51–82	46–61
Fusarium semitectum	3–6	90–96	90–96	20–31	41–92	41–51
Puccinia recondita f. sp. *tritici*[d]	90–95	25–53	16–28	204–660	41–428	41–153
Septoria tritici	50–65	4–10	0	51–92	30–41	···
Ustilago tritici[d]	90–96	90–96	90–96	12–38	10–38	8–30
Verticillium dahliae	48–62	70–85	70–85	30–71	26–31	20–31

[a] Range of two tests of two replicates per test.
[b] Isolate from oats.
[c] Isolate from sorghum.
[d] Spores cast on potato-dextrose agar with extract incorporated in agar. All other fungi assayed on glass slides in moist chambers.

Table 2. Growth rates of fungal colonies on potato-dextrose agar amended with guar or cotton root extract[x]

Fungus	Incubation time (days)	Mean radial growth rate (mm/day) of colonies								
		Guar extract					Cotton extract			
		mg extract/ml H_2O in medium								
		0	2.5	5	10	15	2.5	5	10	15
Bipolaris sorokiniana	5	4.8 a	1.7 e	1.3 f	1.3 f	1.4 f	4.9 b	4.3 b	3.8 c	3.4 d
Cladosporium sp.	7	1.5 a	1.5 a	1.2 b	1.0 bc	0.9 c	...	...	...	...
Colletotrichum graminicola[y]	4	4.9 a	1.3 f	0.8 g	0.8 g	0.9 g	4.5 b	4.2 c	3.7 d	3.3 e
Colletotrichum graminicola[z]	4	5.0 a	1.5 b	1.6 b	1.4 b	1.3 b	...	...	...	...
Colletotrichum trifolii	4	3.4 b	1.2 c	0.9 d	0.8 d	0.7 d	3.5 ab	3.7 a	3.7 a	...
Drechslera avenacea	4	5.3 a	1.7 c	1.2 c	1.6 c	2.0 c	3.5 b	3.7 b	4.0 b	...
Drechslera turcica	5	2.3 c	1.4 d	1.5 d	1.6 d	1.6 d	2.6 bc	3.1 a	3.0 a	2.9 ab
Fusarium oxysporum f. sp. *vasinfectum*	5	3.9 c	4.8 a	4.1 bc	4.6 ab	4.4 abc	...	...	...	...
Fusarium moniliforme	5	4.8 a	2.5 bc	2.7 b	2.6 bc	2.4 c	5.0 a	5.0 a	4.9 a	4.8 a
Fusarium semitectum	5	5.0 a	2.9 b	2.5 c	1.9 d	2.5 c	...	...	...	...
Neocosmospora vasinfecta	7	2.2 a	2.1 a	1.8 b	1.6 bc	1.5 c	...	...	...	...
Phymatotrichum omnivorum	7	3.8 a	1.0 d	0.5 e	0 f	0 f	3.4 ab	3.3 b	2.8 c	...
Rhizoctonia solani	3	8.3 a	4.7 b	2.8 c	2.6 c	2.4 c	8.3 a	8.2 a	8.3 a	...
Sclerotium rolfsii	4	5.9 a	2.3 b	2.3 b	2.9 b	3.0 b	5.8 a	5.7 a	6.2 a	...
Verticillium dahliae	7	1.1 b	1.1 b	0.9 c	0.7 d	0.7 d	1.6 a	1.7 a	1.6 a	1.2 b

[x] Mean of three replicates. Values in the same horizontal row not followed by the same letter are significantly different ($P = 0.05$) as determined by Duncan's multiple range test.
[y] Isolate from oats.
[z] Isolate from sorghum.

stored in a desiccator at 22–25 C.

Concentrations of 7.5 and 15 mg of guar extract/ml of water were used for germination assays of conidia on glass slides (2). Germination of conidia was also determined after inoculation of spores on Difco potato-dextrose agar (PDA) in 3.5 × 1 cm petri dishes with the extract incorporated into the medium at 7.5 and 15 mg/ml of water.

The effect of different concentrations of guar root extract on the radial growth of fungi was determined on PDA amended with extract at levels of 0, 2.5, 5, 10, and 15 mg/ml of water. The inhibitory effect of guar root extract was compared with that of an extract from the bark of cotton roots prepared in the same manner and at the same concentrations. Three 6 × 1.5 cm petri dishes with each extract and concentration were centrally inoculated with either a 2 × 2 mm mycelial plug from a culture of each test fungus or a sclerotium, as was the case with *S. rolfsii*.

The effect of the extract from guar roots on the formation of sclerotia by *P. omnivorum* was tested in soil. Houston black clay (150 g, dry weight) was mixed with 100 ml of water in 250-ml flasks. Sorghum seed (5 g) was placed on the surface of the mixture, and the mixture was autoclaved 30 min. Then 0, 1.25, 2.5, 5, and 10 mg of guar root bark extract per gram of soil (in 5 ml of water) were added to flasks and autoclaved 20 min. Three flasks per concentration were prepared. Each flask was then inoculated with a 3 × 3 mm mycelial plug of *P. omnivorum*. The fungus was incubated at 22–25 C for 48 days and then sclerotia were recovered from the soil by washing soil through a 1.00-mm sieve. The sclerotia were then dried and weighed.

