Volatiles From Soil Influencing Activities of Soil Fungi

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ABSTRACT

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Volatile compounds were detected in natural soils. Among these, dosage-response curves for acetone, ethylene, ammonia, and formaldehyde were developed. Concentrations of ethylene and acetone considerably above those detected in soil were necessary to inhibit germination of conidia of test soil fungi. Formaldehyde was detected in some soils at concentrations high enough to inhibit spore germination. Inhibition of conidium germination of *Aspergillus flavus* by formaldehyde was nullified by exogenous nitrogen and carbon, but the same phenomenon was not observed for conidia of *Fusarium solani* f. sp. *phaseoli*. The ED_{50} values for conidium germination of *Penicillium chrysogenum* and *Gonatobotrys simplex* were below 1 μg ammonia/g air. Reduced germination due to volatiles from five soils, limed and not limed, was correlated directly with concentrations of volatile ammonia accumulated for 24 hr above these same soils. At certain concentrations, ammonia, ethylene, and acetone stimulated growth and/or germination of some of the test organisms. Ammonia must be considered a prime candidate for a volatile inhibitor in soil. Formaldehyde may inhibit some fungi in certain soils. Bioassays, however, provided evidence for another volatile component present in the atmospheres of acid soils. So far, it has not been identified by chemical analysis.

Additional key words: spore germination, soil fungistasis.

A volatile fungistatic factor in soil has been implicated in the inhibition of germination of fungal propagules in soil (7, 21). Lockwood (15) recently reviewed literature in this and other areas of research on soil fungistasis.

Certain properties and characteristics can be assigned to the inhibitory volatile on the basis of literature reports: (i) it is volatile at ambient temperatures and is water extractable (8); (ii) emanations from soils may occur over extended periods, but their effects may be nullified by small quantities of nutrients (6, 21); (iii) the factor is typically adsorbed on activated charcoal; (iv) inhibition of spore germination by the volatile(s) decreases with increasing depth of soil (20); (v) increasing alkalinity of soil increases inhibition of germination (10, 11) and may indicate increases in release of at least one of several possible fungistatic chemicals; and (vi) inhibitory volatiles may be produced by soil microorganisms (9). In general, these observations suggest that the factor might be one or more low-molecular-weight compounds.

Although there appear to be sufficient data in the literature supporting the participation of inhibitory chemicals in the phenomenon of soil fungistasis (2, 7, 12, 13, 21, 24), the emanation rates from soils are generally very low. Thus, the identification of the factor requires collecting sufficient quantities nondestructively to permit identification. Despite this problem, a number of compounds have been suggested as inhibitory volatiles in soil. Smith (24) found emanations of ethylene from soils fungistatic to propagule germination. Balis (1) was unable to confirm the inhibitory properties of ethylene, but suggested it might induce formation of a fungistatic compound tentatively identified as allyl alcohol. Ko and Hora (14) implicated ammonia as an inhibitor of spores in certain alkaline soils. Few of these reports, however, relate quantitative data on volatiles originating from aerobic raw soil to dosage-response measurements for the suspected volatile inhibitors. We present in this paper qualitative and quantitative studies of volatiles in soil and their dosage-response relationships for inhibition of germination of spores of several soil fungi.

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MATERIALS AND METHODS

Trapping methods.—Because of the generally low emanation rates of the fungistatic factor(s) in soils, the experimental approach devised for their identification included means for nondestructive isolation and preconcentration under fairly representative soil conditions. To accomplish this, soil samples (see Table 1) were purged under conditions to be described while using the following collection/preconcentration systems. The first was a cryosorption trap of activated charcoal [Nuchor WV-G, 1.85-0.50 mm particle size range (12-40

mesh)] cooled in dry ice-acetone or liquid nitrogen baths. This unit was efficient for the collection of numerous compounds of low molecular weight. The second was a trap of boric acid solution equipped with a gas dispersion frit so that the purge gas could be scrubbed free of compounds such as ammonia.

Preparation of soil samples.—All soils were air-dried and ground to pass a 2-mm sieve. If the soil pH was to be increased, 1:1 $Ca(OH)_2$:$CaCO_3$ was mixed with soil in a twin-shell blender in amounts appropriate to raise the pH to 8.5. The amounts required were determined by the method described by Hora and Baker (10). All soils were moistened subsequently with distilled water to approximately 60-75% of the 1/3-bar percentage values after being placed in the soil column of the purging system used for removal of the volatile factors. Sample size was typically 40 g of dry soil.

