Effect of Amendments and Fungicides on Aphanomyces Root Rot of Peas

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Accepted for publication 23 September 1970.

ABSTRACT

Cruciferous amendments such as leaves and stems of cabbage, kale, and mustard at 0.5% of the oven-dry weight of soil, gave a considerable reduction of the root rot of peas caused by *Aphanomyces euteiches* in the greenhouse. Kale was also effective in the field. Several NH_4-N sources (NH_4Cl, NH_4OH, NH_4HCO_3, NH_4NO_3), urea, and 20:20:20 fertilizer greatly reduced root rot severity in the greenhouse. Hydrated lime, S, and several Na and Ca sources ($NaNO_3$, Na_2CO_3, Ca cyanamide) were ineffective. Attempts to control the diseases with the nonvolatile fungicides 2-(3-thiazolyl)benzimidazole, 2,6-dichloro-4-nitroaniline (Botran), methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl), and zinc ethylenebis (dithiocarbamate) (zineb) failed. Only the fungicide *p*-dimethylaminobenzenediazo sodium sulfonate (Dexon) was effective in the greenhouse and in the field at 100 ppm.

Very good control of *A. euteiches* was obtained in both greenhouse and field with methylisothiocyanate (MIT) and other fungicides that decompose to MIT in natural soil. Sodium *N*-methyldithiocarbamate (metham), 3,5-dimethyl-tetrahydro-1,3,5,2H-thiadiazine-2-thione (DMTT-I, dazomet), DMTT-II (Mico-fume, a dazomet formulation), and DMTT-III (Plant Bed Fumigant, a dazomet formulation) controlled root rot effectively in the greenhouse and field at 50-200 ppm. These fungicides were effective with several pea cultivars and at a soil temperature range of 17-32 C. Lower concentrations of these materials were required to suppress root rot when the soil containers were closed in polyethylene bags for 2 weeks after fumigant application than when left uncovered. Phytopathology 61:215-220.

Cruciferous amendments such as stems and leaves of cabbage, kale, mustard, and turnip added to soils suppressed root rot of peas (*Pisum sativum* L.) caused by *Aphanomyces euteiches* Drechs. (15). Volatile substances, similar to those reported by Lichtenstein et al. (10), may be produced as a result of decomposition of the cruciferous amendments. Papavizas (15) postulated that volatile toxic substances may adversely affect several vital phases in the life cycle of the pathogen before or after host penetration.

Recently, Lewis & Papavizas (8) obtained direct evidence that crucifers decomposed in soil with the formation of the volatile sulfur-containing (S-containing) compounds methanethiol (CH_3SH), dimethyl sulfide [$(CH_3)_2S$], and dimethyl disulfide [$(CH_3)_2S_2$]. None of the above volatile materials was evolved from decomposing corn tissue in soil. We also (8) detected methanol, ethanol, acetone, acetaldehyde, and unknown aldehydes or ketones from both decomposing cabbage and corn. In addition to sulfides, isothiocyanates have been detected by others in vapors, distillates, and extracts of fresh or cooked cabbage (1, 3, 10). Sulfides and isothiocyanates, especially $(CH_3)_2S_2$ and methylisothiocyanate ($CH_3N{=}C{=}S$) (MIT) were extremely toxic to *A. euteiches* even at concn as low as 0.04 ppm (9).

No commercially available fumigants are available that have $(CH_3)_2S_2$ or $(CH_3)_2S$ as their active ingredients (5). Sodium *N*-methyldithiocarbamate (SMDC, metham), 3,5-dimethyl-tetrahydro-1,3,5,2H-thiadiazine-2-thione (DMTT-I, dazomet), DMTT-II (Mico-fume, a dazomet formulation), DMTT-III (Plant Bed Fumigant, a dazomet formulation), and other fumigants decompose to MIT in soil. Turner & Corden (19) showed that SMDC through an oxidative process decomposes in dilute aq solutions at alkaline pH to MIT and elemental S. In acid solutions, CS_2, H_2S, *N*,*N*′-dimethylthiuram disulfide, methylamine, and MIT are formed.

