

Control of Pythium Blight on Bean with Ethazol and Prothiocarb

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ABSTRACT

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In vitro toxicity of the experimental systemic fungicide, N-(3-dimethylaminopropyl) thiocarbamic acid S-ethyl ester hydrochloride [DTEH, SN41703, prothiocarb (proposed)] against 11 *Pythium* spp. was quite uniform; the dosage response curves were linear and generally steep up to 1.0-2.0 µg active ingredient (a.i.)/ml, with ED₅₀ values ranging from 0.1 to 1.0 µg/ml. When ED₁₀₀ values were considered, *P. irregulare*, *P. butleri*, *P. arrhenomanes*, *P. debaryanum*, and *P. myriotylum*, in the order given, were the most sensitive species. The in vitro effectiveness of DTEH was pH-dependent, with the best suppression obtained at about pH 6.2-7.4. The efficacy of ethazol, DTEH, and a DTEH analog [propyl-N-(γ-dimethylaminopropyl) carbamate (SN39744, PDAC)] were compared as seed treatments with other fungicides in the greenhouse against damping-off and

Pythium blight of bean caused by *P. aphanidermatum*, *P. myriotylum*, and *P. ultimum*. Only the systemic fungicide DTEH completely prevented Pythium damping-off and blight when applied directly to the seed. It also was completely effective when applied to the seed by solvent infusion with water or ethanol as the solvent. With ethazol, the best control of damping-off and blight was obtained when the fungicide was applied to the seed with acetone or dichloromethane as solvents. The organic solvents alone neither increased nor reduced Pythium blight and seed germinability. The only fungicide applied to the seed that reduced Pythium blight and resulted in significant yield increases in a heavily infested field near Salisbury, Maryland was DTEH.

Additional key words: seed treatment, organic solvent infusion technique, *Phaseolus vulgaris*.

Pythium aphanidermatum (Edson) Fitzp., *P. myriotylum* Drechs., and *P. ultimum* Trow are the most important *Pythium* spp. in the epidemiology of blight of bean (*Phaseolus vulgaris* L.) on the eastern shore of Maryland (8, 10) and elsewhere in the United States (2, 5, 6, 9, 11). When environmental conditions are favorable, Pythium blight may cause up to 30% losses or even completely destroy an established stand (10).

Despite the importance of this disease, no adequate control measures have been developed. Although some progress has been made in finding white-seeded snapbean breeding lines resistant to seed decay and damping-off caused by *P. ultimum* (2), no bean cultivars resistant to all three pathogens are available (4, 6). Neither do we know of any specific biological or cultural-control measures for Pythium blight.

Little work has been reported on the efficacy of various fungicides for control of Pythium blight of bean. None of the presently registered systemic fungicides, applied in small amounts on the seed, controls diseases caused by Phycomycetes. According to Sanders et al. (14), the fun-

gicides 1,4-dichloro-2,5-dimethoxybenzene (chloroneb), 5-ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole (ethazol, ETMT), and sodium *P*-(dimethylamino) benzenediazot-sulfonate (BAY 22555, Dexon) currently are among the best protectant materials, but require repeated applications for control of Pythium blight on Penncross creeping bentgrass. In field-scale fungicide trials in Wisconsin against three bean root-rotting fungi, including *Pythium* spp. (6), only BAY 22555 provided good control when added to soil with a modification of the in-furrow spray application. No significant yield increases over the untreated control were obtained.

Recently, a new systemic fungicide N-(3-dimethylaminopropyl) thiocarbamic acid S-ethyl ester hydrochloride [DTEH, SN41703, prothiocarb (proposed)] has become available for experimentation with Phycomycetes (15). This material has been used experimentally on *Phytophthora cinnamomi* on ornamental conifers (17) and *P. cactorum* (1) and *P. fragariae* (16) on strawberry plants.

The experiments reported here were undertaken (i) to determine the toxicity of DTEH to *Pythium* spp; (ii) to determine the efficacy of DTEH, applied to the seed, against Pythium blight of bean in the greenhouse and

field; and (iii) to compare DTEH with ethazol, a systemic fungicide recommended for control of some diseases caused by phycomycetous fungi.

