Survival of Endoconidia and Chlamydospores of Thielaviopsis basicola as Affected by Volatile Soil Fungicides

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ABSTRACT

Methylisothiocyanate (MIT) and volatile materials from the decomposition of 3,5-dimethyl-tetrahydro-1, 3,5,2H-thiadiazine-2-thione (DMTT) and sodium *N*-methyldithiocarbamate (SMDC) in soil reduced germinability of endoconidia and chlamydospores of *Thielaviopsis basicola*. Fungicides with low vapor pressures in aqueous solutions or in suspensions did not produce vapors in soil toxic to spores of *T. basicola*. The toxicity of the vapors of MIT, DMTT, and SMDC to the propagules in soil was enhanced by increased concentrations and length of exposure. SMDC was more effective than MIT and DMTT. Endoconidia of *T. basicola* were slightly more sensitive than chlamydospores to the vapors from SMDC. The three volatile fungicides were fungicidal rather than fungistatic. Alfalfa hay, kaolinite, and NH_4Cl, added to soil with DMTT, reduced the effectiveness of the fungicide. Alfalfa hay added to soil 3 and 9 weeks before the fumigant, and montmorillonite added with the fumigant, did not reduce the effectiveness of the fungicide. *Thielaviopsis basicola* inoculum in undecomposed or partially decomposed bean hypocotyls was less sensitive to vapors from DMTT than was free chlamydospore inoculum in soil. The effectiveness of DMTT to reduce germinability of chlamydospores and black root rot of bean varied with the inoculum density of the pathogen in soil.

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Black root rot of economic plants caused by *Thielaviopsis basicola* (Berk. & Br.) Ferr. can be suppressed by the use of fumigants (3, 11, 12) and nonvolatile fungicides (3, 10, 11, 13). Recently, Papavizas & Lewis (10) obtained good control of black root rot of bean (*Phaseolus vulgaris*) and tobacco (*Nicotiana tabacum*) with the volatile fungicides methylisothiocyanate (MIT), 3,5-dimethyl-tetrahydro-1,3,5,2H-thiadiazine-2-thione (DMTT), and sodium *N*-methyldithiocarbamate (SMDC). The present study was undertaken to evaluate the effects of the volatile fungicides on the survival of endoconidia and chlamydospores of *T. basicola* in soil.

MATERIALS AND METHODS.—Rumford loamy sand was used throughout. Unless otherwise indicated, the soil pH was 5.3. The soil contained 1.6% total C, 0.13% total N, and had a water-holding capacity (WHC) of 30%. Before use the soil was air-dried, passed through a 5-mm sieve, and mixed thoroughly. All amounts of soil are given on the dry weight basis.

Apparatus.—Apparatus A (Fig. 1-A) was used for the study of the effects of volatile fungicides on spore germinability. It consists of a 1-liter Erlenmeyer flask (Y) containing 500 g soil and the volatile fungicide. A plexiglass cylinder (X) (15 × 2.5 cm) is connected to the flask by glass tubing through a hole in the flask rubber stopper. Glass wool (2 to 3 cm thick) is placed in the bottom of the tube to facilitate gas dispersion. Fifty-g samples of soil infested with endoconidia or chlamydospores of *T. basicola* are placed in the tubes, and the tubes are stoppered with perforated rubber stoppers. Compressed moist air, regulated to flow at ca. 480 ml/hr, is passed through an inlet (Z) to sweep the volatile substances emanating from the treated soil in Y through the pathogen-infested soil in X.

The apparatus used to study the effect of volatile substances on the inoculum density of *T. basicola* in relation to black root rot of bean caused by this