We undertook the present study to evaluate in the greenhouse and field several cruciferous and noncruciferous amendments and several fungicides that decompose to MIT in soil for their effectiveness to control *Aphanomyces* root rot of peas. For comparison, we also tested several nonvolatile fungicides, including *p*-dimethylaminobenzenediazo sodium sulfonate (DASS, Dexon), a compound known to be effective against *A. euteiches* (11, 12), and various mineral salts of Na and N found to be effective against *Aphanomyces* root rot of peas (4, 6).

MATERIALS AND METHODS.—Codorus loam (CL), Galestown-Evesboro loamy sand (GELS), and Rumford loamy sand (RLS) were used. CL (pH 6.7) contained 0.76% total C, 0.074% total N, and had a water-holding capacity (WHC) of 53%. GELS (pH 5.0) contained 1.2% total C, 0.13% total N, and had a WHC of 30%. RLS (pH 5.9) contained 1.6% total C, 0.13% total N, and had a WHC of 30%. Large batches of the natural soils were infested with zoospores of isolates A6 and 7 of *A. euteiches* and cropped with Early Alaska peas, until the soils were thoroughly infested. Isolates A6 and 7, both very pathogenic on peas were obtained from Adele S. Lawyer, Calif. Packing Corp., and W. Schroeder, Cornell Univ., respectively. Methods and materials used to prepare zoospore inocula, and to maintain the desired temp and soil moisture before and after soil infestation, have been previously described (17).

Mineral salts of N and Na, Ca cyanamide, urea, and the N-P-K fertilizers 10:10:10 and 20:20:20 (containing both NH_4-N and NO_3-N) were added at a rate to give 200 ppm additional N in the soil. Other materials used were hydrated lime [$Ca(OH)_2$] at 400 lb./acre;

S at 600 lb./acre; Na_2CO_3 at 600 lb./acre; 2,6-dichloro-4-nitroaniline (DCNA, Botran), methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl), and zinc ethylenebis (dithiocarbamate) (zineb), each at 100 ppm active.

The following amendments were used: mature leaf and stem tissue of oats (*Avena sativa* L.); corn (*Zea mays* L.); soybean (*Glycine max* [L.] Merr.); Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* DC.); collards (*Brassica oleracea* L. var. *acephala* DC.); watercress (*Radicula nasturtium-aquaticum* [L.] Britt. & Rendle); turnip (*Brassica rapa* L.); mustard (*Brassica nigra* [L.] Koch); kohlrabi (*Brassica oleracea* var. *caulo-rapa* DC.); kale (*Brassica oleracea* var. *acephala* DC.); and cabbage (*Brassica oleracea* var. *capitata* L.). All plant materials were air-dried, ground in a hammer mill, and incorporated in soil at 0.5% of the dry wt of soil and amendment before planting at time lengths specified in each particular experiment.

DMTT-I was mixed with talc (4% DMTT-I in talc), and the mixture added to soil. DMTT-I, DMTT-II, and DMTT-III were added to soil dry. MIT and SMDC were mixed with water and added to soil as drenches. The nonvolatile fungicides 2-(4′-thiazolyl)-benzimidazole (TBZ), DCNA, benomyl, and zineb were also mixed with water, added to soil, and mixed thoroughly before planting. In greenhouse experiments, the fungicide DASS was added at 100 ppm active of 70% wettable powder and in the field experiment at 100 ppm of 5% granular material. All fungicides were added to soil at the lengths of time before planting, and at concn specified in each particular experiment.