MATERIALS AND METHODS

Laboratory experiments.—Fungitoxicity in vitro of DTEH to 11 *Pythium* spp. (Table 1) was evaluated on the basis of inhibition of mycelial growth on solid (20 ml/petri dish) or liquid (40 ml/250-ml Erlenmeyer flask) Basal Medium 1 (BM-1), a synthetic medium described previously (12). Initial pH of BM-1 was adjusted to 7.4 before sterilization and buffered at that value with phosphate buffer (in ml/liter: 0.33M KH_2PO_4 , 3; and 0.33M Na_2HPO_4 , 17). Potassium and P were added to the medium with the buffer. The pH of the medium after autoclaving was 6.9 - 7.0.

The DTEH [70% active ingredient (a.i.) in water], an ethanol- and water-soluble fungicide, was diluted in sterile water and added in appropriate quantities (on a w/v basis) to BM-1 before it was dispensed to petri dishes or flasks. Concentrations of DTEH (micrograms a.i./ml of BM-1) tested in liquid and solid medium were 0.0 (control), 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, and 2.0. In solid medium, concentrations of 2.5, 5, 10, 50, 100, and 300 $\mu\text{g}/\text{ml}$ also were tested. Inoculum of the *Pythium* spp. tested was grown on nonamended BM-1. Disks 5-mm diameter were cut from the periphery of actively growing colonies and transferred to the center of petri dishes or to flasks. The flasks were shaken on a reciprocal shaker at 90 strokes/min. Colony radii on solid media were measured at 3, 7, and 14 days. Dry weights of mycelial mats of flask cultures were determined at 7 and 14 days. Values of ED_{50} were derived from the percentage inhibition of growth as compared to the control. Each treatment was replicated three times and all experiments were performed twice.

Greenhouse experiments.—All tests in the greenhouse were performed in soil from a Salisbury, Maryland field naturally infested with *P. myriotylum*, *P. aphanidermatum*, and *P. ultimum* (10). Snapbean seeds (cultivars Early Gallatin and Blue Lake) were planted in 1,500 g of soil in 2-liter stainless steel beakers (10

seeds/beaker), and the beakers were incubated in constant temperature baths at 21 C for 1 wk. At that time, percentage seedling emergence was determined. The tanks then were covered with plastic to increase the relative humidity and the temperature was raised to 32 C for an additional wk. The beakers then were removed from the tanks and placed on a bench in a greenhouse compartment kept at 27 ± 3 C. Bean damping-off and *Pythium* blight were evaluated 3 wk after planting by determining final plant stand and percentage of plants blighted. In some experiments plant height also was recorded at harvest time.

The following fungicides were used for seed treatments: *N*-(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide (captan); ethazol (ETMT 25 EC, Truban); pentachloronitrobenzene (PCNB)+ETMT (L-21, 22.8% PCNB+11.4% ETMT); PCNB+ETMT (L-205, 23.2% PCNB+5.8% ETMT); PCNB+ETMT (SD-205, 20% PCNB+5% ETMT); PCNB+ETMT (Zn 2055, 20% PCNB, 5% ETMT, 5% zinc 2-pyridinethiol 1-oxide); thiophanate-methyl+ETMT (Banrot, 25% thiophanate-methyl+15% ETMT); 6,8-dichloro-3-methyl-chromone oxime (U-34910 analytical); ethazol technical 99%; DTEH 70% aqueous; propyl-N-(γ -dimethylamino-propyl)carbamate (PDAC); and 2-iodobenzanilide (IBA, Benodanil), an experimental fungicide for the control of *Rhizoctonia solani*.

Fungicides were applied to bean seed either by direct fungicide application (DFA) or with the solvent infusion technique (SIT) described previously (7, 13). With the DFA method, the liquid materials were added to small batches of seeds, and the seeds were tumbled in glass jars for 5 min and immediately allowed to dry before planting. The wettable powders were mixed with graphite and applied to slightly moist seed (1 mg graphite:1 g seed), the seeds were tumbled for 5 min and immediately air-dried. With the SIT method, U-34910 and ethazol were dissolved in acetone or dichloromethane (DCM) in amounts indicated in each particular experiment. The PDAC was dissolved in water or acetone and DTEH in water or 95% ethanol. Bean seeds were immersed in the solutions for lengths of time indicated in each experiment.