Removal and collection of volatiles.—Carrier gases were passed through the soil column to remove the volatiles. Air was used to remove volatiles from the soils under aerobic conditions and prepurified nitrogen was used to produce anaerobic conditions. The carrier gases were passed first through a charcoal cryosorption trap to remove nutrients. Then they were passed through distilled water and thence to the soil column and a magnesium perchlorate trap to collect water prior to the collection trap. Air flow was maintained at 3 cc/min while the nitrogen flow was 5 cc/min. The cryosorption trap was held at the dry ice-acetone temperature (-78 C), when air was used as the purge gas to avoid blockage of the trap by freezing of constituents in air. The trap was held at the liquid temperature of the nitrogen (-196 C) when nitrogen was used as the purge gas. Prior to each use, the charcoal trap was conditioned for 30-60 min at 210 C while being flushed with prepurified nitrogen. Collections were carried out at ambient temperature (about 25 C) for periods of 6-12 days. The traps were designed so they could be sealed, removed from the system, stored in an appropriate cold bath until analysis, and replaced by another trap for continuation of the collection if that was desired. Prior to their removal, however, each trap was sealed at its influent end and evacuated from its effluent end to a pressure of about 2-5 mm Hg to remove excess noncondensable gases.

Removal of volatiles from the cryotrap.—To remove the volatiles from a cryotrap, the trap was connected to an evacuated 150-cc flask equipped with a vacuum stopcock at the point of trap connection and an outlet sealed with a silicone septum. The opposite side of the trap was connected to a tank of prepurified nitrogen. After raising the temperature of the sealed trap to 90 C in a silicone oil bath and allowing 5 min for desorption of the sorbed volatiles, the trap was opened and the nitrogen allowed to flow through it thereby purging the volatiles into the evacuated, volume-calibrated flask. Thus, the volatiles collected during the 6-12 day purge period were concentrated into 150 cc of prepurified nitrogen in a sealed system from which gas samples could be withdrawn for analysis.

Analysis of condensible volatiles.—The identification and determination of the volatiles collected were based largely on gas chromatographic analyses. Most analyses were made with a 305 cm × 0.32 cm Poropak-Q column [293-177 μm particle size range (50-80 mesh)] operated isothermally at 100 C with a helium carrier flow of 22 cc/min. Confirmatory identifications of most of the volatiles were made with a 183 × 0.32 cm deactivated alumina (25, 26) column (Alcoa alumina, F-20) operated at 82 C with a helium carrier flow of 22 cc/min. Both columns were preconditioned for 24 hr at 230 C with helium flow. In either case, detection was with a flame ionization detector operated on hydrogen (28 cc/min) and air (370 cc/min).

Gas samples (5 cc) were withdrawn through the silicone septum of the calibrated collection flask described previously with a gas-tight syringe. Prepurified nitrogen was used to replace the withdrawn sample. Samples were injected directly into the gas chromatograph. Compounds were identified by matching retention times with those of pure gases of known identity and verified by matching of retention times on the second column whenever feasible. Quantitation was based on calibration with measured amounts of pure compounds diluted with measured volumes of prepurified nitrogen and analyzed under the same conditions.

Ammonia and acetone were also identified by expanding the gases collected in a cryosoption trap into an evacuated infrared cell (10-cm path length) and running their infrared absorption spectra.

Collection and determination of ammonia.—Ammonia was purged from the soil columns and passed into a boric acid (0.162 M) scrubber trap. A KOH (pellet) trap was used between the soil column and the boric acid trap to remove other gases that might interfere with the ammonia analysis. The injection of known amounts of ammonia verified that it passed through the KOH trap and was collected quantitatively. After collection, the amount of ammonia was determined by titration with standard HCl. The end point was determined potentiometrically against a blank containing the same initial amount of boric acid.

Dosage-response determinations.—Various test organisms were exposed to known concentrations of the volatiles, considered to be fungistatic factors and detected in soils.