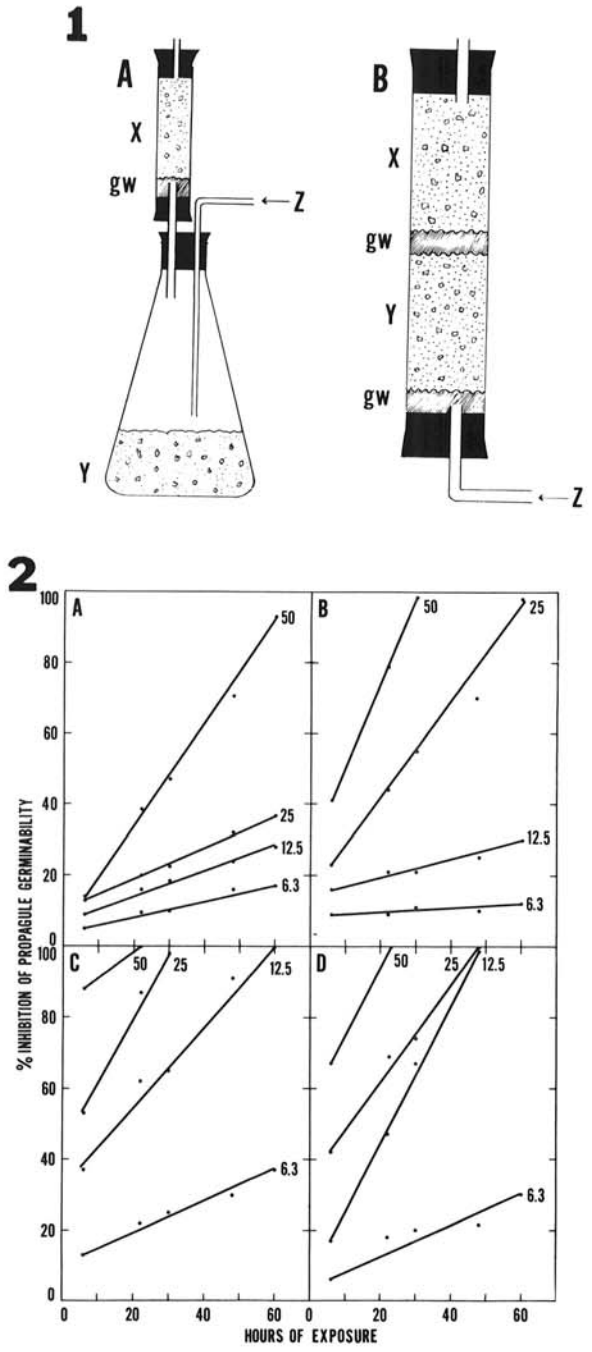

Fig. 1-2. 1) Schematic diagram of apparatus A and B used for fumigating 50 and 600 g of soil, respectively. X = plexiglass cylinder containing soil infested with *Thielaviopsis basicola* propagules; gw = glass wool; Z = air inlet; Y = Erlenmeyer flask in A or cylinder in B containing soil and fumigant. **2)** Effect of volatile fungicides on germinability of propagules of *T. basicola*. The numbers at each line of the slope of inhibition represent μg/g soil (active material). **A)** Methylisothiocyanate on chlamydospores. **B)** 3,5-dimethyl-tetrahydro-1,3,5,2H-thiadiazine-2-thione on chlamydospores. **C)** Sodium *N*-methyldithiocarbamate (SMDC) on endoconidia. **D)** SMDC on chlamydospores.

pathogen is shown in Fig. 1-B. It consists of a plexiglass tube (51 X 7 cm) containing 600 g soil that had been treated with the volatile fungicides (Y), and 600 g soil infested with endoconidia or chlamydospores (X). The soils are separated by glass wool (10 cm thick) into two portions. After fumigation, the soil in the upper portion may be transferred to a 4-inch pot for growing beans.

Fungicides.—MIT and SMDC were applied to soil as aqueous solutions at concentrations calculated to add the desired amount of fumigant and to bring the soil moisture to about 45 to 50% of the WHC. A DMTT preparation (DMTT:talc, 1:25 w/w) was mixed with the soil before adding water. Benomyl, 2-(4-Thiazolyl)-benzimidazole (TBZ), nabam, captan, and 2-methylsulfonyl-6-nitrobenzothiazole (MSNB) were mixed with the soil as aqueous suspensions. The nonvolatile fungicides were added to soil at 200 μg/g soil (active material). The volatile fungicides were added at concentrations specified in each experiment.

Inoculum.—Endoconidia and chlamydospores of *T. basicola* were obtained as previously described (9) from 3-week-old cultures grown on Czapek-Dox agar containing 0.25% yeast extract. Chlamydospores were added to soil as aqueous suspensions, and the soil was kept at ca. 40 to 50% of its WHC for 10 days. The soil was then air-dried, mixed thoroughly for 30 min in a Patterson-Kelley twin shell blender with an intensifier to assure complete and uniform distribution of inoculum, and kept at 4 to 5 C. This procedure insured the breaking of chlamydospore chains into individual chlamydospores. When needed, chlamydospore-infested soil was brought to 45 to 50% of its WHC and used for the tests. Aqueous suspensions of endoconidia were added to dry soil immediately before use so that the soil moisture was increased to about 45 to 50% of WHC.