All greenhouse experiments were done in No. 5 plastic pots, and the equivalent of 1 kg of air-dry soil was used/pot. In the field we used plastic pails (20 × 35 cm) with 10 holes (13 mm diam) in the bottom for drainage. Twenty kg of soil were used/pail. Large holes were dug in the field, and the soil-filled pails were placed in the holes in a randomized complete block design in such a way that the surface of the soil in the pails was at the same level as that of the field. Ten and 30 pea seeds were planted in the pots and pails, respectively. Unless otherwise indicated, Early Alaska peas were used. After a 5-week growing period, the seedlings were removed, counted, examined for disease symptoms, and rated on a scale of 0 (no visible symptoms) to 4 (severe symptoms). A disease severity index (DSI) of 0 to 100 was calculated from the individual ratings by the method of Sherwood & Hagedorn (18). In some experiments, a second planting was made and the same procedure of disease rating was followed. Five replications were used throughout, and the experiments were performed twice.

To determine evolution of MIT from DMTT-I, 50-g portions of GELS, amended with 100 ppm DMTT-I, were placed in 250-ml Erlenmeyer flasks. The flasks were fitted with rubber stoppers pierced by glass tubing capped with serum bottle closures. Five-ml samples of headspace air were withdrawn periodically with a syringe.

We used an F and M Scientific Corp., Model 810 gas chromatograph with a flame ionization detector and stainless steel packed column (6 ft × 0.25 inch inside diam). The stationary phase was Carbowax 20 M, 20% on 80/100 mesh chromosorb (white diatomite, acid-washed). Injection port and detector temp were 185 C; helium flow rate, 25 ml/min; hydrogen flow rate, 25 ml/min; and air flow rate, 200 ml/min. Column temp was maintained at 125 C during analysis. Micrograms of MIT/5 ml sample of headspace air were calculated from a standard curve.

RESULTS.—*Effect of amendments on Aphanomyces root rot.*—All cruciferous amendments tested and soybean tissue gave a considerable reduction of the root rot of peas caused by *A. euteiches* (Table 1). DSI values of 5 or less were observed for all cruciferous amendments in the table. Control (no amendment) and corn had DSI of 93 and 85, respectively. The cruciferous amendments added to soil 3 weeks before planting did not reduce stand.

Effect of mineral salts and fungicides on Aphanomyces root rot.—The fungicide DASS, urea, and all NH_4-N sources greatly reduced root rot severity in the greenhouse (Fig. 1-A). No statistically significant differences were observed among NH_4-N treatments. The fertilizers reduced disease severity effectively, with 20:20:20 being superior to 10:10:10. The Na and Ca salts and $Ca(OH)_2$ were ineffective. Sulfur and the fungicides DCNA, benomyl, and zineb were also ineffective, and are not included in Fig. 1-A.

Effect of fungicides that decompose to MIT on Aphanomyces root rot.—An initial experiment was performed in the greenhouse to evaluate the ability of several fungicides that decompose to MIT in natural soil (5, 19) to suppress *A. euteiches* root rot of peas. MIT and two DMTT formulations were added to *Aphanomyces*-infested GELS at 25, 50, 100, and 200 ppm; SMDC at 65, 130, 185, and 260 ppm; and the fungicide TBZ was added at 25, 50, 100, and 500 ppm for comparison. First planting of Early Alaska peas was

TABLE 1. Effect of amendments on root rot severity in Alaska peas in the greenhouse grown in Galestown-Evesboro loamy sand infested with *Aphanomyces euteiches*

Amendment[a]	Disease severity[b]	Stand
	Index	%
None (control)	93 a[c]	80 b
Corn	85 a	70 a
Brussels sprouts	5 b	85 b
Collards	5 b	80 b[c]
Soybean	5 b	85 b
Cress	3 b	92 c
Turnip	0 b	90 c
Mustard	0 b	85 b
Kohlrabi	0 b	80 b
Kale	0 b	82 b
Cabbage	0 b	90 c

[a] Amendments were added at 0.5% of the oven-dry wt of soil and amendment 3 weeks before planting.

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

[c] Means with the same letter are not significantly different at the 5% level.

done 4 weeks and the second planting in the same soil 9 weeks after soil treatment.