TABLE 1. Toxicity of *N*-(3-dimethylaminopropyl) thiocarbamic acid *S*-ethylester hydrochloride (DTEH) to *Pythium* spp. in solid synthetic medium BM-1^a

Isolate no.	<i>Pythium</i> species	ED_{50}^b ($\mu\text{g}/\text{ml}$)		ED_{100}^b ($\mu\text{g}/\text{ml}$)
		1 wk	2 wk	
ATCC ^c 12531	<i>P. arrhenomanes</i>	0.1 - 1.0	0.1 - 1.0	5
ATCC 26081	<i>P. aphanidermatum</i>	0.1 - 0.25	0.75 - 1.0	>300
ATCC 10930	<i>P. butleri</i>	0.5 - 0.75	1.0	5
ATCC 10393	<i>P. debaryanum</i>	0.1 - 1.0	0.1 - 1.0	10
ATCC 16970	<i>P. irregulare</i>	0.1 - 0.25	0.75 - 1.0	2
Pma-C62 ^d	<i>P. mamillatum</i>	0.1 - 1.0	0.1 - 1.0	>300
ATCC 26082	<i>P. myriotylum</i>	0.1	0.1 - 0.25	50
Pp-A5 ^d	<i>P. paroecandrum</i>	0.1 - 1.0	0.1 - 1.0	>300
ATCC 26083	<i>P. ultimum</i>	0.25 - 0.5	0.5 - 0.75	>300
P-1A ^c	<i>Pythium</i> sp.	0.1 - 1.0	0.1 - 1.0	>300
P-2C ^c	<i>Pythium</i> sp.	0.1 - 1.0	0.1 - 1.0	>300

^aBasal Medium 1 (12).

^bDosage for 50 or 100% inhibition of growth.

^cAmerican Type Culture Collection.

^dIsolated from Salisbury soil in 1973.

^eIsolated from soybean roots in Maryland in 1975.

The solvent was allowed to evaporate in a fume hood and the seeds were planted in stainless steel beakers. Controls included nontreated seed and seed treated with solvent only.

To determine which of the fungicide application methods (DFA or SIT) resulted in more fungicide accumulation in the cotyledons, Tendercrop bean seeds were coated (DFA method) with ethazol (Truban, 25 EC), or immersed for 15 and 30 min in a solution of 5% ethazol in acetone (SIT). Treated and nontreated seeds were kept dry in the laboratory for 1 wk and then washed five times with acetone and 45 min with running sterile water to remove the fungicide from the seed surface. The seed coats were aseptically removed, and four cotyledons were placed on V-8 juice agar equidistantly from each other, 3 cm from a centrally placed inoculum plug of *P. ultimum*. The dishes were examined periodically to see whether the pathogen was inhibited near the cotyledons.

Field experiment.—Treated and nontreated Tendercrop snapbean seeds were planted 11 May and 4 August 1976 in a field heavily infested with *Pythium* spp. located near Salisbury, MD (10). Seeds were planted in rows 7.6 m long and the rows were spaced 91 cm apart.

Each row comprised a replication. Treatments were replicated five times, with 140 seeds per replication. Plant stand and *Pythium* blight severity were estimated at 2, 3, and 9 wk after planting. At 9 wk, all plants were harvested and fresh vine and pod weights were determined. Pod weight per plant was estimated by dividing the total pod weight per plot by the number of apparently healthy plants.

RESULTS

Laboratory experiments.—In general, little variation in mycelial growth response to DTEH in solid BM-1 was observed among the 11 *Pythium* spp. tested. The ED₅₀ values for the 11 species tested were 1 µg/ml or less (Table 1). The dose required for complete inhibition ranged from 2 to more than 300 µg/ml. Steep linear dosage-response curves were obtained with *P. aphanidermatum*, *P. irregulare*, and *P. ultimum* as the rate of DTEH increased from 0 to 1 µg/ml (Fig. 1). Further increases in concentration of the fungicide up to 300 µg/ml had very little additional effect. A similar dosage-response curve to DTEH was produced in liquid BM-1 with *P. irregulare* (Fig. 2-A). No bimodal response curves, such as those shown with pyroxychlor (14), were observed with the species tested.