Agar disks were washed as previously described (6, 12) to remove nutrients. Two hundredths of a milliliter of the following conidium concentrations were pipetted on the surface of each disk: *Fusarium solani* (Mart.) Appel & Wr. f. sp. *phaseoli* (Burk.) Snyd. & Hans., 7×10^5 conidia/ml; *Gonatobotrys simplex* Corda, 3×10^6 conidia/ml; *Penicillium chrysogenum* Thom, 3×10^6 conidia/ml; and *Verticillium albo-atrum* Reinke & Berth., 3×10^6 conidia/ml. The disks with conidia were placed in desiccator jars and various concentrations of candidate volatile fungistatic factors were introduced through a rubber septum with a Hamilton microliter syringe. Concentrations were based on micrograms of volatile compound per gram of air. Laboratory-grade ethylene gas (Matheson Lyndhurst, NJ 07071) and reagent grade formaldehyde solution (Mallinckrodt, St. Louis, MO 63132) were used. Since methyl alcohol is the solvent used for formaldehyde solutions, separate dosage-response curves were developed using the anhydrous reagent grade methanol (Mallinckrodt). Reagent grade acetone was obtained from Eastman (Rochester, NY 14650).

After 20 hr of incubation at room temperature (24-26

C), germination of conidia was recorded and expressed as percentages of nontreated controls. These were converted to dosage-response curves based on the log-probit transformation.

RESULTS

Analysis of volatiles from soils.—Ethylene was consistently identified as one of the volatile compounds in the gaseous mixtures emanating from the soils. Since ethylene was reported previously as fungistatic and identified in soil air (24, 25, 26), initial attention was focused on it. Total ethylene emanations from three representative soils was measured at intervals of 24-48 hr over a 12-day period at their virginal pH levels and after adjustment to pH 8.5. Measurements also were made under aerobic and anaerobic conditions to determine if the increase of inhibition due to volatiles (9, 10, 11), of soil pH on inhibition (and therefore the rate of ethylene release) was influenced by oxygen. Under aerobic conditions, liming of the soils did not affect significantly the rate of ethylene emanation. Under anaerobic conditions, liming actually decreased the rate of ethylene release by 20-40% over the period of study. Cumulative amounts of ethylene released over a 6-day period varied from 0.7 to 35 ηg/g of soil for the three soils studied. The rates of emanation, however, were not the same for aerobic and anaerobic conditions for each soil.

Twelve different soils (nine of which are characterized in Table 1) with pH values ranging from 5.1 to 8.6 and exhibiting varying degrees of fungitoxicity were subsequently selected for study. These were moistened, placed in sealed chambers and the amounts of volatiles emitted were determined as functions of time with and without the addition of lime. Although an extensive amount of information was accumulated, it is summarized in Table 2. Five classes of compounds were detected. In each instance, the specific compounds released were divided into two categories: those found to be emitted by all study soils and those detected for some soils but not others. Failure to detect a particular compound for a particular soil does not indicate its total absence, but merely that the amounts released were below detectable levels for the methods used; i.e. less than approximately 0.01 ηg/g air. The results of these studies are summarized below.

Inorganic compounds.—Raising the pH of the soils enhanced the emanation of ammonia, but the reverse was true for carbon dioxide and nitrous oxide. Inhibition of germination of *F. solani* conidia by volatile agents from soils was nullified by a KOH trap (6) but it is unlikely that carbon dioxide was responsible because of its ubiquitous nature and since the Fusaria are tolerant to this gas. Thus, carbon dioxide was not considered as a likely volatile fungistatic agent. Emission of nitrous oxide was observed only during the early stages of incubation and its rates,

TABLE 1. Properties of soils used in chemical assays and bioassays for volatile fungistatic activity

			Concentrations										
										DPTA[d] extractable micronutrients			
Soil designation	Soil pH[a]	Origin and texture	Organic[b] matter (%)	Lime[c]	NO_3^--N (μg/g)	NH_4^+-N (μg/g)	P_2O_5 (μg/g)	K_2O (μg/g)	Na (μg/g)	Zn (μg/g)	Fe (μg/g)	Mn (μg/g)	Cu (μg/g)
BH	5.1	Colorado loam	2.6	Medium	51	ND[e]	30	85	ND	2.40	106.0	ND	2.90
DP	6.1	Colorado clay loam	4.2	Low	165	ND	8	393	ND	2.20	27.8	ND	41.00
49	5.7	Colorado loam	2.9	Low	225	19.0	16	315	10.4	8.70	63.0	10.0	0.28
98	6.2	Iowa loam	3.1	Medium	5	5.7	14	216	16.1	72.5	50.2	52.0	0.69
74	6.4	Colorado loam sand	0.7	Low	74	9.6	16	260	5.8	1.26	12.4	24.0	0.52
22	7.0	Colorado sandy clay loam	1.5	Low	5	ND	6	650	ND	2.20	5.9	ND	1.03
91	7.5	Colorado sandy clay loam	7.8	High	16	ND	9	480	ND	5.50	16.0	ND	0.80
110	7.8	Colorado clay loam	0.7	High	>100	ND	360	70	ND	1.40	8.5	ND	0.96
CS	8.6	Colorado clay loam	3.4	High	62.2	29.0	24	500	ND	10.0	10.0	6.5	1.8

[a]Hydrogen ion concentration determined in a suspension of 1:2 soil:$CaCl_2$ solution (0.01 M) at the end of 24 hr of incubation at 25 C.