During both plantings, SMDC at 130, 185, and 260 ppm; DMTT-I at 100 and 200 ppm; and DMTT-II at 150 ppm suppressed root rot greatly (Fig. 1-B). To simplify the data, all concn of chemicals used are not shown in Fig. 1-B. DMTT-I at 50 ppm and DMTT-II and MIT at 50 and 100 ppm were less effective in reducing root rot severity during the first planting, but very effective during the second planting. For instance,

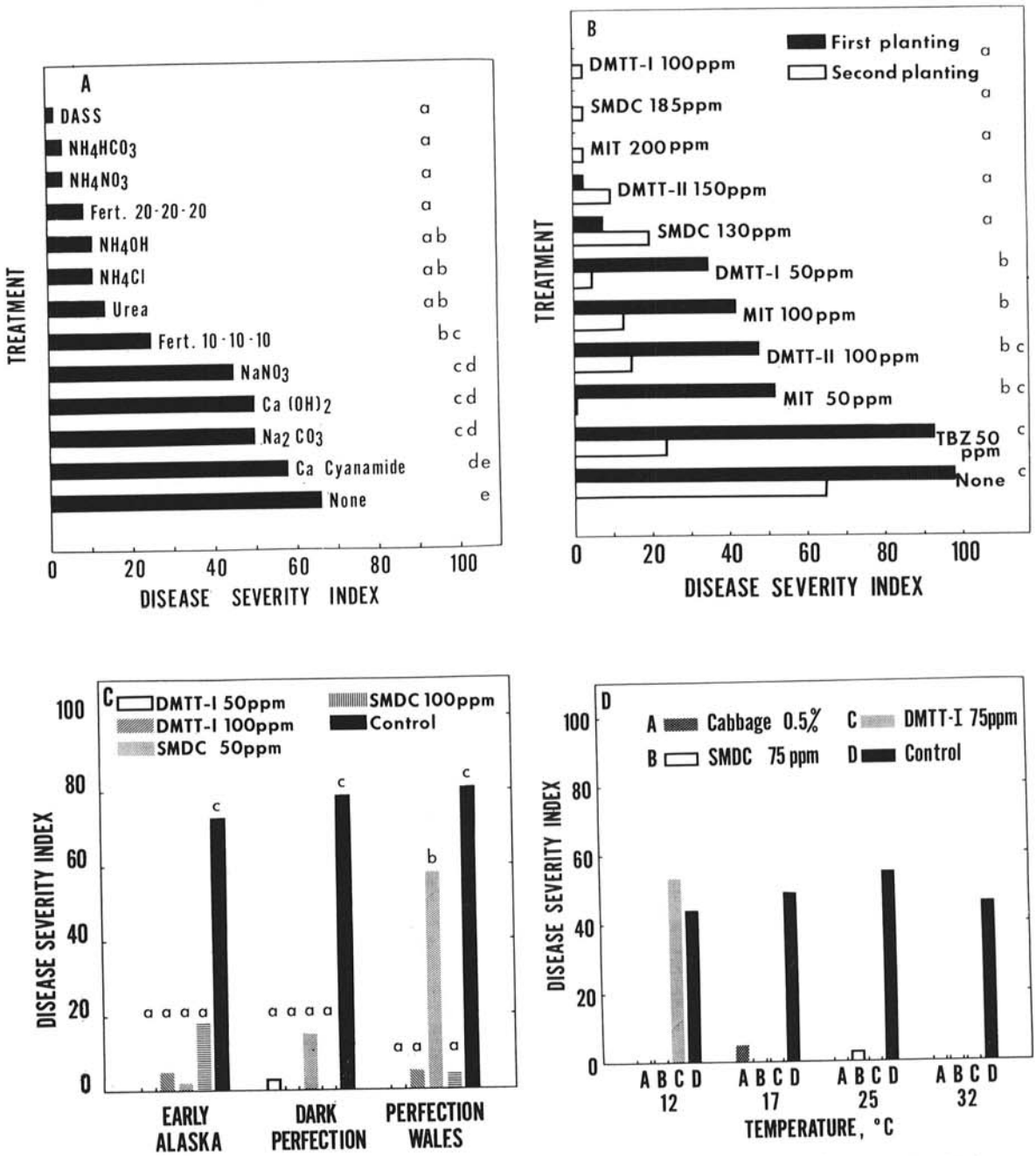

Fig. 1. Root rot severity in peas grown in soil infested with *Aphanomyces euteiches:* **A)** in Early Alaska peas as affected by nonvolatile fungicides, fertilizers, nitrogen sources, and lime; **B)** in Early Alaska peas as affected by various fungicides (that decompose to methylisothiocyanate [MIT] in soil) added to soil 4 weeks before the first planting; **C)** in three pea cultivars as affected by two concn of fungicides that decompose to MIT; **D)** in Early Alaska peas as affected by soil temp during cabbage and fungicide decomposition in soil. Means with the same letter are not significantly different at the 5% level. (Explanation of abbreviations: DASS, *p*-dimethylaminobenzenediazo sodium sulfonate; DMTT, 3,5-dimethyl-tetrahydro-1,3,5,2H-thiadiazine-2-thione [dazomet]; SMDC, sodium *N*-methyldithiocarbamate [metham]; TBZ, 2-[4′-thiazolyl]benzimidazole).

MIT at 50 ppm had a DSI of 54 and 2 during the first and second planting, respectively. TBZ at 25 and 50 ppm was somewhat effective during the second but not during the first planting. The higher concn of TBZ used were phytotoxic to peas. None of the chemicals reduced stand during both plantings. In fact, some fungicides (MIT, DMTT-I, DMTT-II, and SMDC) increased pea stand considerably over that of the control.

