Effect of Sulfur-Containing Volatile Compounds and Vapors from Cabbage Decomposition on Aphanomyces euteiches

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ABSTRACT

Vapors from several sulfur-containing compounds adversely affected various processes in the life cycle of *Aphanomyces euteiches*. Isothiocyanate vapors were more effective than those of sulfides in inhibiting growth, zoospore formation, motility, and zoospore germination. For example, mycelial growth and zoospore formation and germination were prevented by vapors of 0.04, 0.10, and 0.30 parts per million (ppm) of allylisothiocyanate (AIT), respectively, and the same processes were prevented by vapors from 20, 100, and 350 ppm of $(CH_3)_2S_2$. Sublethal amounts of the volatiles had no effect on oospore formation. Pea root rot was reduced by more than 90% when soils were fumigated with CS_2, $(CH_3)_2S_2$, AIT, methylisothiocyanate (MIT), or CH_3SH. Materials not as effective in disease reduction included butylisothiocyanate (BIT), phenethylisothiocyanate (PhIT), and $(CH_3)_2S$. Vapors from the decomposition of cabbage tissue, an amendment which suppressed disease, adversely affected morphology of the fungus, development of oospores, and mycelial growth. Vapors arising from the decomposition of corn tissue, an amendment which did not suppress disease, had no effect on the fungus. Phytopathology 61:208-214.

Root rot of peas caused by *Aphanomyces euteiches* Drechs. can be appreciably reduced in the greenhouse by the incorporation into soil of stem and leaf tissue of crucifers such as cabbage, kale, mustard, turnip, and Brussels sprouts (20, 21). In the greenhouse, cabbage tissue used as an amendment also significantly reduced root rot of bean (22, 24) and root rot of sesame (P. B. Adams, *unpublished data*), both caused by *Thielaviopsis basicola*. Cabbage tissue was not effective in reducing root rot of beans and peas caused by *Rhizoctonia solani* (17, 20).

One mechanism of pea root rot suppression by cruciferous amendments (20, 21) may be the adverse effect on *A. euteiches* by volatile substances evolved during decomposition of the amendment. Crucifers contain an abundance of sulfur-containing (S-containing) materials. The S-containing volatiles reported to be present in cabbage, or obtained from its decomposition, include mercaptans (14, 16), sulfides of various types (2, 9, 16), and isothiocyanates (2, 5, 12, 16). Recently, we found that materials such as methanethiol (CH_3SH), dimethyl sulfide ($[CH_3]_2S$), and dimethyl disulfide ($[CH_3]_2S_2$) were evolved from decomposing cabbage tissue in soil (14). Most of the S-containing volatiles, however, have been found in crucifers either as a result of chemical extraction (5), distillation (2, 9), or in vitro enzymatic hydrolysis of extract components (1, 2, 12).

Toxicity of S-containing volatiles has been demonstrated with many fungi. Mercaptans, for example, prevent germination of sclerotia of *Sclerotium cepivorum* (6), and mercaptans and sulfides are toxic to *Colletotrichum circinans* and *Botrytis allii* (26). Isothiocyanate vapors also adversely affected various other plant pathogens (11, 18).

The purpose of this study was to determine the effects of several known S-containing volatiles and vapors from decomposing cabbage on various stages in the life cycle of *A. euteiches* and on the root rot caused by this fungus. A preliminary report has been published (13).

MATERIALS AND METHODS.—Isolate A7 of *A. euteiches*, obtained from Adele S. Lawyer, Calif. Packing Corp., was used throughout. Corn broth was prepared by heating 100 g of corn kernels in 1 liter of distilled water for 15 min in flowing steam. After cooling, the volume of the filtrate was adjusted to 1 liter and, when required, agar (1.5%) was added. Inoculations were made with 5-mm discs of the fungus cut from 5-day-old petri dish colonies. Cultures were incubated at 25 C.

The soil used was a natural Galestown-Evesboro loamy sand, pH 5.6. The soil was air dried and passed through a 10-mesh screen before use. Ground, mature leaf and stem tissue of air-dried cabbage (*Brassica oleracea* var. *capitata* L.) and air-dried corn (*Zea mays* L.) were used as amendments. Corn was chosen because its incorporation into soil does not suppress pea root rot (10, 21). Additional properties of the soil have been reported (14).

