Extraction of a Volatile Factor from Soil-Inducing Fungistasis

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ABSTRACT

A volatile factor extracted in water from soil significantly inhibited germination of conidia in 8 of 12 species of test fungi. Presence of conidia in water during extraction was necessary for inhibition of spore germination.

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Lockwood (10) reviewed evidence for the presence of inhibitory chemical substances contributing to fungistasis in soil and pointed out that soil extracts rarely retained inhibitory activity after filter sterilization, although attempts to demonstrate such retention have been made many times. He concluded "that these results were characterized by variable and inconclusive data". Recently, an inhibitor was detected, particularly in neutral or alkaline soil (5). The volatile nature of this factor may explain the difficulty of obtaining uniform inhibitory extracts. This paper reports the successful extraction in water of a volatile inhibitor from soil.

In preliminary experiments, a clay loam soil of pH 7.5 (determined colorimetrically in 1:2 soil: 0.01 M $CaCl_2$ suspensions, 13) with the following properties was used: organic matter, 1.0%; NO_3^--N, 29 μg/g; lime, 4.5%; P_2O_5, 95 μg/g; K_2O, 500 μg/g; Fe, 8.0 μg/g; Mn, 12.6 μg/g; and Cu, 1.72 μg/g. The soil was stored air-dried and moistened to 50-55% water-holding capacity for 24 hr before use. Air was washed in concentrated H_2SO_4 at 25 C, passed through a 1-kg soil sample packed in a 1,000-cc capacity burette at 25 C and bubbled for 24 hr at a flow rate of 6-8 cc/min (monitored by a Fisher flowmeter) into a filtering tube containing a fungal spore suspension in 2 ml of sterile distilled water held at 2-3 C. Conidia did not germinate when agitated during the extraction. After 24 hr, a drop of the spore suspension was placed on 2% water agar (Difco purified) discs (8 mm-diam and 5-6 mm thick) supported by a sterile glass slide in a moist chamber, and incubated for 20-24 hr at room temperature. Percentage spore germination was determined from a total of 400-500 conidia observed in three fields (140-160 conidia/0.032 cm^2) for each test organism. Controls were processed without soil, but otherwise under identical conditions. The results for 10 test fungi are presented in Table 1. Significant reduction was evident in conidial germination of *Aspergillus flavus* Link, *A. ochraceous* Wilhelm, *Claviceps purpurea* (Fr.) Tulane, *Gonatobotrys simplex* Corda, *Trichoderma viride* (Fr.) Pers. and *Zygorhynchus vuilleminii* Namaslowski.

Test fungi used in preliminary experiments were representative of those with small spores, usually requiring exogenous nutrients for germination (8). Since agar can be a source of nutrients essential for fungal spore germination, thus masking the fungistatic activity of the inhibitor, two organisms with large spores, *Helminthosporium sativum* Pam., King & Bakke and *H. victoriae* Meehan & Murphy (kindly supplied by D. L. Yoder, Michigan State Univ.), not requiring an external source of nutrients for germination, were included in subsequent tests.

Volatiles were extracted from a swale soil (pH 8.8) using the above method. Properties of this soil have been described (12). Fungi with small spores were tested on water agar discs, whereas the two large spore-producing fungi were tested for germination on sterile glass slides in moist chambers. Results for *H. sativum* and *H. victoriae* (large spores), *T. viride* (a

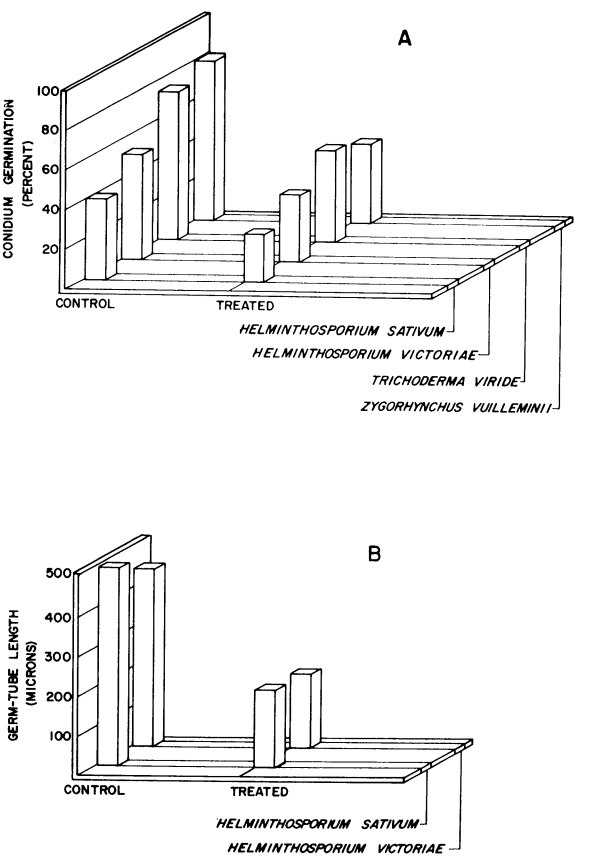

Fig. 1. Influence of a volatile inhibitor extracted in water from soil on (A) fungal spore germination and (B) germ tube length.