In a second greenhouse experiment, DMTT-I and SMDC were added to *Aphanomyces*-infested RLS at 50 and 100 ppm, and the soil was kept moist in uncovered pots for 3 weeks before planting. The pea cultivars Early Alaska, Dark Perfection, and Perfection Wales were then planted without chemical seed treatment. With the exception of Perfection Wales, which had a DSI of 58 in soil treated with 50 ppm of SMDC, all three varieties had very little root rot in soil treated with the two fungicides that decompose to MIT in soil (Fig. 1-C). In the control soil, the three cultivars had a DSI of more than 70.

Effect of soil temp during cabbage and fungicide decomposition on their effectiveness in the control of root rot.—To determine whether soil temp before planting would reduce the effectiveness of cabbage and fungicides to suppress root rot, GELS amended with cabbage tissue or treated with SMDC or DMTT-I at 75 ppm was distributed into quart crocks. The crocks were covered with plastic film (Saran) and incubated in soil temp tanks at 12, 17, 25, and 32 C for 2 weeks; the soil from the crocks was transferred to pots and maintained moist and uncovered at 24 ± 2 C for 1 week before planting. We observed considerable differences in plant stand. The per cent stand for control soil at 12, 17, 25, and 32 C was 27, 45, 43, and 27%, respectively; for SMDC it was 98, 100, 92, and 95%; for DMTT-I it was 83, 100, 79, and 100%; and for cabbage per cent stand at 12, 17, 25, and 32 C it was 95, 100, 95, and 98%, respectively.

Incubation of the treated soil at 17, 25, and 32 C before planting did not reduce the effectiveness of cabbage, SMDC, and DMTT-I to suppress *Aphanomyces* root rot (Fig. 1-D). The effectiveness of DMTT-I, however, was not demonstrated when infested soil treated with this material was incubated at 12 C for 2 weeks before planting. Effectiveness of cabbage and SMDC was not reduced at 12 C.

Gas chromatographic analysis of headspace in flasks containing DMTT-amended GELS showed that decomposition of DMTT and production of MIT proceeded at a more rapid rate at 26 than at 10 C. During the first day of incubation, 9 μg MIT/5 ml of headspace were liberated at 26 C, and only 4 μg at 10 C. After 6 days of incubation, 3 μg MIT/5 ml of headspace were released vs. 1 ml at 10 C.