Germinability of the two spore forms of *T. basicola* was assayed by the propagule assay method (8) on a medium developed by Papavizas (7) for the detection and isolation of *T. basicola*. The ability of 400 propagules/soil treatment per collection date to germinate on the agar at 20 to 22 C was determined by microscopic examination. Five-g soil samples were used from tube X of apparatus A after fumigation, and a standard germination time of 16 to 18 hr was adopted from previous experience (9).

To find out whether the effectiveness of the volatile fungicides may be reduced in a given soil when the inoculum is protected in plant tissue, bean hypocotyls infected with *T. basicola* were washed with running tap water, cut into 0.5-cm segments, and added to soil at the rate of 1.3 g/50 g soil. The bean hypocotyl segments were not dried prior to addition to soil. In some experiments, undecomposed bean hypocotyl inoculum was used immediately after washing. In other experiments, partially decomposed bean hypocotyl inoculum was used. Partially decomposed hypocotyl inoculum was obtained by incubating the segments in moist soil at 22 to 23 C for 1 month prior to use in tube X of apparatus A.

In all cases where infected hypocotyl segments were used as inoculum, survival of *T. basicola* in the

segments was assayed after fumigation as follows: The 50-g soil samples containing the hypocotyl inoculum, fumigated with DMTT or SMDC, were comminuted in 450 ml sterile tap water in a blender for 1 min at ca. 2,500 rpm. Appropriate dilutions were made from the comminuted samples on the isolation medium. Without fumigation, 1.3 g segments/50 g soil resulted in ca. 20,000 to 25,000 colonies/g of soil.

RESULTS.—With the exception of MIT, DMTT, and SMDC, the fungicides did not produce vapors in soil toxic to chlamydospores of *T. basicola* (Table 1). Vapors from MIT, DMTT, and SMDC were extremely toxic even after 48 hr of exposure.

Effect of MIT, DMTT, and SMDC on germinability of spores.—MIT was included in these tests because it is known to be produced, along with other products, during the decomposition of DMTT and SMDC in soil (4, 5, 6, 14). Per cent inhibition of propagule germinability was plotted against time of exposure for each concentration of the fumigant used. The slopes of the dosage response curves for MIT, DMTT, and SMDC with chlamydospores, and SMDC with endoconidia, increased with increasing concentration and exposure time (Fig. 2). The dosage-response depended on the kind of fumigant and propagule used.

An ED_{50} value for chlamydospores was obtained with MIT only at 50 μg/g soil in flask Y and about 30 hr of exposure (Fig. 2-A). DMTT was more effective than MIT at 25 and 50 μg/g, but not at 6.3 and 12.5 μg/g (Fig. 2-B). An ED_{50} value was obtained with 25 μg/g soil of DMTT after ca. 25 hr of exposure. SMDC was the most effective of the three volatile fungicides used. Even 12.5 μg/g of SMDC resulted in 50% inhibition of both chlamydospore and endoconidial germinability after 20 hr of exposure (Fig. 2-C, D). Endoconidia were slightly more sensitive to the vapors from SMDC than were chlamydospores. Results obtained with 100 μg/g soil of the volatile compounds were not included in Fig. 2. This high concentration was so toxic to spores that none of them survived after 6 hr of exposure.

After these experiments were terminated, the remainder of the *T. basicola*-infested soil from tube X of apparatus A was transferred to 250-ml beakers and kept at ca. 40% of the WHC for 3 weeks at 20 C. Subsequent bioassays with the isolation medium showed that the vapors from MIT, DMTT, and SMDC had killed the propagules.

Effect of soil amendments on the effectiveness of DMTT.—The following materials were added to soil in flask Y of apparatus A with, or before, the addition of DMTT (at 50 μg/g soil) (active):kaolinite or montmorillonite at 1 and 5% (w/w) each; NH_4Cl at 200 μg/g N added to acid soil (pH 5.3) and to alkaline soil [soil adjusted to pH 7.6 with $Ca(OH)_2$ 3 weeks before use]; and dry, ground alfalfa hay at 1% (w/w) added 0, 3, and 9 weeks prior to DMTT incorporation. Kaolinite, montmorillonite, and NH_4Cl were added to soil together with DMTT.