Isothiocyanates, sulfides, and disulfides from commercial sources were used without additional purification. They included methylisothiocyanate (MIT), butylisothiocyanate (BIT), allylisothiocyanate (AIT), phenethylisothiocyanate (PhIT), carbon disulfide CS_2, methanethiol CH_3SH, dimethyl sulfide $(CH_3)_2S$, and dimethyl disulfide $(CH_3)_2S_2$. Four replications were used throughout, and all experiments were repeated at least twice.

Growth.—The effect of volatile isothiocyanates and sulfides on growth of *A. euteiches* was determined as follows: Forty ml of liquid medium in 125-ml Erlenmeyer flasks were inoculated with 5-mm agar discs from a 3-day-old culture of the fungus, and four of these flasks were placed in 3.5-liter jars each containing a 10-ml beaker with an appropriate dilution of the volatile material. Quantities of the materials were chosen so that, assuming complete volatilization, the atmospheres of the jars contained 0.01 to 1,000 ppm of the S-containing volatiles. No S-containing volatiles

were added to control jars. The jars were sealed with plastic household wrap and metal screw caps. Cultures were incubated in this condition for 14 days, after which time the mycelial dry wt were determined. Preliminary results indicated that oxygen depletion was not a limiting factor with this method.

To determine the effect of volatiles from decomposing cabbage and corn tissues in soil on growth of the fungus, 1-kg portions of soil were amended with the tissues at a rate of 5% and brought to approx 50% of the moisture-holding capacity. Control soils contained no amendments. The soils were placed in desiccators (250 mm, internal diam) and covered. At periodic intervals the soils were mixed, and inoculated petri dishes (100 mm, outer diam) of the corn decoction agar were placed on the surface of the soil. Periodic mixing of the soils prevented oxygen depletion. Fungal growth rates were determined by daily measurements of the colony radius.

Zoospore formation, motility, and germination.— Zoospores were prepared by the method of Cunningham & Hagedorn (8) and their concn were determined with a hemacytometer. To determine the effects of vapors from isothiocyanates, sulfides, and disulfides on zoospore formation, the flasks with the mycelial mats in tap and distilled water were placed in closed 3.5-liter jars containing 10-ml beakers with various dilutions of the S-containing materials. To determine the effects of volatiles released from the decomposition of cabbage and corn tissues in soil on zoospore formation, materials in the atmospheres above amended soils were trapped in water during a 10-day period. Amendments were added to soils at rates of 1.0, 2.0, and 5.0%. The liquids were then mixed with an equal amt of the tap and distilled water containing the mycelial mats.

To observe the effects of the S-containing volatiles on zoospore motility, 5-ml aliquots of the zoospore suspension were pipetted into 50-mm glass petri dishes which were then placed in the 3.5-liter jars which contained beakers holding various dilutions of the S-containing volatiles. To determine the effect of volatiles from the decomposing amendments on motility, the water which contained trapped vapors was added to an equal amt of zoospore suspension. Periodically, samples from all treatments were withdrawn and placed in a hemacytometer. The per cent zoospore motility was determined by counting the nonmotile zoospores initially present in the preparation and substracting the amt from the total zoospore population. The total population was counted after application to the hemacytometer containing the suspension of slight heat to eliminate motility.

To study the effect of S-containing volatiles on zoospore germination, droplets of zoospore suspensions were placed on petri dishes containing 10 ml of the corn decoction agar. Dishes were then placed in closed jars containing dilutions of the S-containing volatiles in beakers. We studied the effect of volatiles evolved from decomposing cabbage and corn tissues on zoospore germination by placing petri dishes of agar containing droplets of a zoospore suspension on the surface of amended soils in desiccators. These plates were allowed to incubate under the indicated conditions for 24 hr before the per cent germination was determined.

