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Inhibition of Mycelial Growth of Pythium graminicolum by Soil Fungi

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Inhibition of Mycelial Growth of *Pythium graminicolum* by Soil Fungi[1]

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Abstract: Cultures of twenty-five selected soil fungi were assayed for fungistatic effects on *Pythium graminicolum* mycelium. All cultures demonstrated some degree of inhibition. Several were strongly inhibitory. Cultures of six of the more fungistatic fungi were enclosed in dialyzer tubing, and their effects on growing *P. graminicolum* mycelium observed. All retarded growth markedly.

INTRODUCTION

Field soils have been shown to have an inhibitory effect on many fungi. Dobbs and Hinson (2) reported inhibition of fungus spore germination and postulated a widespread fungistasis in soils. Chinn (1) observed that natural soils inhibited germination of spores of several fungi. Jackson (6) found several soils in Nigeria prevented spore germination. Lockwood (7) reported that *Streptomyces* species were fungistatic and lytic to fungus mycelium and postulated the production of an antibiotic factor which inhibited fungus growth. Griffin (3) found some soil fungi to be inhibitory to conidial germination and hyphal growth of *Gliocladium fimbriatum* Gilman and Abbott.

Many observers have noted that autoclaving or otherwise sterilizing soil reduces or destroys the fungistatic properties of soils. This is further circumstantial evidence that the fungistasis may be an effect of other living organisms.

Previous work by Knaphus (4, 5) showed that samples of field soil from several sites in Iowa were fungistatic to *Pythium graminicolum* Subr. Presence of field soils enclosed in dialyzer tubing inhibited growth of mycelium of *P. graminicolum* on petri plates of 2% water agar.

FUNGISTASIS BY SOIL FUNGI

Methods and Procedures. It is probable that this fungistasis is the effect, in whole or in part, of metabolic activity of other living organisms. This experiment was an attempt to determine possible inhibitive effects of some common soil fungi on the growth of *P. graminicolum.* Cultures of twenty-five soil fungi were obtained from Dr. Lois H. Tiffany. Petri plates of 2% water agar were inoculated about 1 cm from the edge with small blocks

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of three-day-old *P. graminicolum* water agar cultures. Diametrically opposite the *P. graminicolum* inoculation, the petri plate was also inoculated with a block of potato-dextrose agar with a three-day culture of one of these soil fungi. There were five replicates of each combination of *P. graminicolum* and the respective soil fungi. As checks, five plates of 2% water agar were inoculated with *P. graminicolum* alone. Four days after inoculation, the plates were examined, and the following distances measured:

1. Radial extent of mycelium of the soil fungus.
2. Distance between edge of soil fungus colony and *P. graminicolum* colony.
3. Radial extent of mycelium of *P. graminicolum.*

"Radial extent" and "distance between" in all instances were measured on a line between inoculation loci (Table 1). Two of these measurements are likely to indicate inhibitory effects: the radial extent of *P. graminicolum* mycelium and the distance between the soil fungus mycelium and the *P. graminicolum* mycelium. Similar measurements were again recorded after five days (Table 1).

Results. Immediately noticeable is the apparently antagonistic effect of several fungi. There was a distance of at least 0.6 cm between colonies of *P. graminicolum* and colonies of *Emericellopsis humicola* (Cain) Cain, *Talaromyces spiculosporus* (Lehman) Benjamin, *Penicillium rubrum* Stoll, *Penicillium lanosum* Westling, *Aspergillus niger* van Tiegh. and *Chaetomium globosum* Kunze, as measured after five days. Meanwhile *P. graminicolum* had covered the entire petri plate in the checks.

Of significance also is the restricted growth of *P. graminicolum* mycelium in plates of essentially all fungi tested. The average radial growth of *P. graminicolum* mycelium in the check plates was 5.7 cm after four days and 7.2 cm after five days. In none of the plates containing another fungus did the *P. graminicolum* mycelium grow to this extent. In two sets of petri plates (*Trichoderma lignorum* (Tode) Harz + *P. graminicolum* and *Absidia spinosa* Lendner + *P. graminicolum*) the fungi had grown rapidly and colony edges were joined after four days at the time of the first measurement. *Trichoderma lignorum,* especially, overgrew the *P. graminicolum* mycelium so quickly and with such a mass of mycelium that reliable measurement was impossible.

In plates inoculated with *Rhizoctonia solani* Kühn and *P. graminicolum,* the latter had produced numerous nematosporangia after four days. It is not uncommon for *P. graminicolum* to produce nematosporangia after a few days of growth but it is unusual that so many were produced so soon. This effect was observed in none of the other petri plates.

Table 1. Growth of *Pythium graminicolum* mycelium in proximity to colonies of other common soil fungi