In a separate experiment, medium BM-1 was divided into several portions after autoclaving and adjusted with sterile NaOH or HCl solutions to give pH values of 4.5, 5.5, 6.2, 6.8, 7.4, and 8.2. The media were buffered at these values with phosphate buffer. Before the agar in the medium became solidified DTEH (2 µg/ml) was added to all portions. Batches of BM-1 adjusted to these pH values without DTEH were used as controls. Inhibition of linear growth was maximum from pH 6.8 to 7.4 for all *Pythium* spp. tested (Fig. 2-B). Effect of pH on growth inhibition of *P. butleri* and *P. myriotylum* was identical with that of *P. ultimum* and *P. irregulare*, respectively. Except for *P. aphanidermatum*, considerable growth inhibition also was observed at pH 6.2. No inhibition occurred at pH 4.5 with all species tested and at pH 5.5 with *P.*

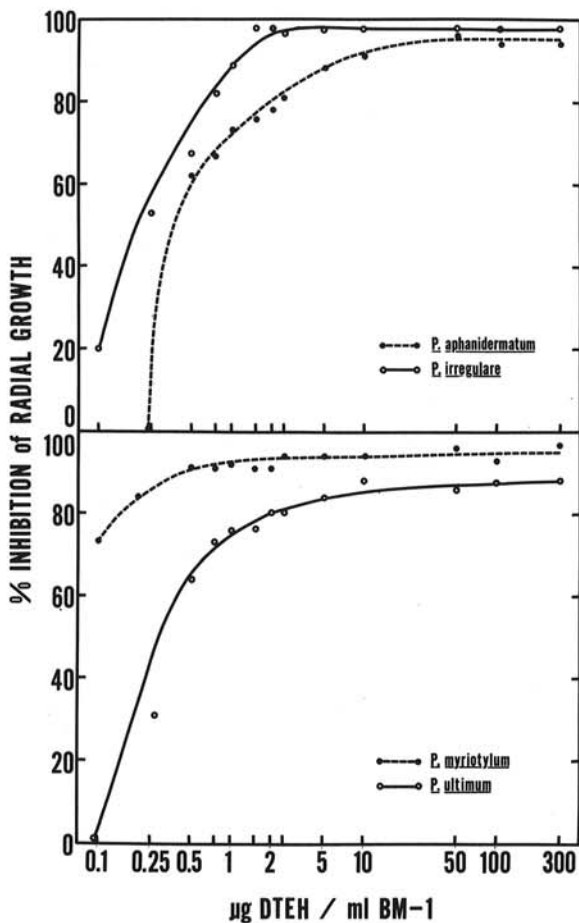


Fig. 1. Rate-response curves of four representative *Pythium* spp. on N-(3-dimethylaminopropyl) thiocarbamic acid S-ethyl-ester hydrochloride (DTEH)-amended solid basal medium 1 (BM-1).

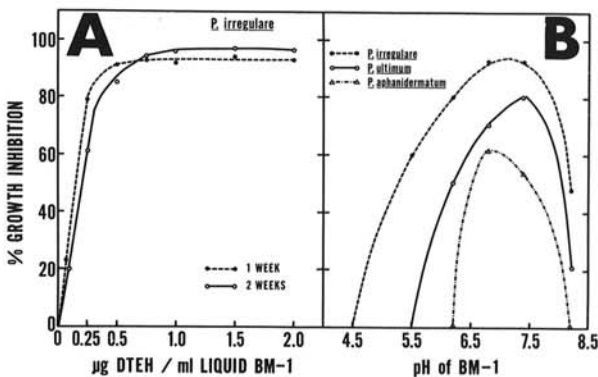


Fig. 2-(A and B). Rate-response curves of three representative *Pythium* spp. on N-(3-dimethylaminopropyl) thiocarbamic acid S-ethyl-ester hydrochloride (DTEH)-amended liquid basal medium 1 (BM-1). A) Effect of DTEH on growth inhibition as measured by dry weight of mycelium of *Pythium irregulare* in liquid BM-1; B) Effect of pH on growth inhibition of three *Pythium* spp. on solidified BM-1 containing 2 µg DTEH a.i./ml.