[b]Organic matter content determined by Walkley-Black method.

[c]Lime; low = <1%; medium = 1-2%; and high = >2%.

[d]DPTA, diethylenetrianine penta-acetic acid extraction.

[e]ND = not determined.

when observed, often were equivalent to several thousand μg/g of soil air. Nitrogen dioxide is produced by the biological reduction of nitrates and nitrites under anaerobic conditions and is ultimately converted to N_2 (3). The concentration of nitrous dioxide typically increases with depth in soils (5) which is counter to the decrease in volatile fungistatic activity previously observed (20). On these bases, the elimination of N_2O appears justified. The special case for ammonia will be discussed below.

Alkenes.—The emission of alkenes was typified by the results on ethylene discussed above.

TABLE 2. Volatile compounds detected in soils

Compound type	Compounds emitted by all soils	Compounds detected for some soils, but not others
A. Alkanes	methane ethane propane	*n*-butane *n*-pentane
B. Alkenes	ethylene propylene 1-butene	*cis*-2-butene *trans*-2-butene 1-pentene[b]
C. Aldehydes		formaldehyde[a,c] acetaldehyde[d] propionaldehyde[d]
D. Ketones	acetone[a,c]	2-butanone
E. Inorganic	carbon dioxide[c] ammonia[a,c]	nitrous oxide

[a]Concentrations emitted were consistently increased by the addition of lime to soils.
[b]Not detectable under anaerobic conditions.
[c]Emanation was initiated by simple addition of water to dry soils.
[d]Detectable only after 15-20 days incubation under anaerobic conditions.

TABLE 3. Concentrations of acetone and formaldehyde in a soil atmosphere during a 3-day incubation period[a]

Soil	Soil	Acetone (μg/g air)			Formaldehyde (μg/g air)		
no.	pH	20 min	24 hr	72 hr	20 min	24 hr	72 hr
49	5.7	3.4	2.4	...[c]	0.76	...[b]	...[b]
	8.5	4.2	3.8	1.5	0.84	...[b]	...[b]
98	6.2	12.0	25.0	4.8	...[c]	...[b]	...[b]
	8.5	14.0	21.0	18.0	0.66	...[b]	...[b]
74	6.4	38.0	41.0	2.8	0.55	...[b]	...[b]
	8.5	44.0	29.0	38.0	1.2	1.8	3.0
BH	5.1	13.0	...[b]	...[b]	...[b]	...[b]	...[b]
DP	6.1	18.0	...[b]	...[b]	...[b]	...[b]	...[b]
22	7.0	4.6	9.2	...[b]	...[b]	...[b]	0.86
91	7.5	3.0	2.0	1.0	...[b]	...[b]	...[b]
110	7.8	3.0	3.2	...[b]	...[b]	...[b]	...[b]

[a]Ambient temperature of soil was 24 C.
[b]Not detectable less than 1.5 μliters/liter (ppm) for acetone and less than 0.5 μliters/liter for formaldehyde.
[c]Not measured.

Alkanes.—These play important roles in the life processes of soil microorganisms and are ubiquitously distributed throughout nature. Liming soils suppressed emanation rates and, when detected, their concentrations were relatively low (3-34 ηg/g soil). These considerations constitute reasonably rational grounds for discounting them as possible volatile fungistatic factors.

Aldehydes.—Propionaldehyde was detected rarely and then at very low concentrations. Formaldehyde was detected only during the early stages of incubation for some soils (Table 3). In one case, (No. 74), the concentration after 72 hr incubation over limed soil reached 3 μg/g air.

Ketones.—Trace amounts of 2-butanone were detected in some soils. Concentrations of acetone from 2.4 to 44 μg/g of soil occurred in all soils tested (Table 3). The addition of lime increased emission of acetone in the three acid soils tested.

