Soil Fungistasis: Behavior of Nutrient-Independent Spores and Sclerotia in a Model System

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ABSTRACT

Conidia or sclerotia of 17 fungi capable of germination without exogenous nutrients were incubated on soil and in a model system that created a nutrient stress. Germination of 15 of the fungi was inhibited similarly in the two systems, suggesting the importance of microbial depletion of endogenous energy substrates as a factor in soil fungistasis.

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Fungistasis of spores requiring exogenous nutrients for germination can be attributed to a deficiency of such nutrients in soil (7). However, germination of propagules without a requirement for exogenous nutrients is also restricted in soil. Ko & Lockwood (7) found that spores of four such species were inhibited when incubated on a membrane filter aseptically leached with slowly dripping distilled water. The system was thought to provide a nutritionally deprived environment similar to that imposed by soil through microbial competition. Subsequently, other nutrient-independent propagules were shown to be inhibited in similar model systems (1, 5).

The purpose of the present investigation was to test a larger number of nutrient-independent, but fungistasis-sensitive,spores and sclerotia in a modified leaching system.

MATERIALS AND METHODS.–The fungi and propagules used are listed in Table 1. Inoculum for all species was produced on potato-dextrose agar (PDA), except for the *Helminthosporium* species, which were grown on moist, sterilized wheat straws, and *Verticillium albo-atrum*, whose microsclerotia were produced on sodium polypectate agar (per liter: sodium polypectate, 1 g; agar, 15 g). Sclerotia of *Macrophomina phaseoli* and *Sclerotium cepivorum* were collected from petri dishes containing PDA covered with noncoated cellophane to facilitate their removal. Sclerotia were treated in 1% sodium

hypochlorite for 1 min to kill attached hyphae. Propagules were washed 3 times in distilled water by refrigerated centrifugation. Ascospores of *Neurospora tetrasperma* were separated from conidia (7) and heat-activated (9) before use.

Spore germination was assayed on Nuclepore (polycarbonate) membrane filters (General Electric Co., 0.5-μm pore size) placed on (i) natural soil; (ii) autoclaved soil; (iii) sand leached to create a nutrient sink; (iv) sand without leaching; and (v) sufficient distilled water to moisten the membrane. Propagules also were incubated directly on natural soil without a membrane filter. The soil, Conover loam, has been described previously (8). It was adjusted to 25% moisture content (moisture tension ca. 0.06 bars) and used with the surface smoothed in petri dishes. The model system for producing a nutrient sink was a modification of a system described previously (8). It was composed of a separatory funnel equipped with a sealed-in dripping tip (Kontes Glass Co., Vineland, N. J.), which served to maintain a constant head. The funnel stem was connected by plastic tubing to a needle valve, then to a glass petri dish fitted with an inlet in the lid and an outlet at the bottom on the opposite side. The dish contained a 5-mm layer of acid-washed silica sand. All glass components were

TABLE 1. Germination of propagules of various fungi on Nuclepore membranes during incubation on Conover loam soil, in distilled water or in an artificial nutrient sink