Results obtained after 1 week of exposure (Fig. 3)

TABLE 1. Inhibition of germinability of chlamydospores of *Thielaviopsis basicola* in soil as affected by the length of exposure to volatile substances liberated during the decomposition of fungicides in soil

Fungicide[a]	Per cent inhibition of germinability after the indicated exposure time in hr[b]		
	48	120	288
Benomyl	0	0	2
Captan	0	0	0
Nabam	0	0	0
TBZ	0	0	0
MSNB	0	0	0
MIT	90	100	100
DMTT	98	100	100
SMDC	100	100	100

[a]All fungicides were added to soil at 200 μg/g soil of active material. TBZ = 2-(4-Thiazolyl)-benzimidazole; MSNB = 2-methylsulfonyl-6-nitrobenzothiazole; MIT = methylisothiocyanate; DMTT = 3,5-dimethyl-tetrahydro-1,3,5,2H-thiadiazine-2-thione; SMDC = sodium *N*-methyldithiocarbamate.

[b]Germinability of chlamydospores from soil was assayed on dextrose-yeast extract agar containing pentachloronitrobenzene and other antimicrobial agents.

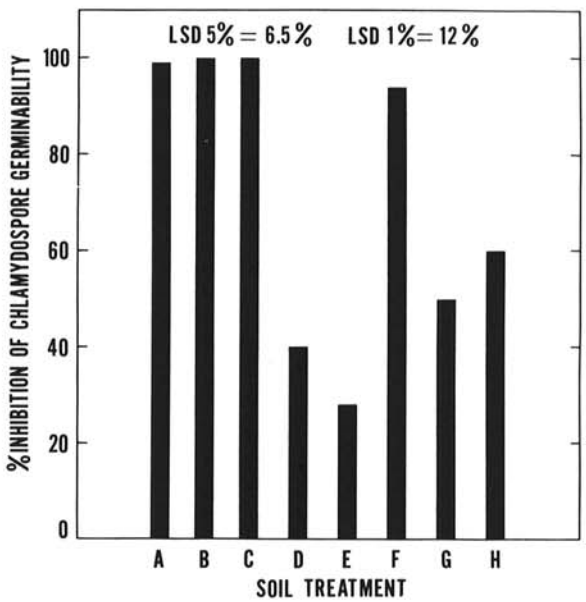

Fig. 3. Effect of soil amendments on the effectiveness of 3,5-dimethyl-tetrahydro-1,3,5,2H-thiadiazine-2-thione (DMTT) against chlamydospores of *Thielaviopsis basicola*. Letters under columns indicate the following: A, DMTT alone at 50 μg/g soil; B, alfalfa hay added to soil 9 weeks prior to DMTT; C, alfalfa hay added to soil 3 weeks prior to DMTT; D, alfalfa hay added to soil concurrently with DMTT; E, kaolinite at 1% added to soil concurrently with DMTT; F, montmorillonite at 1% added to soil concurrently with DMTT; G, NH_4Cl at 200 μg/g N added to acid soil (pH 5.3) concurrently with DMTT; and H, NH_4Cl at 200 μg/g N added to alkaline soil (pH 7.6) concurrently with DMTT. DMTT was added at 50 μg/g soil in all treatments.

show that the effectiveness of vapors from DMTT in reducing germinability of chlamydospores was significantly decreased by kaolinite at 1 and 5% (the 5% concentration is not shown in the graph), NH_4Cl added to either acid or alkaline soil, and alfalfa added to soil alone with DMTT. In a supplementary experiment, no differences in effectiveness of DMTT were observed in soil of pH 5.3 and 7.3 after 24 and 96 hr of exposure. The alfalfa amendment did not decrease the effectiveness of DMTT when the tissue was allowed to decompose in soil for 3 or 9 weeks. Montmorillonite at both concentrations did not reduce the toxic effects of the fungicide. Soil pH did not change appreciably with the various treatments.

Effect of kind of inoculum and mode of DMTT and SMDC application upon fumigant effectiveness.—An experiment was performed with four treatments. In Treatment I, the inoculum consisted of free chlamydospores in soil. Undecomposed bean hypocotyl inoculum was used in Treatment II. Partially decomposed bean hypocotyl inoculum was used in Treatments III and IV. For Treatments I, II, and III, DMTT or SMDC was mixed with the soil in flask Y of apparatus A at 50, 100, and 200 μg/g soil. Therefore, the two fungicides acted exclusively as fumigants. In Treatment IV, the partially decomposed hypocotyl segments and the fumigants were mixed concurrently with the soil, and the mixtures were placed in 150-ml beakers. Assays were performed with the dilution-plate method after 1 week of incubation as described in MATERIALS AND METHODS.