Correlations between volatile accumulation and spore germination above soil.—Concentrations of acetone, formaldehyde, and ammonia above limed and nontreated soils were measured. These soils also were bioassayed by the soil emanation agar (SEA) method (7) for volatile fungistatic activity using *P. chrysogenum*, *G. simplex*, *Trichoderma viride* (Fr.) Pers., and *Zygorhynchus vuilleminii* Namaslowski.

The correlation coefficients for concentration and bioassay results were low for acetone, the highest being 0.14 (Table 4). Formaldehyde was detected in only five of the soils tested. Thus, reliable correlation analyses on these limited data were not possible.

Typical examples of correlation plots of ammonia are presented in Fig. 1. Germination reduction was correlated directly with concentrations of volatile ammonia accumulated for 24 hr above soils. The correlation coefficients (Table 4) were all significant at $P = 0.05$.

Dosage-response relations.—Ethylene was not inhibitory to conidium germination at concentrations between 1-500 μg/g air for any of the four organisms tested (Fig. 2). Decrease in germination for *P. chrysogenum*, *G. simplex*, and *V. albo-atrum* was observed only above 1,000 μg/g air. Germination of macroconidia of *F. solani* f. sp. *phaseoli* was not affected over the concentration ranges of ethylene used. Similarly, acetone at concentrations up to 1,000 μg/g air did not affect conidial germination.

Ammonia between 1 and 5 μg/g air reduced germination of conidia of *P. chrysogenum* and *G. simplex* by 45-90% relative to nontreated controls (Fig. 2).

Conidium germination of *V. albo-atrum* was 13-18% in nontreated controls, but all volatiles tested increased

TABLE 4. Correlation of germination reduction with acetone and ammonia concentrations over soil samples

Test fungi	Correlation coefficients	
	Acetone	Ammonia
Penicillium chrysogenum	0.0029	0.76
Gonatobotrys simplex	−0.13	0.70
Trichoderma viride	0.03	0.89
Zygorhynchus vuilleminii	0.14	0.97

germination especially at lower concentrations of ammonia and ethylene (Fig. 2). Further evidence that these chemicals were capable of stimulating growth of some organisms was obtained by measuring germ tube lengths of *F. solani* f. sp. *phaseoli* conidia after 24 hr. Increases in germ-tube lengths compared to controls was observed for ethylene (1-1000 μg/g air), ammonia (1-100 μg/g air), and acetone (100-1,000 μg/g air) as shown in Fig. 3.

Formaldehyde was inhibitory to conidium germination of *V. albo-atrum* (ED_{50} = 3 μg/g air), *P. chrysogenum* (ED_{50} = 3 μg/g air), and *G. simplex* (ED_{50} = 5 μg/g air) at relatively low concentrations (Fig. 4). The ED_{50} for *F. solani* f. sp. *phaseoli* was 24 μg formaldehyde/g air. Methanol was the depolymerizer in the commercial formaldehyde used in these experiments. At concentrations equivalent to those used in the dosage-response evaluations, however, methanol had no effect on conidial germination of the test organisms.

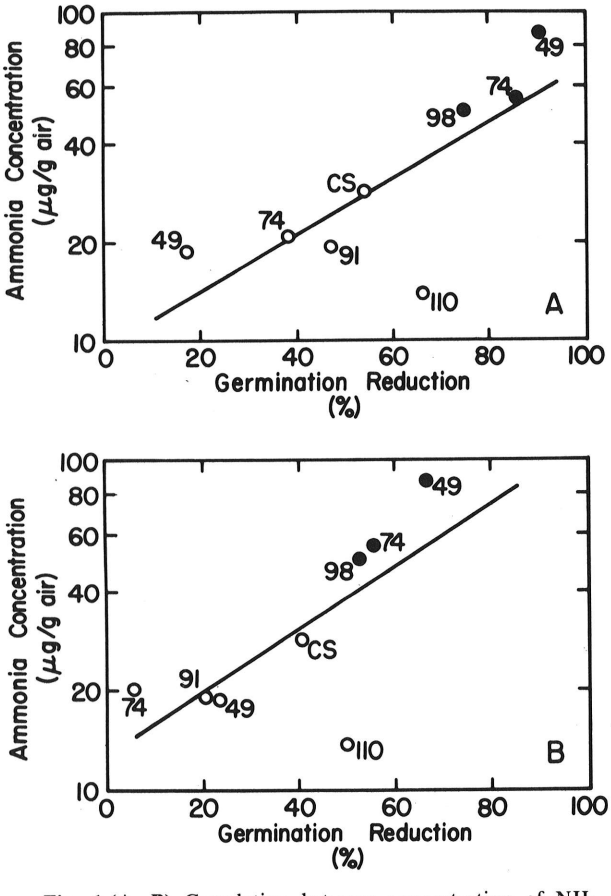

Fig. 1-(A, B) Correlation between concentration of NH_3 accumulated over soils and reduction in germination using the SEA method (7) for **A)** *Penicillium chrysogenum*, and **B)** *Gonatobotrys simplex*. Legend: 0 soil pH not adjusted; and 0 soil pH adjusted to 8.5. Reduction in germination is calculated as percentage germination of conidia exposed to soil volatiles compared with nonexposed controls. Numbers adjacent to points indicate soil designation (Table 1).