The radial growth of *B. sorokiniana, C. trifolii,* and *P. omnivorum* was further tested on PDA with 0, 0.06, 0.13, 0.25, 0.50, 1.00, and 2.00 mg of guar root extract per milliliter water in the medium. A 2×2 mm mycelial plug removed from a culture of each of the three test fungi was used to centrally inoculate agar amended with each of the extract concentrations in 6 × 1.5 cm petri dishes. This test was replicated three times.

Table 3. Growth rates of three fungi on potato-dextrose agar amended with guar root extract[z]

Extract (mg/ml H_2O)	Mean radial growth rate (mm/day) of colonies		
	Bipolaris sorokiniana	*Colletotrichum trifolii*	*Phymatotrichum omnivorum*
0	4.2 a	3.2 a	3.6 a
0.06	3.9 ab	3.0 ab	3.3 a
0.13	3.6 ab	3.0 ab	3.4 a
0.25	3.2 b	2.8 b	3.1 a
0.50	3.0 b	2.8 b	3.3 a
1.00	1.4 c	2.0 c	2.2 b
2.00	1.3 c	1.5 d	1.3 c

[z] Mean of three replicates. Values in the same column not followed by the same letter are significantly different ($P = 0.05$) as determined by Duncan's multiple range test.

Cultures were incubated in the laboratory at 22–25 C. Colony diameters were measured daily and growth rates computed. Data were evaluated by analysis of variance and Duncan's multiple range test.

RESULTS

Germination of conidia of *B. sorokiniana, C. graminicola, C. trifolii, D. avenacea,* and *S. tritici* was less after exposure to guar root extract than germination of conidia in water on glass slides. Germination of conidia by *F. oxysporum* f. sp. *vasinfectum, F. semitectum, P. recondita* f. sp. *tritici, U. tritici,* and *V. dahliae* in contact with guar root extract was either not greatly changed or was increased, compared with conidia not exposed to the guar extract (Table 1).

Germination of conidia of *V. dahliae* was increased, but germ tubes of conidia were shorter in the guar extract than those of conidia incubated in water (Table 1).

The radial growth of most of the fungi was less when grown on the medium with 2.5 mg of guar extract per milliliter of water than on the medium without the extract (Table 2). However, the growth of *Cladosporium* sp., *F. oxysporum* f. sp. *vasinfectum, N. vasinfecta,* and *V. dahliae* was either not changed or was greater on the medium with 2.5 mg of guar extract than on the medium with no extract. The growth rates of all the fungi except *F. oxysporum* f. sp. *vasinfectum* were less at concentrations of 5, 10, and 15 mg of guar extract in the medium than on the medium without the guar extract (Table 2).

There were no apparent differences in the growth rates of fungal colonies on the PDA medium containing cotton root extract and the unamended PDA medium (Table 2).

Radial growth rates of *B. sorokiniana, C. trifolii,* and *P. omnivorum* on agar with 1–2 mg extract per milliliter of water in the medium were half the rate of the

respective fungi growing on the medium without the extract (Table 3).

The dried weights of sclerotia of *P. omnivorum* produced in soil at concentrations of extract at 0, 1.25, 2.5, 5, and 10 mg/g of soil were 1.4, 0.6, 0.7, 0.2, and 0.1 g, respectively. The weight of sclerotia from the soil treatments with the guar extract was significantly lower ($P < 0.05$) than the weight of sclerotia from the untreated soil.

DISCUSSION

The bark of guar roots contained an antifungal substance(s) that inhibited germination of conidia and growth of mycelia of several plant pathogenic fungi. The concentrations of the guar extract needed to inhibit spore germination or mycelial growth may have been lower than that used in this study if the antifungal substance(s) had been separated from other water-soluble materials in the bark of guar roots. Higher levels of the guar root extract appeared to be required to inhibit mycelial growth of *P. omnivorum* than that of *B. sorokiniana* and *C. trifolii* in this study (Table 3).

It is not known if the antifungal substance(s) from guar would effectively reduce the severity or incidence of plant diseases in the field caused by fungi that were sensitive in assays. Rotating guar with other crops may introduce the antifungal substance into an agroecosystem, but it is not known how long the substance may last in the soil. Guar works well in rotation with cotton, grain, sorghum, and vegetables. Yields of cotton rotated with guar have been higher than yields of cotton not rotated with guar (1,3); however, it is not known if the increases in yield were related to a reduction in disease. Wheat following guar had less severe common root rot infections than wheat following other crops or crops not rotated (2). Rotating crops with guar may be effective in reducing the severity of some diseases, but further study is required to investigate this possibility.

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