Properties of a Volatile Fungistatic Factor in Soil

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ABSTRACT

A volatile fungistatic factor (VFF) was detected in neutral to alkaline soils of various textures and properties. Activity of the VFF gradually decreased with increased depth in the soil. The VFF was water-soluble or inactivated by passage through water, and was adsorbed on activated charcoal. Properties of the VFF were similar to those described for soil fungistasis.

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Soil fungistasis has been defined by Dobbs & Hinson (2) as the inhibition of germination of viable fungal spores in soil. Most soil fungi are affected by this phenomenon, although the magnitude of the effect may vary (10, 11). Ko & Lockwood (7) failed to detect an inhibitory factor in soil, and suggested that fungistasis was due solely to the lack of nutrients in soil essential for spore germination.

Recent work (5, 8) suggests that at least under certain conditions there also is a volatile fungistatic factor (VFF) in soil. The objective of the present study was to determine the effects of soil texture, soil depth, water, and activated charcoal on the activity of the VFF as compared to those effects reported in the literature for soil fungistasis.

MATERIALS AND METHODS.—The direct assay and soil emanation (SEA) methods were used to determine fungistatic activity in soils (5). In the direct method, washed "Millipore" membrane filters (pore size 0.45 μ) were impregnated with an aqueous conidial suspension (100-150 spores/0.32 cc) and covered with soil in petri plates. The filters were removed after 15- to 18-hr incubation at room temperatures, stained with lactophenol-cotton blue, and microscopically examined for spore germination. In the SEA method, agar discs 6 to 7 mm in diam and 2 to 3 mm thick were attached to the inside of a petri plate cover. These discs were suspended over 40 g soil in the petri plate for 4 hr at 25 to 27 C. A drop of the aqueous conidial suspension was added to the discs, and the plate cover was replaced over the soil. After 15 to 18 hr, ca. 100 conidia from each of two replications were examined for germination. Controls consisted of the incubation of conidia-impregnated Millipore membrane filters (for the direct method) or agar discs with spore suspensions (for the SEA method) in sterile moist chambers without soil but otherwise under identical conditions. The majority of the tests were conducted in duplicate and repeated twice.

Test fungi used in this study were *Zygorhynchus vuilleminii* Namysl., *Trichoderma viride* Pers., *Penicillium chrysogenum* Thom, *Gonatobotrys simplex* Cda., *Mucor varians* Povah, *Phoma* sp., *Fusarium solani* (Mart.) Appel & Wr. f. sp. *cucurbitae* Snyd. & Hans., race 1, *Fusarium solani* f. sp. *phaseoli* (Burk.) Snyd. & Hans., *Helminthosporium victoriae* Meehan & Murphy, and *Helminthosporium sativum* Pam., King & Bakke. All were grown on 2% potato-glucose agar except *H. victoriae* and *H. sativum*, which were grown on propylene oxide-sterilized (4) oatmeal natural medium. Conidia

TABLE 1. Properties of different textured soils collected from various locations and assayed for fungistatic activity

Texture	Soil separates			Conductivity (salts)	Organic matter	NH_4-N	NO_3-N	pH
	Sand	Silt	Clay					
	%	%	%	*mmhos/cm*	%	*μg/ml*	*μg/ml*	
Loamy sand	87.6	4.2	8.2	0.1	0.3	11.9	1.8	8.0
Sandy loam	78.6	11.2	10.2	0.5	0.5	7.2	3.4	7.4
Loam	48.8	38.4	12.8	1.6	0.9	4.2	32.0	7.5
Loam	44.8	41.2	14.0	1.1	0.8	9.2	18.3	7.7
Loam	28.2	47.2	24.6	1.6	1.7	9.2	37.0	7.5
Clay loam	22.6	40.6	36.8	1.6	1.2	8.0	15.6	8.0
Silty clay loam	18.8	42.8	38.4	38.0	1.7	14.7	223.0	7.6
Clay loam	35.0	26.0	39.0		0.9	10.8	7.9	7.6
Clay	21.6	38.2	40.2	0.9	1.6	9.2	12.5	7.8
Clay	29.6	28.0	42.4	0.9	2.8	8.0	4.7	8.1