Oospore formation.— The effect of the S-containing volatiles on the formation of oospores was determined by placing freshly inoculated petri dishes of the fungus in 3.5-liter jars containing 0.07 mg (0.02 ppm) isothiocyanates or 7.0 mg (2.0 ppm) sulfides or disulfides. These concn were sublethal and allowed some growth of the fungus. The jars were sealed, and after 14 days' incubation the oospore index was determined (23). To study the effect of vapors from decomposing amendments on morphology and oospore formation, inoculated flasks of the chemically defined medium SM-1 (23) were placed in desiccators containing soil (1 kg) amended with cabbage or corn tissues (2%). Amended soils were mixed periodically. Microscopic observation of the mycelium was performed after 20 days of incubation.

Root rot development.—Vapors from 1.0 g of the S-containing materials and those vapors evolved from decomposing cabbage or corn tissues in soil (5% in 1 kg soil) were passed through four 1-kg portions of soil naturally infested with *A. euteiches*. This was accomplished by pumping air through closed vessels which contained the S-containing materials or soils amended with cabbage or corn tissues, and immediately passing this air through infested soil. This was essentially a soil fumigation procedure. After 2 weeks of this fumigation, replicate soils of each treatment were mixed together, placed in No. 4 plastic pots, and allowed to remain in the greenhouse for 3 weeks. The soils were then planted to peas (*Pisum sativum* L. 'Early Alaska') at the rate of 7 seeds/pot. After 5 weeks of growth, plants were harvested and their disease severity indices (DSI) were determined on an infection scale of 0 (no symptoms) to 100 (all roots and epicotyls rotted).

RESULTS.—*Growth.*—The effect of vapors of isothiocyanates and sulfides on the mycelial growth of *A. euteiches* varied with the type of material used (Fig. 1-A). The effective concn of the volatiles which completely prevented growth ranged from 0.04 ppm for AIT to greater than 1,000 ppm for $(CH_3)_2S$. Two distinct sets of responses to the effects of the vapors of the materials were observed. First, in pure culture, vapors of the isothiocyanates were extremely toxic to the fungus. Concentrations of only 0.7 ppm or less of AIT, MIT, BIT, or PhIT in the atmosphere prevented growth. Smaller amt of isothiocyanates with high vapor pressures (AIT or MIT) were more toxic than larger amt of materials with low vapor pressures (BIT or PhIT). Second, vapors of sulfides and disulfides were not as toxic to mycelial growth of *A. euteiches* as were those of isothiocyanates. CS_2, CH_3SH, and $(CH_3)_2S_2$ completely prevented growth at a concn range of 10-30 ppm. At a lower concn (2.0 ppm), however, growth equivalent to 50% of the control occurred. $(CH_3)_2S$ was the least toxic to growth of all the materials used, and even at 1,000 ppm there was growth equivalent to 30% of the control. The fungus did not recover from the effects of volatiles when inoculated flasks with no

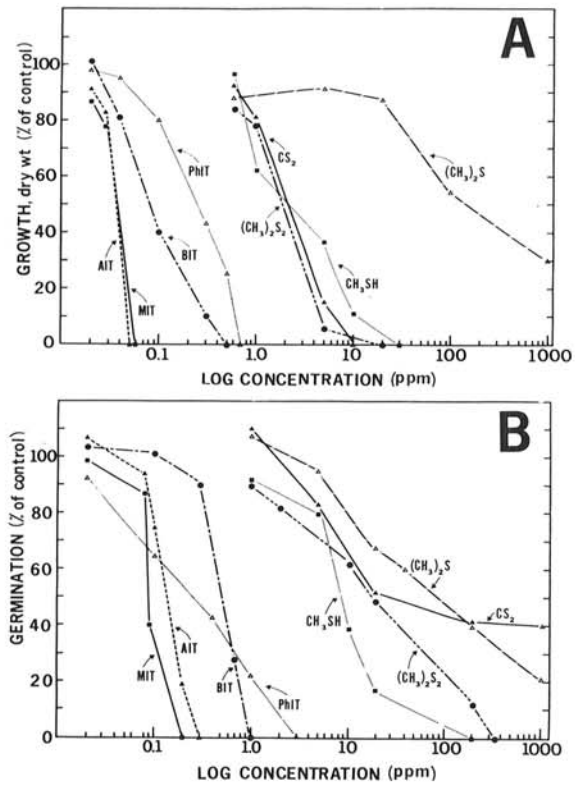

Fig. 1. **A)** Effect of vapor phase of various S-containing volatiles on growth of *Aphanomyces euteiches* in pure culture. Average dry wt of mycelial mat of fungus grown under control conditions was 32.7 mg. **B)** Zoospore germination of *Aphanomyces euteiches* as affected by the vapor phase of various S-containing volatiles. Average germination under control conditions was 67%. Volatiles are abbreviated as follows: allylisothiocyanate (AIT); methylisothiocyanate (MIT); butylisothiocyanate (BIT); phenethylisothiocyanate (PhIT).