Fungus	Propagule	% Germination[b]					
		Sand		Soil			Distilled water
		Leached[a]	Not leached	Natural	Natural[c]	Sterile	
Alternaria tenuis Nees[d]	Conidia	71±3	84±2	54±5	26±5	98±1	95±1
Curvularia lunata (Wakker) Boedijn[d]	Conidia	13±3	90±3	16±4	12±3	99±1	95±2
Fusarium oxysporum Schlecht. f. sp. *lycopersici* (Sacc.) Snyd. & Hans.	Macroconidia	8±1	76±2	4±1	4±1	95±1	90±3
Fusarium oxysporum Schlecht. f. sp. *melonis* (Leach & Currence) Snyd. & Hans.	Macroconidia	5±1	87±4	3±1	6±2	100±0	94±2
Fusarium roseum (Lk. ex Fr.) emend. Snyd. & Hans. f. sp. *cerealis* (Cke.) Snyd. & Hans. 'Culmorum'	Macroconidia	9±1	91±1	20±1	10±2	100±0	94±2
Fusarium solani (Mart.) Appel & Wr. f. sp. *phaseoli* (Burk.) Snyd. & Hans.	Macroconidia	12±3	74±9	51±13	44±13	99±1	81±9
Fusarium solani (Mart.) Appel & Wr. f. sp. *pisi* (F. R. Jones) Snyd. & Hans.	Macroconidia	10±2	87±2	87±3	6±1	100±0	99±1
Helminthosporium maydis Nisik. & Miyake[d]	Conidia	51±4	95±1	50±5	23±2	99±1	97±1
Helminthosporium sativum Pam., King & Bakke[d]	Conidia	49±2	89±3	23±4	11±1	96±1	97±1
Helminthosporium victoriae Meehan & Murphy	Conidia	57±2	70±3	37±4	25±3	94±2	76±3
Macrophomina phaseoli (Maub.) Ashby	Sclerotia	2±1	92±3	4±1	2±1	93±2	87±4
Neurospora tetrasperma Shear & Dodge	Ascospores	84±1	93±2	87±2	82±1	98±1	98±1
Sclerotium cepivorum Berk.	Sclerotia	1±1	68±1	1±1	1±1	89±2	72±2
Sclerotium rolfsii Sacc.	Sclerotia	0±0	75±2	89±3	91±2	91±2	81±3
Stemphylium sarcinaeforme (Cav.) Wiltshire	Conidia	23±3	72±3	27±4	17±2	94±1	90±2
Thielaviopsis basicola (Berk. & Br.) Ferr.[d]	Conidia	5±1	89±3	4±1	3±1	98±1	89±2
Verticillium albo-atrum Reinke & Berth.	Microsclerotia	8±1	72±1	9±2	5±1	98±1	60±2

[a] The artificial nutrient sink (leached sand) consisted of a petri dish containing sand on which were placed membrane filters bearing the propagules. Water or buffer from a separatory funnel dripped onto the sand through an inlet in the dish and drained from an outlet in the opposite side.

[b] Mean of at least three experiments in each of which a minimum of 200 propagules of each fungus were counted. Standard errors of the means are given. Incubation periods were 24 hr for conidia and ascospores, and 2-3 days for sclerotia.

[c] Propagules were placed directly on soil without a membrane.

[d] 0.025 M phosphate buffer, pH 7.0, was used in place of distilled water.

also acid-washed. The separatory funnel was filled with distilled water or 0.025 M phosphate buffer, pH 7.0, and the rate of flow adjusted to ca. 7-8 ml/hr with the needle valve. When nonleaching conditions were desired, saturated sand was used in an ordinary petri dish. Incubation times were 24 hr for conidia and 2-3 days for sclerotia.

After incubation, the propagules on Nuclepore membranes were stained with aqueous rose bengal (1% rose bengal, 5% phenol, 0.01% $CaCl_2$), after which the membranes were destained on water-saturated sand, air-dried, and mounted on glass slides in clove oil. Spores incubated directly on the soil were stained and removed as previously described (9). At least 100 propagules of each fungus were counted in each of duplicate plates. Propagules were scored as germinated when the germ tube length equaled half the length of the spore or sclerotium. The tests were done at least 3 times. Aseptic conditions were used unless otherwise specified.

RESULTS.–Conidia or sclerotia of 17 different fungi were tested (Table 1). Propagules of all species germinated 60% or more in water or phosphate buffer; all but three germinated more than 80%. Germination of all propagules directly on natural soil was strongly inhibited when compared with germination in distilled water or buffer, with two exceptions: (i) the activated ascospores of *Neurospora tetrasperma*; and (ii) sclerotia of *Sclerotium rolfsii*. Germination on soil in most instances was similar with or without the membrane. Where exceptions occurred; e.g., with *Alternaria tenuis* and *Fusarium solani* f. sp. *pisi*, germination directly on soil was less than on the membranes on soil.