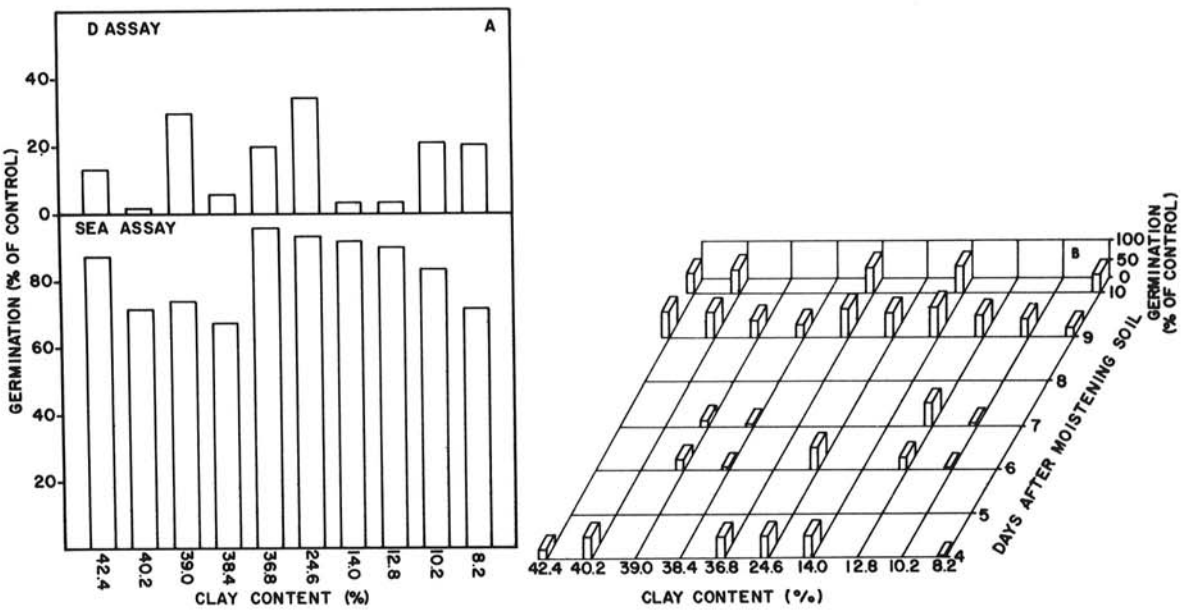

Fig. 1. A) Germination of conidia of *Fusarium solani* f. sp. *cucurbitae* as influenced by the fungistatic factor in soils having varying clay contents 6 days after moistening. D assay, direct method; SEA assay, soil emanation agar method. **B)** Germination of conidia of *Zygorhynchus vuilleminii* as influenced by the fungistatic factor in soils having varying clay contents using the soil emanation agar method.

were transferred from the surface of a culture to sterile distilled water. A drop of this suspension was then applied, using a 1-ml sterile pipette, to the surfaces of the agar discs, or suction was used to impregnate the Millipore membrane filters with the suspension.

All soils were stored air-dried and moistened to 50 to 60% field capacity prior to use. Moistened soils were incubated at room temperatures in Erlenmeyer flasks with foam rubber stoppers. Assays were performed at various intervals after moistening.

RESULTS.—Surface soil samples of various textures from different locations were assayed to determine fungistatic activity. Some of their properties are given in Table 1.

In the direct assay, spore germination of *F. solani* f. sp. *cucurbitae* in these soils ranged from 2 to 34% of the control, whereas the range was 67 to 96% in the SEA assay (Fig. 1-A). There was a trend toward lower germination in the SEA assay with soils of high or low clay content. Germination inhibition of *Z. vuilleminii* conidia in the SEA assay was greater than that recorded for *F. solani* f. sp. *cucurbitae* (Fig. 1-B). Some soils with lower clay content tended to induce greater inhibition than soils with a high clay content; however, inhibition also was high in the silty clay loam (38.4% clay) and in a clay loam (39.0% clay).

Samples of two soils, taken throughout the soil profile extending over a depth range of 0 to 1.40 meters, were assayed for fungistatic activity with *T. viride*. Properties of these samples are given in Table 2. Although there was a greater reduction in conidial

TABLE 2. Properties of two soil types collected from different depths of the soil profile

Soil type	Depth	Texture	Conductivity (salts)	Organic matter	NH_4-N	NO_3-N	pH
	meters		*mmhos/cm*	*%*	*μg/ml*	*μg/ml*	
Apishapa clay	0-.18	Clay loam	2.1	2.5	24.44	5.8	7.7
	.18-.30	Clay	1.1	2.6	11.85	2.3	7.6
	.30-.41	Clay	1.1	2.1	9.23	1.7	7.7
	.41-.81	Clay	1.4	1.9	6.52	0.9	7.6
	.81-1.40	Clay loam	1.4	0.7	6.52	0.0	7.6
Limon silty clay	0-.30	Silty clay	4.0	2.5	10.32	33.0	7.7
	.30-.56	Silty clay	6.5	1.2	17.93	5.2	7.7
	.56-1.02	Silty clay	4.5	1.1	19.01	6.2	7.6