TABLE 2. Effect of fungicides applied to Early Gallatin snapbean seed with conventional methods or with the organic solvent infusion technique on the severity of blight caused by *Pythium* spp. in a greenhouse soil test

Fungicide ^b	Concentration (g active ingredient/kg seed)	Duration of immersion (min)	Plant stand ^a (%)	Pythium blight ^a (%)
None (control)			80	100
Acetone (control)		30	80	100
Dichloromethane (DCM) (control)		30	75	100
Ethanol		30	74	100
Solvent infusion technique (SIT):				
U-34910 5% in acetone		30	64	100
Ethazol 5% in acetone		30	92	30
Ethazol 5% in DCM		30	73	35
DTEH ^c 2.8% in ethanol		12	88	5
Direct fungicide application (DFA) ^b :				
Captan	1.9		85	95
ETMT ^d (Truban 25 EC)	0.9		85	85
PCNB ^e -ETMT (L-21)	3.4		75	100
PCNB-ETMT (L-205)	3.4		80	100
PCNB-ETMT (SD-205)	1.9		63	100
PCNB-ETMT (Zn 2055)	1.9		85	100
Thiophanate-methyl+ETMT	1.9		65	95
DTEH (SN 41703) ^f	1.4		100	0

^aAt 3 wk after planting.^bThe liquid fungicides of this group were added directly to the seed. The wettable powders were mixed with graphite before application to slightly moist seed (1 g seed:1 mg graphite).^cN-(3-dimethylaminopropyl) thiocarbamic acid S-ethylester hydrochloride (DTEH).^d5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole (ethazol).^ePentachloronitrobenzene.^fApplied directly to the seed as 70% aqueous solution.TABLE 3. Effect of two concentrations of ethazol in acetone applied to Blue Lake snapbean seed on the severity of blight caused by *Pythium* spp. in a greenhouse soil test

Technical ethazol in acetone	Duration of immersion (hr)	Plant stand ^a (%)	Pythium blight ^a (%)
None (control)		95 a ^y	100 a
Acetone (control)	0.5	94 a	100 a
	1	90 abc	98 a
	2	93 ab	100 a
	3	94 ab	100 a
Ethazol 5%	0.5	90 abc	89 a
	1	93 ab	23 b
	2	83 abcd	24 b
	3	83 abcd	15 b
Ethazol 10%	0.5	78 bcd	3 b
	1	70 d	14 b
	2	80 abcd	18 b
	3	75 cd	3 b

^aAt 3 wk after planting.^yNumbers followed by the same letter do not differ significantly ($P = 0.05$) by Duncan's multiple range test.

aphanidermatum, *P. butleri*, and *P. ultimum*. At pH 8.2 *P. irregulare* and *P. myriotylum* were inhibited by about 50% and *P. ultimum* by 22%. The other two species were not inhibited at pH 8.2.

Greenhouse experiments.—In the first experiment, only DTEH applied to Early Gallatin seeds directly as 70% aqueous solution at 1.4 g a.i./kg seeds prevented blight completely and allowed a 100% healthy stand. All

other fungicide treatments applied with the DFA method were ineffective in controlling blight and less effective in preventing damping-off than was DTEH (Table 2). Of the fungicides applied to the seed with the SIT, U-34910 in acetone solution was completely ineffective in controlling blight. Ethazol in acetone or DCM (30-min immersion) reduced *Pythium* blight considerably. The DTEH was equally effective in reducing blight when applied with either method. Acetone and DCM controls did not differ from the untreated controls. The concentrations used and the duration of soaking did not reduce plant height nor cause any other visible toxic effects on this cultivar. Ethazol and DTEH were selected for further experiments in the greenhouse.

An experiment was performed with seeds of the snapbean cultivar Blue Lake to test the effect of increasing ethazol concentrations in acetone and increasing duration of immersion in the fungicide-acetone mixture. Unlike the data with Early Gallatin, no significant control was observed when seeds of Blue Lake were immersed for 30 min in a 5% ethazol acetone solution (Table 3). However, *Pythium* blight was significantly reduced by 5% ethazol in acetone at 1, 2, and 3 hr immersion and with 10% ethazol at 0.5, 1, 2, and 3 hr immersion. The higher concentration of ethazol, however, resulted in lower stands and some plant toxicity (hypocotyl curvature). The combination of ethazol 5% in acetone and 0.75-1.0 hr immersion was selected for further studies. The effect of 5% ethazol in acetone (30-min immersion) on the cultivar Tendercrop is shown in Fig. 3.