growth were transferred from an atmosphere of the volatiles to air at 25 C.

The effect of volatiles from the decomposing amendments on radial growth of *A. euteiches* is indicated in Fig. 2. As early as 2 days after the beginning of amendment decomposition, volatiles from decomposing cabbage completely prevented growth of the fungus. This effect lasted for 26 days. *Aphanomyces euteiches* did not recover when the plates were transferred from the atmosphere above decomposing cabbage to atmospheric air at 25 C. Volatiles from decomposing corn did not prevent growth of the fungus.

Zoospore formation, motility, and germination.— Vapors of isothiocyanates were more inhibitory to zoospore formation than were sulfides (Table 1). AIT and MIT were also more inhibitory than BIT or PhIT. For example, AIT and MIT completely prevented zoospore formation at concn of 0.1 ppm. BIT and PhIT completely inhibited zoospore formation at concn 10 times higher than those of AIT and MIT. One thousand times more of the sulfides and disulfides were needed than MIT and AIT to obtain total inhibition of zoospore formation.

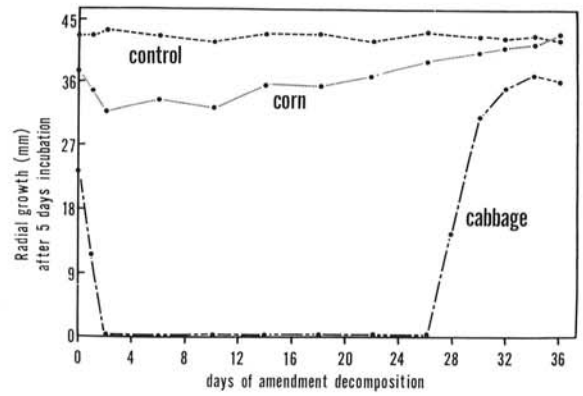

Fig. 2. Effect of vapors from decomposing amendments (5%) on linear growth of *Aphanomyces euteiches*. Inoculated plates were placed on surface of soils for total of 5 days at indicated days of amendment decomposition.

The volatiles from cabbage and corn tissues decomposing in soil and trapped in water had little effect on zoospore formation. Although the trapped volatiles from a high concn (5%) of decomposing cabbage decreased zoospore formation by 13%, the decrease was not significantly different from the control. S-containing volatiles from decomposing cabbage were evidently trapped in the water, because constituents similar to CH_3SH, $(CH_3)_2S$, and $(CH_3)_2S_2$ were detected by gas chromatographic analysis in the aq solutions (14).

Vapors of the isothiocyanates, sulfides, and disulfides also appreciably reduced motility of the zoospores (Table 2). Isothiocyanates were more inhibitory than the other materials, and after a period of 2-6 hr, vapors from 0.2 ppm of the isothiocyanates stopped motility completely. AIT and MIT were more effective than BIT or PhIT. CH_3SH, CS_2 and $(CH_3)_2S$ stopped motility after 6, 12, and 24 hr, respectively. $(CH_3)_2S_2$ allowed considerable motility even after 24 hr. Volatiles trapped in water, from both decomposing cabbage and corn tissues, had little effect on motility. Motility was not reduced 1 hr after the tests began, and even after 24 hr, motility in liquids from control, cabbage, and corn amended soils was 41, 28, and 36%, respectively.