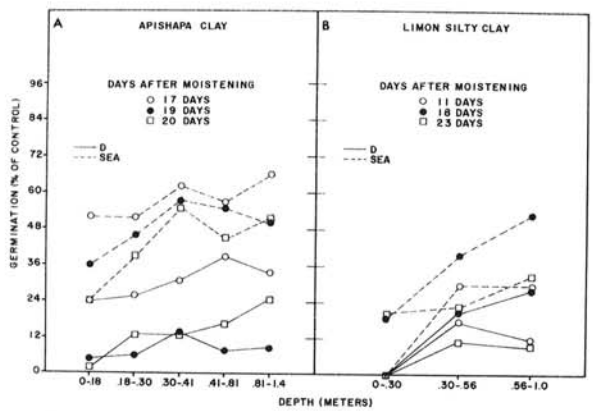

Fig. 2. A) Germination of conidia of *Trichoderma viride* as influenced by soil depth in Apishapa clay soil. **B)** Germination of conidia of *Trichoderma viride* as influenced by soil depth in Limon silty clay soil. D, direct method; SEA, soil emanation agar method.

germination in the direct assays as compared to the SEA assays, both assays showed a gradual loss of fungistatic activity with increased depth in the soil (Fig. 2-A, B).

Air-dried swale soil with the following properties: texture, clay; conductivity (salts), 48 mmhos/cm; organic matter, 2.4%; NH_4-N, 5.6 μg/ml; NO_3-N, 61.7 μg/ml; depth, 0 to 35 cm; and pH, 8.7 was moistened and then incubated 3 days. The apparatus in Fig. 3 was used as a modification of the SEA method to study the effect of water on the activity of a VFF from this soil. When air was passed through the soil without water in flask E, conidial germination of several fungi was reduced (Table 3). When air was passed through water in flask E, germination was comparable to controls in which the air was passed through water only.

The same apparatus (Fig. 3) was used to determine if a VFF could be adsorbed on activated charcoal. Air-dried swale soil was moistened and then incubated for 5-11 days. Air passed through the soil was fungistatic to conidia of *Z. vuilleminii* and resulted in an average for all experiments of 38% germination (expressed as per cent of controls in which air was passed through water only). When activated charcoal was mixed with the soil, there was

TABLE 3. Effect of water on the activity of a volatile fungistatic factor from a swale soil on conidial germination of nine test fungi[a]

Test fungus	Germination (% of controls)	
	Air passed through soil only	Air passed through soil and water
Zygorhynchus vuilleminii[b]	51	97
Trichoderma viride	30	89
Penicillium chrysogenum	44	91
Gonatobotrys simplex	66	96
Mucor varians	35	94
Helminthosporium sativum	71	93
Helminthosporium victoriae	55	84
Fusarium solani f. sp. *phaseoli*	51	92
Phoma sp.	74	100

[a]Assays conducted 3 days after moistening for *Zygorhynchus vuilleminii*. Assays were performed 24 hr after moistening for the other test fungi.

[b]Means of five tests.

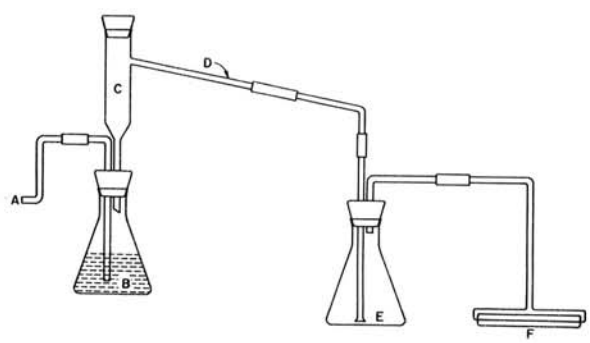

Fig. 3. Apparatus used to determine the effect of water and activated charcoal on the inhibition of conidial germination by a volatile fungistatic factor. Air applied at A (5 to 7 cc/min) was washed and saturated in water (B) and passed into a tube (C) containing 20-g soil. In some experiments, the arm of the tube (D) was filled with activated charcoal. From the tube, the air was passed through an Erlenmeyer flask (E), eventually passing over spore suspensions on two agar discs in a petri plate (F).

an increase in germination to an average of 89% of the control. There was also a germination increase (average of 85% of control) when air from the soil was passed through activated charcoal in tube D. Activated charcoal without soil in the tube did not influence germination.

DISCUSSION.–Many of the properties of the VFF correlate well with those reported in the literature for soil fungistasis. Both are found in many soils (2, 9). Previous work indicates that inhibition is greatest in alkaline soils, lowest in acid soils (5, 10). In both instances, activity decreases with depth (2, 3, 6), the fungistatic factors are water-soluble or inactivated in water (1, 2, 12), and are adsorbed on activated charcoal (2). The most obvious difference between the VFF and soil fungistasis is the decrease in activity of the latter immediately after the wetting of dried soil followed by an increase in activity (2), whereas the activity of the VFF decreases with time (5).

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