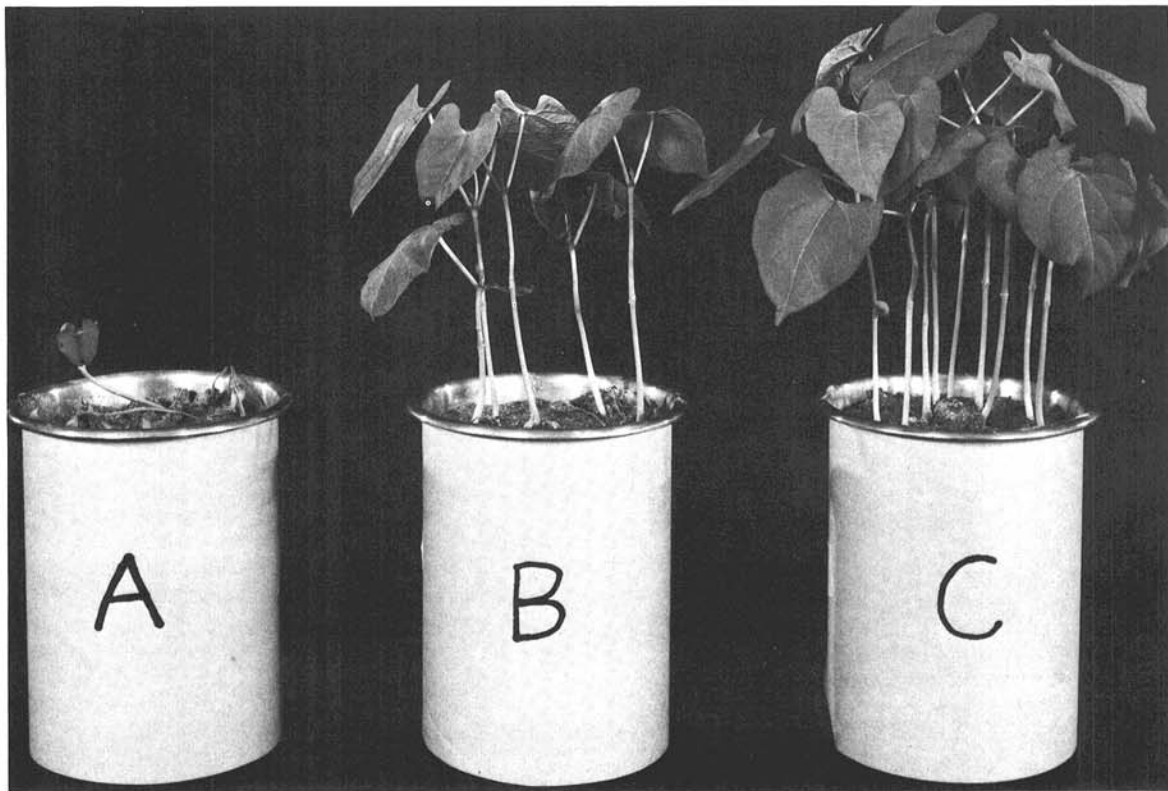


Fig. 3. Effect of ethazol and N-(3-dimethylaminopropyl) thiocarbamic acid S-ethylester hydrochloride (DTEH) on *Pythium* blight of Tendercrop snapbean in the greenhouse. Treatments of seed: (A) untreated seed; (B) 5% ethazol in acetone, 30 min immersion; and (C) 2.8% DTEH in ethanol, 30 min immersion.