When the inoculum of *T. basicola* was protected, at least to some extent, within undecomposed or partially decomposed bean hypocotyl tissue, 50 μg/g of DMTT did not reduce viability by fumigation (Fig. 4-A, Treatments II, III). The effectiveness of DMTT at 50 μg/g soil was higher when the fungicide was added directly to the soil containing the inoculum (Treatment IV) than when used strictly as fumigant in apparatus A. DMTT was most effective when the inoculum consisted of chlamydospores free in soil (Treatment I). At 100 and 200 μg/g of DMTT, no differences were observed among kinds of inoculum or between the two modes of application. Similar results were obtained with SMDC.

Relationship of inoculum density and fumigant efficiency.—An experiment was performed with apparatus B to establish whether there is a relationship between inoculum density of *T. basicola*, root rot severity of bean, and fumigant efficiency. Soil heavily infested with chlamydospores was divided into three portions and diluted with increasing amounts of uninfested soil. The low inoculum portion contained 1.5×10^3 chlamydospores/g soil. The medium and high inoculum portions contained 2.4×10^4 and 9.6×10^4 chlamydospores/g soil, respectively. Six hundred g of each soil were placed in tube X of apparatus B. Six hundred g of uninfested soil containing DMTT at 25 or 100 μg/g were placed in tube Y. After 1 week of fumigation, 10-g samples from tube X were used for inoculum density determinations with the dilution-plate method. The remainder of the soil in tube X was transferred to 4-inch plastic pots and planted to beans. A disease severity index was calculated as described previously (11).

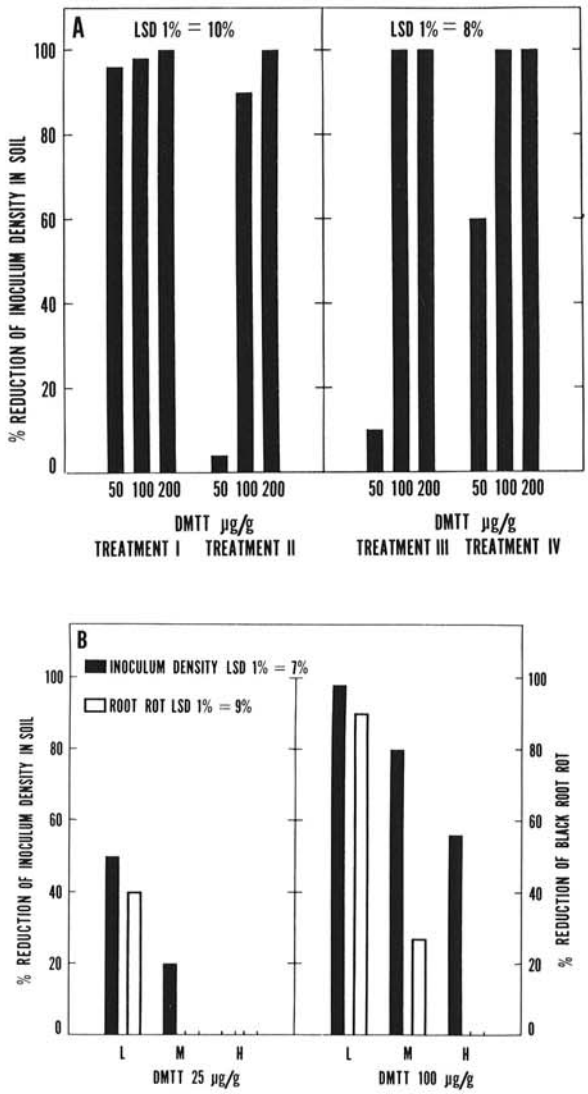

Fig. 4. A) The effect of the kind of inoculum and mode of application of 3,5-dimethyl-tetrahydro-1,3,5,2H-thiadiazine-2-thione (DMTT) on the effectiveness of DMTT against chlamydospores of *Thielaviopsis basicola.* Treatment I, free chlamydospores in soil; Treatment II, undecomposed bean hypocotyl inoculum; Treatments III and IV, partially decomposed bean hypocotyl inoculum. DMTT was used strictly as a fumigant in Treatments I, II, and III. In Treatment IV, DMTT was mixed with the soil and inoculum. **B)** Relationship of inhibition of inoculum density of *T. basicola* and of root rot of bean with two concentrations of DMTT. Letters under columns indicate the following: L, low inoculum (1.5×10^3 chlamydospores/g soil); M, medium inoculum (2.4×10^4 chlamydospores/g soil); H, high inoculum (9.6×10^4 chlamydospores/g soil).