A property of the volatile fungistatic factor (6) as well as the phenomenon of soil fungistasis (15) is that nutrients nullify inhibition of germination. Several levels of glucose were added to solutions containing various concentrations of formaldehyde. *Aspergillus flavus* Link ex Fries, the test organism, has an exogenous requirement of nitrogen as well as carbon for conidium germination (19); therefore these solutions were supplemented with 0.15 ηg N/g of carbon in the form of NH_4Cl. Additions of these nutrients to formaldehyde solutions overcame inhibition from the formaldehyde (Fig. 5). In a similar experiment, inhibition of macroconidium germination of *F. solani* f. sp. *phaseoli* was not influenced by nutrients added at the same levels.

DISCUSSION

Volatiles identified from soils in this investigation at concentrations sufficient to have potential influences on spore germination were ammonia, formaldehyde, acetone, and perhaps ethylene.

Our results confirm those reported earlier (1, 4, 16) that relatively high concentrations of ethylene are required to inhibit germination of many soil fungi. Indeed, ethylene at concentrations of 1-5,000 μg/g air stimulated conidium germination of *V. albo-atrum* (Fig. 2) and increased germ tube elongation of *F. solani* f. sp. *phaseoli*. Further, cumulative amounts of ethylene emanated over soils in a 6-day period were only 0.7-35 ηg/g soil under both anaerobic and aerobic conditions. These results support Lockwood's (15) conclusion that serious doubts exist regarding the role of ethylene in soil fungistasis.

Balis (1) postulated that ethylene might induce the production of another volatile inhibitor, allyl alcohol. However, this conclusion was based on gas chromatography and absorption spectrophotometric analyses which must be interpreted as circumstantial evidence rather than direct verification. We consistently failed to detect allyl alcohol from the soils analysed for volatiles in these investigations.

While acetone was detected in relatively large concentrations and increased when soils were limed, it had little fungistatic activity (Fig. 2). In addition, its concentration in soils was not correlated with volatile fungistatic activity (Table 4). Thus, its role appears negligible.

Precise identification of formaldehyde as a volatile fungistatic factor based on the present soil emanation data is not possible. It was detected in some soils at concentrations (Table 3) presumably high enough to inhibit germination of conidia of some of the test organisms used for bioassay (Fig. 4). Correlations between formaldehyde detected in soils and their volatile fungistatic activity, however, were not possible because of the low frequency of detection of the chemical among soils. Bioassays of the effects of nutrients in overcoming inhibition of germination by formaldehyde gave different responses according to the test organism used. Nullification of germination inhibition of *A. flavus* by formaldehyde was directly correlated with increasing nutrient concentration, whereas no nutrient effect was observed for *F. solani* f. sp. *phaseoli*. It is possible that formaldehyde contributes to fungistatic activity, but