TABLE 4. Effect of N-(3-dimethylaminopropyl) thiocarbamic acid S-ethylester hydrochloride (DTEH, SN41703) and propyl-N-(γ -dimethylaminopropyl) carbamate (PDAC, SN39744) on the severity of blight^a caused by *Pythium* spp. when applied to Blue Lake snapbean seeds

Treatment	Concentration (g active ingredient/kg seed)	Pythium blight ^y (%)	Plant stand ^y (%)
None (control)		97 a ^z	42 c
Direct fungicide application (DFA):			
PDAC	1.4	83 ab	36 c
	2.8	82 ab	38 c
	4.2	63 b	70 b
	5.6	17 c	94 a
DTEH	1.4	0 c	92 a
	2.8	2 c	94 a
	4.2	0 c	92 a
	5.6	0 c	90 a
Solvent infusion technique (SIT):			
PDAC 2.8% in water (12-min immersion)		85 a	84 ab
5.6% in water (12-min immersion)		57 b	88 a
2.8% in acetone (45-min immersion)		0 c	86 ab
5.6% in acetone (45-min immersion)		0 c	88 a
DTEH 2.8% in water (12-min immersion)		0 c	90 a
5.6% in water (12-min immersion)		0 c	92 a
2.8% in ethanol (30-min immersion)		0 c	88 a
5.6% in ethanol (30-min immersion)		0 c	86 ab

^aPythium blight attributed primarily to *P. myriotylum*.

^yAt 3 wk after planting.

^zNumbers followed by the same letter do not differ significantly ($P = 0.05$) by Duncan's multiple range test.

In another experiment, PDAC directly applied to the seed significantly reduced blight and the incidence of damping-off only when added at 5.6 g a.i./kg seed (Table 4). When PDAC was applied to seed with the SIT, significant control of blight was obtained with the 5.6% concentration in water or with 2.8% in acetone. The DTEH was very effective at all concentrations used with both methods of application (Table 4, Fig. 3). The solvents used in this experiment did not affect *Pythium* blight. Although plant height was not affected by the treatments, phytotoxicity (hypocotyl curling) was observed when seeds had been immersed for 30 min in 5.6% PDAC or DTEH in an organic solvent; however, no phytotoxicity was observed when seeds were immersed

for 12 min in 5.6% PDAC or DTEH in water.

In an experiment performed to detect penetration and accumulation of ethazol in Tendercrop bean cotyledons, the most pronounced zones of inhibition to *P. ultimum* were produced around cotyledons from seeds immersed in 5% ethazol in acetone for 15 or 30 min (Fig. 4). Cotyledons from seeds treated with ETMT 25 EC (Truban) (DFA) and from untreated seeds produced no zones of inhibition.

Field experiments.—Incidence and severity of *Pythium* blight in the field was very low during the first planting in May when temperatures were relatively mild. On the other hand, disease incidence, caused primarily by *P. myriotylum*, was very high in the August planting when hot, damp weather prevailed. Determination of plant stand at 3 and 9 wk after planting indicated that only the DTEH seed treatments appreciably improved stand (Table 5). Application of DTEH by the SIT method or directly (with or without IBA, a fungicide used with DTEH to protect plants from *R. solani*) resulted in more than 60% stand. Results with other fungicides were not significantly different from the control. Although vine weight per plot and pod weight per plant were not affected by the treatments, immersion treatment in DTEH + IBA increased pod yield per plot over the control by about 40-60%.

DISCUSSION

Our laboratory results indicate that the response of 11 *Pythium* spp. to the new systemic fungicide DTEH is quite uniform. The ED₅₀ values with all species tested on liquid and agar media did not exceed 1.0 µg DTEH (a.i.)/ml. The uniformity of response to DTEH of the *Pythium* spp. tested is in contrast to the response of *Pythium* spp. to pyroxychlor, another systemic fungicide selective for Phycomycetes (14). Pyroxychlor differs considerably in structure from DTEH. Moreover, the *Pythium* spp. and isolates used in the present study differed from those studied by others with pyroxychlor (14). Although the ED₅₀ values were quite uniform for all 11 species tested, their ED₁₀₀ values differed markedly; the solutions ranged from 2 to more than 300 µg a.i./ml DTEH.