Effect of covering of containers on fungicide efficiency.—One-kg portions of *Aphanomyces*-infested CL and GELS in pots were treated with DMTT-II and DMTT-III at 25, 50, and 100 ppm and with SMDC at 50 and 100 ppm. The pots were immediately enclosed in plastic bags for 2 weeks and kept at 25 ± 1 C; then they were uncovered, allowed to remain in the air for 2 more weeks, and planted to Early Alaska peas.

Although there were some differences between covered and uncovered pots of the same treatment, covering of the containers in general did not increase the effectiveness of the three fumigants in CL, a heavy soil (Fig. 2-A). In GELS, however, a sandy soil, enclosure of pots containing treated soil greatly increased the ef-

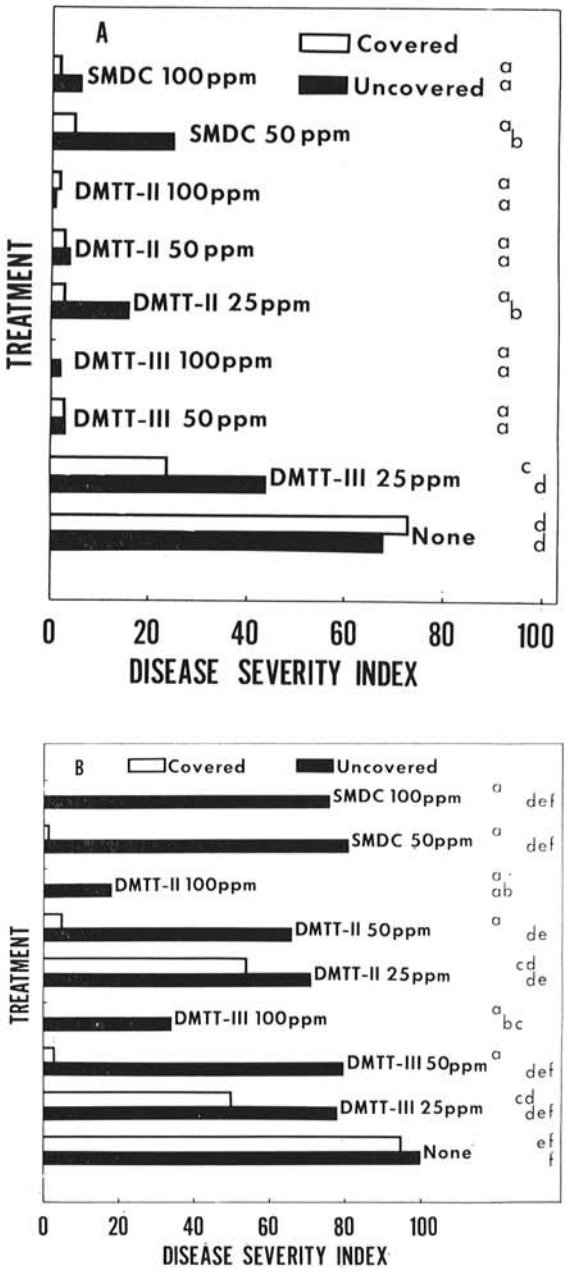

Fig. 2. Root rot severity in Early Alaska peas grown in soil infested with *Aphanomyces euteiches* as affected by various concentrations of fungicides and covering of the containers for 2 weeks after fungicide addition: **A)** in Codorus loam; **B)** in Galestown-Evesboro sandy loam. Means with the same letters at the right-hand side of the graphs are not significantly different at the 5% level. (Explanation of abbreviations: DMTT, 3,5-dimethyl-tetrahydro-1,3,5,2H-thiadiazine-2-thione [dazomet]; SMDC, sodium *N*-methyldithiocarbamate [metham]).

fectiveness of the fungicide tested (Fig. 2-B). With the exception of DMTT-II and DMTT-III at 25 ppm, all three materials at all concn were more effective against *Aphanomyces* root rot when the pots were enclosed in plastic bags than when they were left uncovered. In the uncovered soil, only DMTT-II at all three concn and DMTT-III at 100 ppm were significantly effective against the root rot.