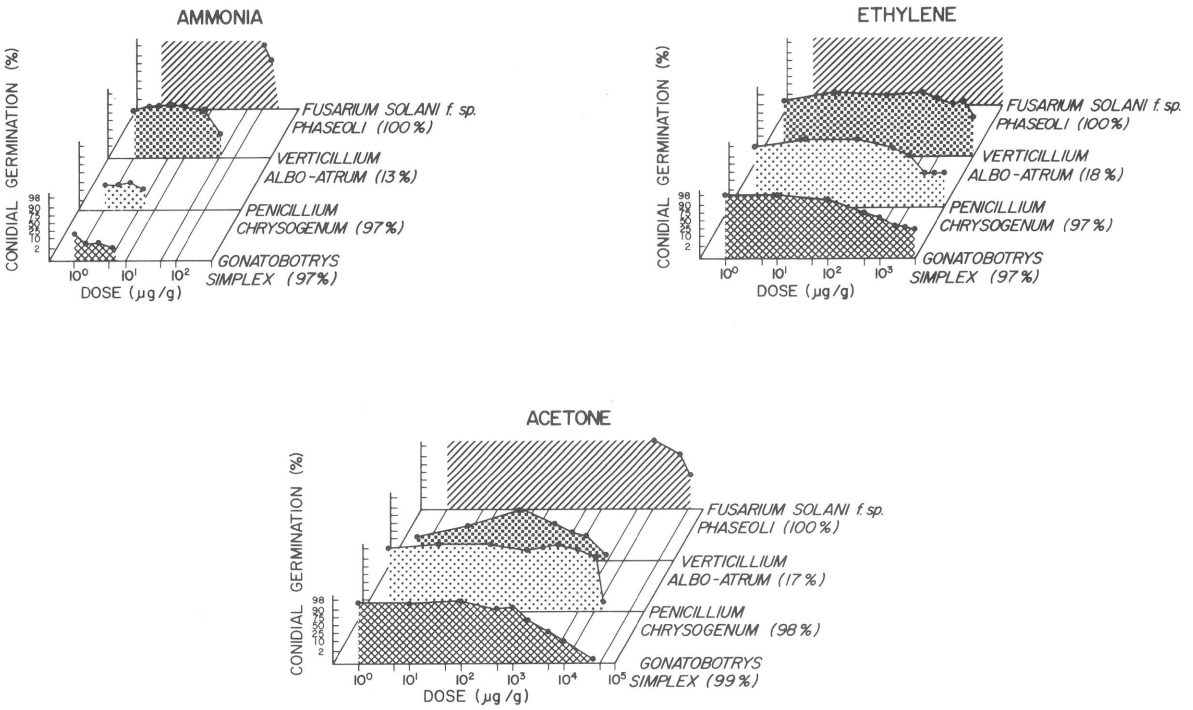

Fig. 2. Dosage response curves for ammonia, ethylene, and acetone (μg/g air) and spore germination for four test organisms. Percentages after species names indicate germination in nontreated controls.

perhaps only in some soils under certain conditions and with specific microorganisms.

Our results confirm the reports of others (14, 23) implicating ammonia as a volatile fungistatic factor in soil. The ED_{50} values measured were below 1 μg/g air for conidial germination of *P. chrysogenum* and *G. simplex* (Fig. 2). Correlations between concentrations of ammonia evolved from soils and germination of conidia of test species of fungi were significant in all cases. Qualitative evidence also can be cited; germination of *F. solani* f. sp. *phaseoli* was not inhibited over the Colorado Swale soil (generating relatively high quantities of ammonia, Fig. 1) even when an extremely sensitive bioassay was used (21). This same fungus was not inhibited by concentrations up to 100 μg ammonia/g air (Fig. 2).

Emissions of ammonia, acetone, and formaldehyde were enhanced when soils were limed. If ammonia is an inhibitor of germination for many soil fungi, this enhancement provides an explanation for increased fungistatic activity resulting from elevation of pH (9, 10, 11) or in alkaline soils (7). Soluble inorganic (22) and organic (17, 18) ammonium salts common to soils are the potential sources of the ammonium ion. In an aqueous medium such as the soil solution, the equilibrium between ammonia and the ammonium ion is described by the following reaction:

$$NH_3 + H_2O = HN_4^+ + OH^-;\ K_b = 1.86 \times 10^{-5}$$

Thus, increasing the pH by lime addition shifts this equilibrium toward ammonia (NH_3). Any such increase in the partial pressure of this compound in solution must be accompanied by a concomitant increase in the amount of ammonia released into the soil air. An excess of base (lime) should favor continued ammonia release until the soil sources of the ammonium ion are exhausted. This also provides an explanation for the abiotic generation of the volatile factor (10); i.e., the generation can be chemical as well as biological.

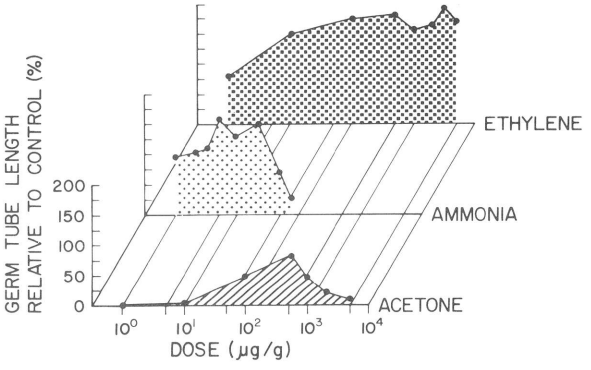

Fig. 3. Germ tube lengths of germinating macroconidia of *Fusarium solani* f. sp. *phaseoli* after 20 hr of incubation in the presence of ethylene, ammonia, and acetone.