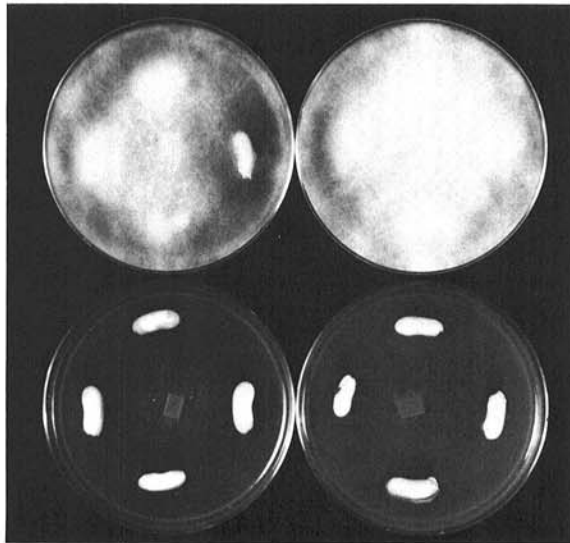


Fig. 4. Inhibition of *Pythium ultimum* on V-8 juice agar by ethazol in bean cotyledons. Upper row (left to right), untreated seed and seed treated with ethazol 25 EC (Truban); lower row (left to right), seed immersed in 5% ethazol in acetone for 15 and 30 min, respectively.

TABLE 5. Effect of six different fungicides applied to Tendercrop snapbean seeds on the severity of blight caused by *Pythium* spp. in a field at Salisbury, Maryland

Fungicide	Concentration (g active ingredient per kg seed)	Plant stand (%)		Pod wt (kg/plot)
		3 wk	9 wk	
None (control)	...	38 c ^u	39 b	1.9 b
Captan	1.9	40 c	38 b	1.7 b
Thiophanate-methyl+ETMT ^v	1.9	43 c	42 b	1.7 b
ETMT (Truban 25 EC)	1.9	41 c	39 b	1.8 b
Ethazol 5% in acetone ^w	...	45 c	46 b	2.1 ab
DTEH ^x	1.4	60 b	65 a	2.6 ab
DTEH 2.8% in water ^y	...	62 b	63 a	2.7 a
IBA 5% in acetone+DTEH ^z	1.4	76 a	68 a	3.1 a

^uNumbers followed by the same letter do not differ significantly ($P = 0.05$) by Duncan's multiple range test.

^v5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole (ETMT, Truban 25 EC) applied directly to the seed.

^wTechnical ethazol in acetone. Seed was immersed in the solution for 45 min.

^xN-(3-dimethylaminopropyl) thiocarbamic acid S-ethylester hydrochloride (DTEH) applied to seed directly.

^ySeed was immersed in the solution for 12 min.

^zSeed was immersed for 45 min in a 5% 2-iodobenzanilide (IBA)-acetone solution, followed by a direct DTEH application.

Another interesting point with the in vitro studies is the observation that toxicity of DTEH to *Pythium* spp. appeared to increase at pH values of 6.5 or higher, a phenomenon also observed by others (15). The degree of pH-dependency, however, was based on the particular isolate tested. From the in vitro results it appears that DTEH may control diseases caused by several *Pythium* spp. equally well in the greenhouse and field.

Our greenhouse results also indicate that it is possible to partially or completely control Pythium blight of bean with the systemic fungicides ethazol and DTEH, and to achieve partial control with DTEH's acetone-soluble analog PDAC. The degree of control with ethazol was far superior when this material was applied to the seed with the solvent-infusion technique than when added to the seed directly. The DTEH was equally effective with the SIT and the DFA method. The success of the solvent infusion technique with some fungicides may be a function of the ability of the solvent-fungicide mixture to penetrate the seed and accumulate in the cotyledons. Some evidence of ethazol accumulation in bean cotyledons that had been treated with the solvent infusion technique was demonstrated (Fig. 4). Further evidence indicates that organic solvents may facilitate movement of fungicides (3, 13) or other substances (7) into dormant plant seeds.

Pythium blight was controlled by DTEH in the greenhouse and also in a very heavily infested field near Salisbury, Maryland. Pod yields of Tendercrop snapbean were increased about 40-60% by DTEH, despite the fact that it did not result in increased vine weight per plot or productivity per plant. The increase in pod yields is attributed to DTEH protection of the plants from Pythium blight during the first 2-3 wk after planting, thus allowing a stand twice as large as that in control plots. Lastly, lower disease incidence and maximum yields in the field were obtained with very small amounts of DTEH (e.g., 1.4 g a.i./kg seed), corresponding to about 115 g a.i./ha. Further studies are now in progress with other *Pythium* spp. and with *Phytophthora* spp.

LITERATURE CITED

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