Fungus opposite *P. graminicolum* in petri plate	Four days after inoculation			Five days after inoculation		
	Length of other soil fungus	Distance between fungal growths	Length of *P. graminicolum* mycelium	Length of other soil fungus	Distance between fungal growths	Length of *P. graminicolum* mycelium
	cm	cm	cm	cm	cm	cm
Emericellopsis humicola	0.1[a]	2.2	2.6	0.2	2.1	2.6
Talaromyces spiculosporus	0.2	1.2	3.5	0.3	1.1	3.5
Penicillium rubrum	0.6	1.1	3.5	0.6	0.9	3.7
Penicillium lanosum	0.2	1.0	3.9	0.2	0.8	4.0
Chaetomium globosum	0.3	0.8	3.9	0.4	0.7	4.0
Aspergillus niger	0.6	0.8	3.7	0.9	0.6	3.8
Myrothecium verrucaria	0.3	0.9	3.5	0.4	0.5	3.8
Aspergillus terreus	0.2	0.6	4.1	0.3	0.4	4.1
Circinella spinosa	0.1	0.5	4.4	0.2[b]	0.4	4.4[b]
Aspergillus flavus	0.1	0.4	4.6	0.2	0.2	4.8
Mortierella species	0.5	0.4	4.1	0.5	0.2	4.3
Phoma species	0.3	0.3	4.5	0.4[b]	0.2	4.6[b]
Cladosporium herbarum	0.1	0.3	4.8	0.2[b]	0.2	4.9[b]
Penicillium solitum	0.3	0.2	4.5	0.4[b]	0.2	4.5[b]
Fusarium moniliforme	0.7	0.5	3.6	1.0	0.1	3.8
Stemphylium botryosum	0.6	0.2	3.7	0.7[b]	0.1	3.9[b]
Aspergillus nidulans	0.2	0.5	3.9	0.4[b]	0	4.3[b]
Rhizoctonia solani	2.0	0.2	3.0	2.4[b]	0	2.6[b]
Zygorhynchus heterogamus	0.6	0.2	4.2	0.8[b]	0	4.4[b]
Penicillium cyclopium	0.4	0.1	4.8	0.5[b]	0	5.0[b]
Absidia spinosa	1.5[b]	0	3.5[b]	[b]	0	[b]
Actinomucor repens	0.5	0	4.6	0.6[b]	0	4.8[b]
Coniothyrium species	0.5	0	4.3	0.7[b]	0	4.3[b]
Mucor mucedo	1.2	0	3.9	1.3[b]	0	4.4[b]
Trichoderma lignorum	3.2[b]	0	3.2[b]	[b]	0	[b]
Check. *Pythium graminicolum*			5.7			7.6

[a] Each datum is an average of five measurements.

[b] On these plates fungi had grown together.

Fungistasis Through Dialyzer Tubing

Dialyzer tubing has been observed to allow the fungistatic property of soil contained in it to affect growth of *P. graminicolum* outside and beyond the membrane (4, 5). An experiment was designed to determine whether the fungistatic property of cultures of fungi would similarly be effective through this membrane.

Methods and procedures. In this experiment, blocks of potato-dextrose-agar 1.5 cm in diameter were wrapped in dialyzer tubing and placed about one cm from the edge of a 2% water agar petri plate. These blocks were from seven-day-old cultures of *Emericellopsis humicola, Penicillium rubrum, Aspergillus niger, Chaetomium globosum, Talaromyces spiculosporus, Trichoderma lignorum* and *P. graminicolum. Trichoderma lignorum* was included because the dialyzer tubing restricts the growth of this rapidly growing fungus and thus allows assay of its fungistatic properties. The remainder were fungi which had shown an apparent antagonism to *P. graminicolum* in the previous experiment.

Inoculation of 2% water agar plates with *P. graminicolum* and placement of fungi enclosed in dialyzer tubing was followed by measurements of radial extent of *P. graminicolum* mycelium after three and five days (Table 2).

Table 2. Radial extent of *Pythium graminicolum* mycelium growing toward one growth of several fungi enclosed in dialyzer tubing. Measurements three and five days after inoculation and placement of package

Fungus in dialyzer tubing	Length of *P. graminicolum* after three days	after five days
	cm	cm
Emericellopsis humicola	2.8	3.0
Penicillium rubrum	2.4	2.6
Trichoderma lignorum	2.2	2.4
Talaromyces spiculosporus	2.1	2.2
Chaetomium globosum	2.7	2.9
Aspergillus niger	1.9	2.0
Pythium graminicolum	3.6	5.3[a]
Check (none)	3.5	6.5

[a] Mycelium had grown to dialyzer tubing package.

Results and conclusions. Of pertinence is the restricted growth of *P. graminicolum* mycelium on each of the plates on which a previously adjudged "antagonistic" fungus was contained in dialyzer tubing. *Trichoderma lignorum,* which grew so rapidly that measurement was difficult in the previous experiment, also restricted growth of *P. graminicolum. P. graminicolum* in dialyzer tubing does not restrict growth of other *P. graminocolum* mycelium growing in 2% water agar under it.

Fungus cultures may have varied effects on the growth of other propinquous fungi. These effects include:

1. A strong antagonism such as was shown by *Emericellopsis humicola* against *P. graminicolum.* Lockwood (7) reported *Streptomyces spp.* demonstrated this type of antagonism to soil fungi.

2. A debilitating effect in which the mycelium continues growth but at a reduced rate. In association with several species in plates, *P. graminicolum* mycelium continued growth at a reduced rate. Griffin (3) reported similar effects.

3. Inhibition of fungus spore germination.

4. Unusual development of sporangia. An unusually large number of sporangia were produced by *P. graminicolum* grown on the plates with *Rhizoctonia solani.*

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Microbiological Studies on the Vitamin B_6 Antagonist Found in Flaxseed[1]

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Abstract: Extensive investigations were conducted on the development of a microbiological assay of a vitamin B_6 antagonist found in flaxseed. It is the only known naturally occurring inhibitor of vitamin B_6. By means of a screening procedure, ten microorganisms were found to be inhibited by preparations of the B_6 antagonist. One of these, *Bacillus polymyxa,* was investigated more fully. Correlations of the *B. polymyxa* and live chick assays were excellent. All column chromatographic fractions of inhibitor which reduced Tollen's reagent inhibited *B. polymyxa.*

Vitamin B_6 does not competitively reverse the bacterial inhibition. Inhibition of *B. polymyxa* by high levels of Vitamin B_6 and the B_6 antagonist may suggest that the antagonist is an analogue of the vitamin.

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