TABLE 1. Effect of S-containing volatiles on formation of zoospores of *Aphanomyces euteiches*

Volatile[a]	Zoospores produced ($\times 10^4$/ml)[b] at indicated volatile concn (ppm)							
	0.02	0.10	0.20	1.0	2.0	10	20	100
AIT	3.9	0						
MIT	4.6	0						
BIT	5.3	3.0	1.7	0				
PhIT	4.0	2.6	2.0	0				
$(CH_3)_2S_2$					5.2	3.9	0.2	0.0
$(CH_3)_2S$					5.0	4.7	4.3	0.3
CH_3SH					4.5	5.3	5.1	0.0
CS_2					4.7	4.5	5.4	0.0

[a] Volatiles are abbreviated as follows: allylisothiocyanate (AIT); methylisothiocyanate (MIT); butylisothiocyanate (BIT); phenethylisothiocyanate (PhIT).

[b] 5.1×10^4 Zoospores/ml were produced in the control.

TABLE 2. Effect of S-containing volatiles and vapors from decomposing cabbage and corn tissues on motility of zoospores of *Aphanomyces euteiches*

Volatile[a]	% Motility at indicated hr[b]						
	1	2	4	6	8	12	24
Control[c]	28	32	40	91	57	47	41
MIT	12	0					
AIT	15	0					
BIT	25	18	4	0			
PhIT	27	23	8	0			
CH_3SH	30	17	8	0			
CS_2	28	21	29	56	28	0	
$(CH_3)_2S$	24	28	35	70	56	8	0
$(CH_3)_2S_2$	36	30	42	80	45	27	21
Cabbage	37	40	51	65	42	36	28
Corn	41	37	42	71	53	38	36

[a] Concentration of S-containing volatiles was 0.2 ppm, and vapors from decomposing cabbage and corn tissues (5%) were presumably trapped in water during 10 days of decomposition. Volatiles are abbreviated as follows: allylisothiocyanate (AIT); methylisothiocyanate (MIT); butylisothiocyanate (BIT); phenethylisothiocyanate (PhIT).

[b] Hour at which observations were made after contact with vapors.

[c] 38% of the zoospores were motile at 0 hr.

Motility of zoospores also was not affected by amendment vapors when plates of motile zoospores were placed on amended soils for 24 hr.

Vapors of the known S-containing volatiles affected zoospore germination in the same manner in which they affected its growth, zoospore formation, or motility (Fig. 1-B). Two distinct responses were observed. The isothiocyanates, at concn of 0.2-3.0 ppm, completely prevented germination of the zoospores; more than 100 ppm of the sulfides or disulfides were needed for complete inhibition. Isothiocyanates of high vapor pressure (AIT and MIT) were more inhibitory to germination than those of relatively low pressure (BIT and PhIT). CS_2 was the least toxic material to the fungus allowing germination equivalent to 40% of that of the control in an atmosphere containing vapors from 1,000 ppm of the material. In these experiments, where complete inhibition of zoospore germination occurred, there was no significant recovery when plates with the spore suspensions were placed in air. Zoospores, incubated under conditions which resulted in less than complete inhibition, were subsequently observed to germinate when the suspensions were placed in air. The degree of germination was erratic, however, and the amt of recovery was not consistent. The lengths of the germ tubes were correlated with the degree of germination inhibition. Zoospores that germinated more than 70% of the control had germ tubes of 120-200 μ in length, whereas those that germinated less than 40% of the control had germ tubes less than 40 μ long.

In contrast to the toxic effects of the vapors from the S-containing materials, volatiles from decomposing amendments in soil were not very effective in preventing zoospore germination (Table 3). One day after amendment addition to soil, vapors from both decomposing cabbage and corn tissues significantly reduced zoospore germination. The reduction did not last long, however, and 3 days after amendment addition, germination in both treatments was comparable to that of the control. The length of the germ tubes in all treatments was similar (ca. 100 μ). There was also no effect on zoospore germination when water used to trap vapors from decomposing cabbage and corn tissues was added to a zoospore suspension.

TABLE 3. Effect of volatiles from decomposing cabbage and corn tissues (5%) in soil on germination of zoospores of *Aphanomyces euteiches*

Amendment	% Germination at indicated day[a]			
	1	3	5	10
Control	63	59	70	67
Cabbage	48*[b]	61	58	73
Corn	51*	53	71	64

[a] Days after the beginning of amendment decomposition on which a zoospore suspension was placed on agar in petri dishes on surfaces of soils.