The degree of fungistasis imposed by soil was, in most fungi, mimicked by incubating propagules on sand leached with water or buffer. Exceptions were sclerotia of *Sclerotium rolfsii* that failed to germinate on the model system, and macroconidia of *Fusarium solani* f. sp. *pisi* and *F. solani* f. sp. *phaseoli* that had high germination on membranes on soil but low germination on membranes in the leaching system. Ascospores of *Neurospora tetrasperma* showed little or no inhibition in the leaching system or on soil, as previously reported (7).

We tested germination of five fungi after 24-hr exposure to the artificial nutrient sink by stopping the flow of water, but leaving the spores in situ. Propagules of all five, which were restricted to 20% germination or less during leaching, all germinated more than 88% after leaching was stopped (Table 2). Similar results were obtained when membranes bearing propagules on natural soil were transferred to distilled water.

DISCUSSION.–The results suggest an important role for nutrient deprivation in soil fungistasis, in that nearly all fungi tested behaved similarly on soil as on leached sand, confirming previous results with a few species (1, 5, 7). The modified model, in which the propagules are borne on the surface of a leached substrate, resembles incubation conditions used on soil more closely than previous systems in which the propagules themselves were subjected to leaching from above. Though extensive tests have not been done with nutrient-dependent propagules, their inability to germinate on leached sand should be expected since they fail to germinate in water alone without the additional stress imposed by the leaching system. Limited results confirm this expectation (7, Ko & Lockwood, *unpublished data*). That leaching per se is not detrimental to germination was shown previously; conidia of *Helminthosporium victoriae* and *Neurospora tetrasperma* (7) and sclerotia of *Sclerotium rolfsii* (4) germinated completely when leached with a nutrient solution.

TABLE 2. Germination (i) of propagules after an initial incubation on Nuclepore filters on natural soil or leached sand in an artificial nutrient sink; and (ii) of the same propagules after transfer, respectively, to distilled water or left in situ under nonleaching conditions

		% Germination[a]			
		1st incubation		2nd incubation	
Fungus	Propagule	Leached sand[b]	Soil	Nonleached sand	Distilled water
Curvularia lunata	Conidia	14±3	15±4	91±3	94±2
Fusarium roseum f. sp. *cerealis* 'Culmorum'	Macroconidia	11±2	15±4	91±3	95±3
Helminthosporium sativum	Conidia	19±3	21±4	91±3	96±2
Macrophomina phaseoli	Sclerotia	3±1	3±1	91±3	84±4
Thielaviopsis basicola	Conidia	5±1	4±1	89±3	86±4

[a] Mean of at least three experiments, in each of which a minimum of 200 propagules of each fungus were counted. Standard errors of the means are given. Incubation periods for both first and second incubations were 24 hr for conidia and 2-3 days for sclerotia.

[b] The artificial nutrient sink consisted of a petri dish containing sand on which were placed membrane filters bearing the propagules. Water from a separatory funnel dripped onto the sand through an inlet in the dish and drained from an outlet in the opposite side.

The concept that soil, through potential microbial activity, provides a depleting energy sink is supported by the well-known rapid utilization of energy sources added to natural soil. Moreover, recent data relating known soil microbial populations to the available soil substrate indicate that the bulk of the population must by inactive for a large proportion of the time due to a shortage of available energy (2, 6).

What is needed now is to calibrate the leaching system in terms of the microbial energy sink in soil, and to explain the mechanism whereby nutrient-independent propagules are inhibited by a nutrient stress environment. A means of measuring such an energy sink has been developed which involves determination of the rate of loss of glucose from filter paper discs incubated on soil, or on sand or glass beads in the leaching system (Lockwood, *unpublished data*). A possible mechanism for the restricted activity in soil of propagules without an exogenous energy requirement is the removal, through microbial activity, of spore exudates containing endogenous nutrients that might otherwise be utilized to support germination. This possibility is supported by preliminary investigations (3).

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