Is ammonia the only fungistatic factor consistently found in soils? The positive correlation coefficients

observed between bioassays involving volatile fungistatic activity and concentrations of ammonia from the same soils (Fig. 1, Table 4) using a relatively insensitive method for measuring volatile fungistatic activity (7), indicate a positive response to the question. Other evidence, however, suggests that there may be one or more additional components. For instance, while ammonia suppressed conidial germination of two species of fungi (Fig. 2), it also enhanced germination of *F. solani* f. sp. *phaseoli* at all concentrations used below 50 μg/g air (Fig. 3). Yet this fungus was inhibited in sensitive bioassays by volatiles from soils of pH <7.0 (21). Again, inhibition of the same organism by volatile agents from soils of pH 5.1 and 7.5 was nullified by a potassium hydroxide trap (6). Also, in the same type of experiment, the effect of carbon dioxide removal was to inhibit germination of conidia of *A. flavus* indicating that some acid soils may produce volatiles inhibitory to this fungus. These considerations suggest that there is at least one other volatile fungistatic factor not detected or sufficiently characterized by chemical analyses coupled with bioassay.

The stimulation of conidial germination of *V. albo-atrum* by ammonia, acetone, and ethylene (Fig. 2) provides an explanation for the observed increase in germination observed using the SEA (6) and sterile Nuclepore agar diffusion (12) methods with washed discs incapable of supporting germination before exposure to soil. These volatiles substantially increased germ tube length of *F. solani* f. sp. *phaseoli* over controls (Fig. 3). Thus, volatiles in soils have capacities for decreasing and/or increasing activity of fungi and the same compound may inhibit one species and stimulate another. The concentration and/or emanation rate of the volatile may also determine its impact on biological activity. Overall, the picture is complex and generalizations, if made, have to be restricted with exceptions and reservations.

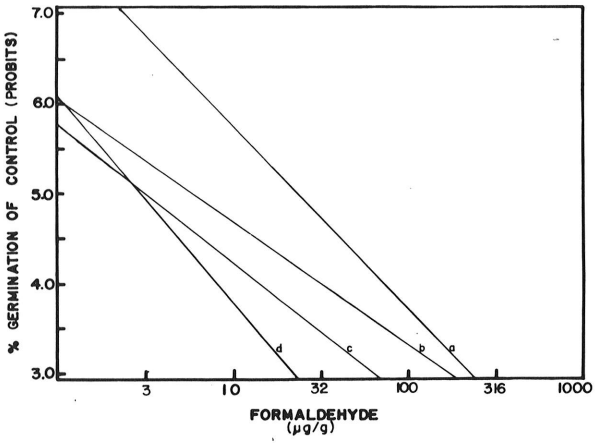

Fig. 4. Dosage-response regression lines for formaldehyde (μg/g air) and four test organisms. Curves represent pooled data from a large number of determinations. Legend: curve a, *Fusarium solani* f. sp. *phaseoli*; curve b, *Gonatobotrys simplex*; curve c, *Penicillium chrysogenum*; and curve d, *Verticillium* albo-*atrum*.

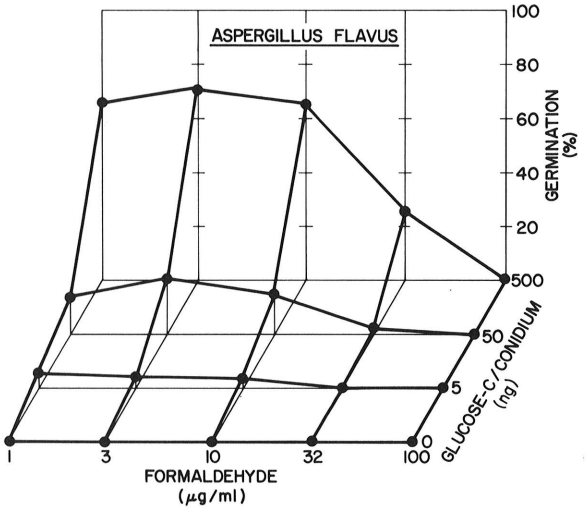

Fig. 5. Germination of *Aspergillus flavus* conidia in response to nutrients added to formaldehyde solutions.

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