Field experiments.—In a field experiment at Beltsville, GELS received 10:10:10 fertilizer (at a rate to give 75 ppm N) immediately before treatments. MIT and DMTT-I were added to soil at 200 ppm each; DMTT-II was added at 150 ppm; DMTT-III at 100 ppm; and SMDC at 195 ppm. The soil-filled pails were set in the field uncovered on 24 April; peas were planted on 15 May and harvested on 13 June for root examinations. Peas were replanted in the same pails on 12 September of the same year.

MIT, SMDC, DASS, and the three DMTT formulations controlled *Aphanomyces* root rot effectively during both plantings (Fig. 3). Kale tissue suppressed disease severity significantly. Cabbage tissue, oat straw, and corn stover amendments were ineffective. During the first planting, the best stand (90-95%) was obtained in soil treated with fungicides that decompose to MIT in soil, and the lowest in control soil (60%).

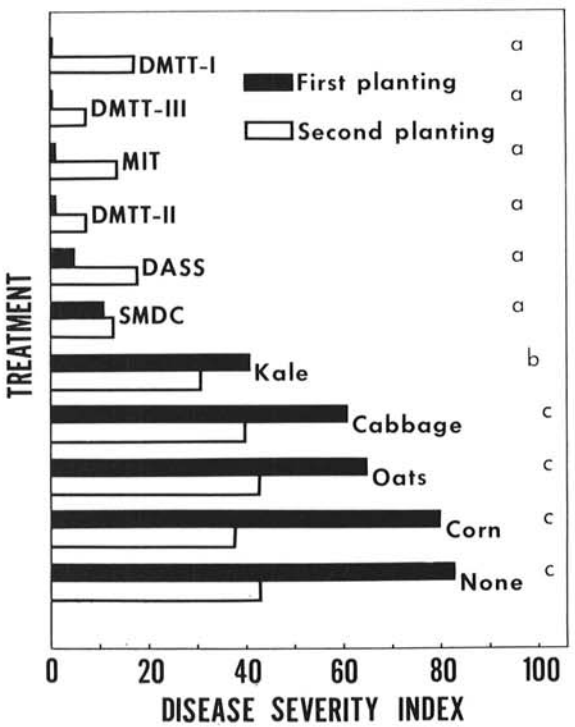

Fig. 3. Root rot severity in Early Alaska peas grown in the field in soil infested with Aphanomyces euteiches as affected by various organic amendments and fungicides added to soil 3 weeks before the first planting. First planting was done 21 days after treatments; second planting, 6 months after treatments. Means with the same letter at the right-hand side of the graph are not significantly different at the 5% level. (Explanation of abbreviations: DMTT, 3,5-dimethyl-tetrahydro-1,3,5,2H-thiadiazine-2-thione [dazomet]; MIT, methylisothiocyanate; DASS, *p*-dimethylaminobenzenediazo sodium sulfonate; and SMDC, sodium *N*-methyldithiocarbamate [metham]).

The field experiment was performed again at Beltsville in 1970 in the same way as in 1969, except that all fungicides were used at 100 ppm and no 10:10:10 fertilizer was used. The results obtained in 1970 were essentially the same as those obtained in 1969. MIT, the three formulations of DMTT, SMDC, and DASS gave excellent control of *A. euteiches*. Kale and cabbage reduced the disease by 50 and 40%, respectively. No control was obtained with corn or oat amendments.

Discussion.—With the exception of *p*-dimethylaminobenzenediazo sodium sulfonate (DASS) (11, 12, 16), no nonvolatile fungicides are known to be effective against *A. euteiches*. This observation holds true in the present study. Our results also suggest that the fungicides DMTT and SMDC that decompose to MIT in soil are very effective against *A. euteiches* in the greenhouse and field at concn of 50-100 ppm, and that their effect in the field may last for at least 6 months. Although the DMTT and SMDC have been commercially available for many years, to the best of our knowledge they have not been tested before against *Aphanomyces* root rot of peas. It may be significant to note that our studies to unravel the mechanism of biological control of *A. euteiches* by cruciferous amendments (specifically involving S-containing volatile substances evolved during crucifer decomposition in soil [8] or in crucifer vapors and distillates [1, 3, 10]) led us to the use of DMTT and SMDC. These two fungicides are known to liberate MIT during their decomposition in soil (13, 14, 19). Isothiocyanates and sulfides adversely affected many phases in the life cycle of *A. euteiches* (9).