TABLE 1. Fungistatic activity of a volatile inhibitor extracted in distilled water from clay loam soil (pH 7.6)

	Conidial germination[a]	
	Control (water)	Treatment (water extract)
	%	%
Actinomucor repens Schostakowitsch	30	30
Aspergillus flavus Link	70	52[b]
Aspergillus ochraceous Wilhelm	45	30[b]
Cladosporium sp.	90	90
Claviceps purpurea (Fr.) Tulane	50	25[b]
Gonatobotrys simplex Corda	90	70[b]
Mucor varians Povah	25	20
Penicillium chrysogenum Thom	100	100
Trichoderma viride (Fr.) Pers.	55	28[b]
Zygorhynchus vuilleminii Namaslowski	90	35[b]

[a] Conidia of test fungi suspended in water and water extract were tested for germination on 2% water agar discs.

[b] Significantly different from the control at $P = .01$.

different isolate than the one used in the preceding experiment), and *Z. vuilleminii* (small spores) are shown in Fig. 1-A. There was 30-50% reduction in conidial germination of test fungi. In *H. sativum* and *H. victoriae*, there was a concomitant reduction in germ tube length of the germinated spores (Fig. 1-B).

Conidia of test fungi were placed in the extract after air from soil had been passed through water for 24 hr. Germination was not reduced. Thus, the presence of conidia in water during the passage of air through the soil column was essential for inhibition. This confirms the ephemeral nature of the extracted inhibitors reported by other workers (2, 3, 4, 9, 11, 14, 15, 16).

If fungistatic volatiles can be extracted from other soils reported to be fungistatic (1, 5, 7, 12), then the two criteria originally suggested (10) for the existence of inhibitory chemical substances in soil as a basis for fungistasis appear to have been satisfied. These are: "(i) demonstration of inhibitory activity in extracts made with mild reagents from a wide range of soil; and (ii) demonstration of inhibitory activity in such extracts against a wide range of fungi". These criteria, however, are not adequate to establish the role of volatile inhibitory substances in soil fungistasis. We suggest three additional criteria as follows: (iii) isolation of volatile inhibitory compounds from soil and their identification; (iv) demonstration of a dosage response indicating that these compounds are inhibitory at concentrations actually present in soil solution; and (v) evidence that these inhibitors are stable long enough in soil to be fungitoxic. We are currently exploring means of satisfying these additional criteria.

Reduced spore germination and growth of fungi in soil may "appear to be explicable in terms of nutrient relations and (do) not require an assumption that fungistatic substances are involved" (6). Nevertheless, such substances have been detected in many soils (1, 5, 7, 12), and should be considered as possible factors in soil micro-ecological relationships.

LITERATURE CITED

1. BALIS, C., & V. KOUYEAS. 1968. Volatile inhibitors involved in soil mycostasis. Ann. Inst. Phytopathol. Benaki, N.S. 8:145-149.
2. BUBLITZ, W. 1954. Über Keimhemmende und antibacterielle substanzen in Bodenwasser der Fichtenstreu. Naturwissenschaften 41:502-503.
3. GRIFFIN, G. J. 1964. Long term influence of soil amendments on germination of conidia. Can. J. Microbiol. 10:605-612.
4. HESSAYON, D. G. 1953. Fungitoxins in soil II. Trichothecin, its production and inactivation in unsterilized soils. Soil Sci. 75:395-404.
5. HORA, T. S., & R. BAKER. 1970. Volatile factor in soil fungistasis. Nature 225:1071-1072.
6. HSU, S. C., & J. L. LOCKWOOD. 1971. Responses of fungal hyphae to soil fungistasis. Phytopathology 61:1355-1362.
7. KO, W. H., & F. K. HORA. 1972. The nature of a volatile inhibitor from certain alkaline soils. Phytopathology 62:573-575.
8. KO, WEU-HSIUNG, & J. L. LOCKWOOD. 1967. Soil fungistasis: relation to the fungal spores. Phytopathology 57:894-901.
9. LINGAPPA, B. T., & J. L. LOCKWOOD. 1962. Fungitoxicity of lignin monomers, model substances, and decomposition products. Phytopathology 52:295-299.
10. LOCKWOOD, J. L. 1964. Soil fungistasis. Annu. Rev. Phytopathol. 2:341-362.
11. NEWMAN, A. S., & F. G. NORMAN. 1943. The activity of subsurface soil population. Soil Sci. 55:377-391.
12. ROMINE, MAUREEN, & R. BAKER. 1972. Properties of a volatile fungistatic factor in soil. Phytopathology 62:602-605.
13. SMILEY, R. W., & R. J. COOK. 1972. Use and abuse of the soil pH measurements. Phytopathology 62:193-194.
14. STOVER, R. H. 1958. Studies of Fusarium wilt of bananas. III. Influence of soil fungitoxin on behavior of F. oxysporum f. cubense in soil extracts and diffusates. Can. J. Bot. 33:439-453.
15. VAARTAJA, O. 1967. Mycostasis in extracts from different soils. Bimonthly Res. Notes, Forestry Branch, Dep. of Forestry Rural Development Can. 23:25-26.
16. WINTER. A. G. 1955. Untersuchungen Über Vorkommen und Bedeutung von antimikrobiellen und antiphytotischen Substanzen in naturlichen Böden. Z. Pflanzenernaehr. Dueng. Bodenk. 69:224-237.