[b] Numbers followed by asterisk are significantly different from the control at the 1% level.

Oospore formation and hyphal morphology.—In the concn used, the S-containing volatiles had no significant effect on oospore formation. The materials were used in amt which allowed for growth of the fungus equivalent to 50% of the control. The oospore index in the control was 90; the indices in the presence of vapors from isothiocyanates varied from 83 to 104; and those in the presence of CS_2, CH_3SH, and $(CH_3)_2S_2$ varied from 89 to 110.

The morphology of the sexual structures and mycelium of *A. euteiches* was adversely affected by vapors of decomposing cabbage (2%) in soil. No adverse effects were noted in preparations of the fungus from an atmosphere of decomposing corn. In contrast to the oospores in the control, the oogonia affected by cabbage vapors remained immature, or were deformed, with fingerlike protrusions arising from the structures (Fig. 3-A, B). Several oogonia did show the formation of the characteristic heavy peripheral wall. The effect of vapors from decomposing cabbage on hyphal morphology is shown in Fig. 3-C. The affected hyphae exhibited abnormal protrusions, especially at the hyphal tips.

Root rot development.—Vapors from several of the S-containing materials significantly reduced *Aphanomyces* root rot of peas (Table 4). The thiol, CH_3SH, the two disulfides, CS_2 and $(CH_3)_2S_2$, and the two most volatile isothiocyanates, MIT and AIT, were the most effective fumigants, and almost eliminated the disease completely as indicated in the root systems shown in Fig. 4. Fumigation of the soil with materials which resulted in a low DSI also resulted in plants with healthy root systems. In addition to reducing disease severity, these materials also allowed a greater pea stand than that observed in the control. Vapors from BIT, PhIT, and $(CH_3)_2S$, as well as those evolved from decomposing cabbage and corn tissues, were not very effective in reducing root rot, although all but