At present, it cannot be ascertained whether the use of such compounds as DMTT and SMDC will become agriculturally feasible and economically profitable. Although the two fungicides were more effective when treated soils were covered than when left uncovered, it is encouraging to note that in the field they were very effective without covering or sealing the soil after treatment. Use of plastic cover would undoubtedly add an additional production expense. It is also encouraging to note that these fungicides were successful in suppressing disease with three pea cultivars in the greenhouse. In addition, these materials were generally effective over a wide range of temp (12-32 C) when soils were incubated at these temp after treatment. Good control of *Pythium* root rot and damping-off of peas was also obtained with DMTT and SMDC (G. C. Papavizas & J. A. Lewis, *unpublished data*). The different rates of DMTT decomposition and MIT liberation which we observed at two temp (10 and 26 C) might account for the inability of DMTT to suppress *A. euteiches* at 12 C in the greenhouse temp experiment.

All cruciferous amendments tested with *Aphanomyces*-infested soils were very effective in the greenhouse. In the field, however, results were disappointing to some extent. For two consecutive years, kale reduced root rot by 50% and cabbage tissue significantly reduced root rot during the second trial year by only 40%. Decrease in the effectiveness of the cruciferous amendments in the field may be due to several factors.

Firstly, the soil used in the field was heavily infested with *A. euteiches*. Although cruciferous amendments may not eliminate the fungus, they may reduce root rot of peas in naturally infested soils where the level of infestation is not excessively high. Secondly, there was excessive rainfall in the spring and summer of 1969 at Beltsville. Preliminary results in our laboratory (J. A. Lewis & G. C. Papavizas, *unpublished data*) suggested that the decomposition of crucifers in soils with excessive moisture was not accompanied by the evolution of S-containing compounds in the vapor phase. Under these conditions in the field, the absence of S-containing volatiles, which are toxic to *A. euteiches* (9), may have resulted in the decreased effectiveness of the cabbage tissue. Nevertheless, it is possible that some degree of root rot control may be achieved under normal climatic conditions by turning cruciferous plant residues under in late fall before pea planting in the spring. Results in the greenhouse also suggested that the suppression of disease could be accomplished with cabbage tissue over a wide temp range during the decomposition process (12-32 C). Discouraging results with cruciferous amendments in the field have been reported in Wisconsin (12), but the amt of cabbage tissue used in those tests (550 lb./acre) was less than 0.026% on the basis of 6 inch depth. This amt would be too small to result in any disease suppression under the best of experimental conditions.

The observation that several N sources and commercial fertilizers may reduce *Aphanomyces* root rot of peas is not new. Several workers (4, 6, 7) have reported that N reduced damage caused by *A. euteiches* on peas. A significant point in the present paper is the fact that NH_4-N was more effective in reducing root rot than NO_3-N or Na and Ca salts previously reported to be effective (6). Our data, however, are not in agreement with those of Carley & King (2), who found that NO_3-N supplied as $Ca(NO_3)_2$ tended to suppress the incidence and severity of pea root rot, whereas NH_4-N as NH_4OH or $(NH_4)_2SO_4$ increased it. Lewis (*unpublished data*) has recently observed the suppression of pea root rot in the greenhouse with $CaCl_2$ but not with $Ca(NO_3)_2$. Differences in inocula and soils used and other environmental factors may account for the discrepancies. More studies with various kinds of soils and pathogenic isolates of *A. euteiches* are needed under controlled conditions to determine the importance of the kind of N in the epidemiology of *Aphanomyces* root rot of peas.

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