The relationship between per cent reduction of chlamydospore inoculum, DMTT concentration, and per cent inhibition of black root rot is shown in Fig.

4-B. At 25 μg/g of DMTT, inoculum density was reduced ca. 50, 20, and 0% in the low, medium, and high inoculum density soil, respectively. There was a 40% reduction of root rot in soil fumigated with 25 μg/g of DMTT in the low inoculum soil, but no reduction in the medium and high inoculum soils. Inoculum density was reduced ca. 100, 80, and 60% in the low, medium, and high inoculum soil, respectively, by 100 μg/g of DMTT. Root rot was almost eliminated by 100 μg/g of DMTT in the low inoculum soil, but not in the medium or high inoculum soil. Similar results were obtained with endoconidia.

DISCUSSION.—Although high concentrations and long exposures were used, the decomposition of benomyl, captan, TBZ, and MSNB did not yield any vapors toxic to endoconidia and chlamydospores of *T. basicola* in the effluent air from soil (Table 1). The ineffectiveness of nabam was somewhat unexpected. Munnecke et al. (4) found that CS_2 was produced in detectable amounts from soil by nabam with a peak of production after about 60 to 80 hr from nabam addition to soil. Even if CS_2 were produced, however, no information exists on its toxicity to propagules of *T. basicola*.

MIT was used in our experiments as a reference because it is known as one of the principal compounds in the soil vapors from DMTT and SMDC (4, 5, 6, 14). When the effluent air carried MIT through the tube of *T. basicola*-infested soil (Fig. 1), the germinability of chlamydospores and endoconidia was irreversibly reduced. Although other compounds may have been present, the data in Fig. 2 suggest that MIT was the active fungicidal constituent against *T. basicola* in the vapor phase from DMTT and SMDC. This assumption is further substantiated by evidence with other soil-borne plant pathogens (4). Our data also establish the fact that control of black root rot of economic crops by DMTT and SMDC (3, 10, 11, 12) is probably due to the direct and irreversible toxicity of the vapors from fumigants on the propagules of *T. basicola*. The somewhat reduced effectiveness of MIT at low concentrations (25 μg/g soil or less) may have been the result of the ability of MIT to escape rapidly with the effluent air immediately after the initiation of the experiments. Munnecke & Martin (5) observed that the peak cumulative production of MIT from DMTT in an acid soil (pH 5.3) occurred after about 136 hr.

It has already been established (5) that an increase in clay or organic content of soil decreases the amount of MIT released. In later experiments, Munnecke et al. (6) observed that addition of attapulgite, kaolinite, or bentonite clay to silica sand resulted in an increase in rate of MIT release initially, but the total amount released equaled that released in silica sand alone. Ashley et al. (1) also observed that in a clay soil (pH 6.8) with 6.7% organic matter, all MIT was released in 20 hr. In a high organic-content soil (49.4%), it took more than 1,000 hr. Our experiments with kaolinite and fresh alfalfa hay substantiate these observations. These materials may have reduced the rate of release of MIT, with a consequent reduction in effectiveness. The results with montmorillonite or alfalfa hay added to soil several weeks prior to fumigation, however, indicate that phenomena occurring in the presence of organic matter or clays may be more complicated than one might expect in a model system. Changes in soil microflora may be important in this respect, but have not been studied here. Also, it cannot be explained why montmorillonite failed to decrease the effectiveness of DMTT. In addition to organic matter and clays, many other factors (soil pH, temperature, moisture) are known to influence the rate of release or retention of MIT from DMTT or SMDC (1, 4, 5, 6). On the other hand, the ability of NH_4Cl to reduce the effectiveness of DMTT was expected. NH_4-N is known to combine with MIT to form nonvolatile thioureas (14).

Bald & Jefferson (2) introduced the concept that a dosage of a fungicide reduces the inoculum potential of a pathogen in inverse proportion to the amount of inoculum present. Our results with *T. basicola* (Fig. 4-B) are in line with this concept. Failure to control black root rot with low concentrations of fumigants in heavily infested soils may be accounted for by assuming that the effectiveness of the fungicide is reduced against high inoculum density. Our results in Fig. 4-A introduce another useful concept: namely, that it is not only the inoculum density that may be important, but also the kind and site of inoculum and the presence or absence of protective plant residues. Higher concentrations of fumigant were required to kill inoculum protected by bean hypocotyl residue than inoculum present in a free condition in soil; and it is the protective inoculum that actually exists in soil, at least until infected plant residues are thoroughly decomposed.

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