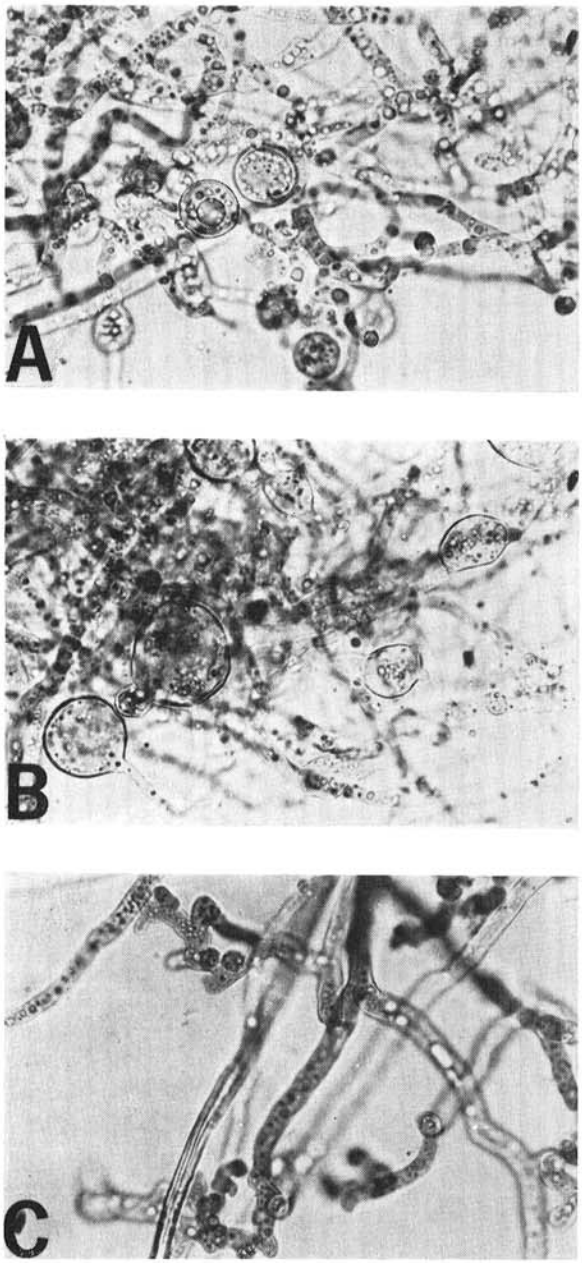

Fig. 3. A) Mycelial preparation of *Aphanomyces euteiches* illustrating normal oospores (×400). **B)** Mycelial preparation of the fungus from an atmosphere of decomposing cabbage in soil (×400). Note immature and deformed oogonia. **C)** Mycelial preparation of the fungus illustrating abnormal hyphal areas due to vapors from decomposing cabbage in soil (×1000).

BIT and $(CH_3)_2S$ resulted in emergence significantly greater than that in the control.

DISCUSSION.—Our results suggest that S-containing volatiles may be implicated in the mechanism of control of *Aphanomyces* root rot of peas by cruciferous amendments. The vapors from various S-containing volatiles at very low concn adversely affected several phases in the life cycle of *A. euteiches*. CH_3SH, CS_2,

TABLE 4. Pea root rot caused by *Aphanomyces euteiches* as affected by S-containing volatiles and vapors from decomposing cabbage and corn tissues[a]

Volatile[b]	Disease severity index[c]	% Stand
CS_2	5 a[d]	100
$(CH_3)_2S_2$	5 a	95
AIT	5 a	75
MIT	5 a	95
CH_3SH	8 a	85
BIT	65 b	65
Cabbage	79 c	75
PhIT	85 c	85
$(CH_3)_2S$	85 c	55
Corn	88 c	75
Control	90 c	55

[a] Vapors from 1.0 g of volatile materials and from decomposing cabbage and corn tissues (5%) in soil.

[b] Volatiles are abbreviated as follows: allylisothiocyanate (AIT); methylisothiocyanate (MIT); butylisothiocyanate (BIT); phenethylisothiocyanate (PhIT).

[c] Based on scale in which 0 indicates all roots apparently healthy and 100 indicates all roots and epicotyls rotted.

[d] Numbers followed by same letter are not significantly different at 5% level.

$(CH_3)_2S$, and $(CH_3)_2S_2$ were effective to various extents in suppressing or preventing growth, zoospore formation, and zoospore motility and germination. All these compounds have been shown to be liberated in appreciably amt during decomposition in soil of cabbage, but not of corn (14).

We have previously observed (14) that the foregoing S-containing volatiles, as well as various other unidentified volatiles, are evolved in detectable amt up to 5 weeks after addition of cabbage to soil. In our greenhouse experiments, peas are usually planted 3 weeks after amendment incorporation. The sulfides evolved from decomposing cabbage tissue in microenvironments could be effective against the zoospores during the first 2 critical weeks following planting. Although the sulfides were not very effective in preventing zoospore germination, they did suppress zoospore formation and motility. CS_2 and CH_3SH, two sulfides particularly effective in stopping motility, also reduced disease severity. Whether these sulfides also prevented

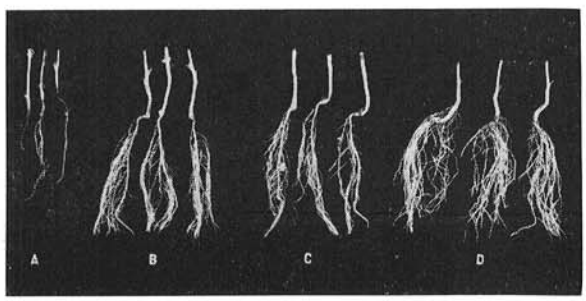

Fig. 4. Root rot of peas caused by *Aphanomyces euteiches* as affected by fumigation with S-containing volatiles. **A)** Root system from plants grown in nonfumigated soil. **B, C, D)** Root systems of plants from methylisothiocyanate (MIT), CS_2, and $(CH_3)_2S_2$ fumigated soils, respectively.

oospore germination in soil cannot be ascertained because no suitable methods exist for in vivo or in vitro germination of oospores in appreciable numbers.

Vapors of the isothiocyanates, especially those of MIT and AIT, were extremely effective in suppressing all phases studied in the life cycle of *A. euteiches*. The most volatile isothiocyanates with the highest vapor pressure (MIT and AIT) were also excellent soil fumigants and significantly reduced root rot of peas. The effectiveness of isothiocyanates against various fungi in relation to the vapor pressures of the materials has been discussed by Hooker et al. (11). Isothiocyanates, however, have not been detected in the headspace above decomposing cabbage in soil (14); nor have isothiocyanate residues been found in soil from decomposing plant tissues. It is assumed, however, that isothiocyanates are produced as decomposition products in nature. In the laboratory, they are enzymatically produced from various thioglucosides found in crucifers by the action of myrosinase (1).

Although the vapors resulting from the decomposition of cabbage tissue in soil were not as inhibitory to *A. euteiches* as were the vapors of the authentic S-containing volatiles, several effects were observed. Vapors of cabbage decomposing in soil adversely altered the morphology and development of oospores and inhibited mycelial growth of the fungus in culture. These effects may be important in the reduction of *Aphanomyces* root rot by cruciferous amendments. The probable importance of oospores to survival of the pathogen has been suggested (25), and abnormal or immature oospores might not produce zoospores necessary for infection. Moreover, deformed or abnormal hyphae, such as those in association with an atmosphere of vapors from decomposing cabbage, may be more predisposed to lysis in soil.

There are two serious questions that cannot be answered by our results here or those of a previous study (14): namely, why the volatiles from cabbage (decomposing in soil) collected in the headspace were not as effective as isothiocyanates or sulfides in adversely affecting certain processes in the life cycle of *A. euteiches* and why isothiocyanates could not be demonstrated in the headspace, although they are known to be evolved in laboratory experiments (1, 2, 5). Several facts are known to at least partly explain the foregoing two difficulties. Components of the vapors from cabbage may be adsorbed to soil particles or converted to other secondary compounds. It is also possible that under the conditions of the experiments, isothiocyanates did not accumulate sufficiently in the headspace to be detected or were adsorbed to colloids or converted to nonvolatile compounds in soil. For example, Munnecke & Martin (19) attributed the small amounts of MIT released from 3,5-dimethyl-tetrahydro-1,3,5,2H-thiadiazine-2-thione (dazomet) in certain soils to the adsorption of the isothiocyanate on the organic matter and clay of the soils. Also, effective concn of S-containing volatiles might have been greater within the soil, where the crucifer decomposed, than in the enclosed space above it. Lloyd (15) also observed that MIT may react with NH_4OH in soil to form nonvolatile, nontoxic thioureas.

Despite the strong circumstantial evidence that S-containing volatiles may be the agents responsible for the control of *A. euteiches* by cruciferous amendment, the foregoing results do not preclude other possibilities. Reduction of disease may also be due, entirely or in part, to increased antagonistic populations, production of antibiotic materials, or increased levels of fungistasis. In our recent experiments, slurries of soil previously amended with cabbage, but not those amended with corn, significantly reduced zoospore germination of *A. euteiches* (J. A. Lewis, *unpublished data*). If a toxicant is involved here, it may be an adsorbed isothiocyanate or antibiotic. These possibilities are now being investigated to explain further the mechanism or mechanisms of *Aphanomyces* root rot suppression by cruciferous amendments.

The results also suggest the possibility of using materials found in cruciferous amendments as fumigants for control of pea root rot. CS_2 and MIT are examples of such fumigants already in use in other situations. CS_2 has been used in soil fumigation to control soil fungi and deep-rooted perennial weeds (3, 4). CS_2 is responsible, at least in part, for the effect of the fungicidally active dithiocarbamic acid derivatives (7). MIT is used in solution with chlorinated hydrocarbons (Vorlex) and is the active material resulting from the decomposition of 3,5-dimethyl-tetrahydro-1,3,5,2H-thiadiazine-2-thione and sodium *N*-methyl-dithiocarbamate dihydrate (metham) (15, 19). All these materials are greenhouse and field soil fumigants effective against damping-off, root rot, and wilt-producing fungi (4). Consideration might also be given to use of fumigants of other volatile materials such as AIT or $(CH_3)_2S_2$, which were effective in reducing pea root rot in the greenhouse.

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