Enhanced Severity of Thielaviopis basicola Root Rot Induced in Soybean by the Herbicide Chloramben

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ABSTRACT

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Of eight herbicides applied at recommended rates to soil infested with *Thielaviopsis basicola* in the greenhouse, chloramben, alachlor, and DNPB resulted in increased severity of soybean root rot, compared with that in plants grown in soil without chloramben. Disease enhancement by chloramben was studied further. In the greenhouse, in soil infested with chloramben at the rate of 2 mg/kg (=3.4 kg/ha =3 lb/acre) disease enhancement was shown with three soybean cultivars, four isolates of the pathogen, and with endoconidia and chlamydospores as inoculum. The effect was expressed over a soil temperature range of 14-26 C. In a field infested with *T. basicola*, disease severity was greater and plant stand and yield were less in soil treated with chloramben than in soil without herbicidal treatment. Germination of spores of *T. basicola* was 2-4 times higher in rhizospheres of chloramben-treated soybean seedlings than in rhizospheres of untreated seedlings. Exudates from roots of soybean seedlings grown axenically with chloramben supported more germination of *T. basicola* spores, and caused development of larger *T. basicola* lesions when applied to soybean seedlings than did exudates from untreated plants. Root exudates from chloramben-treated seedlings contained more electrolytes and amino acids than did control exudates. Carbohydrates, fatty acids, and nucleic acids were increased little or not at all by chloramben. Casamino acids applied to soybean seedlings growing in sterilized soil infested with *T. basicola* increased symptom severity as compared with that in soybeans not so treated. Possibilities of disease enhancement due to direct stimulation of the pathogen, to increased virulence of pathogen, or to changes in populations of other microorganisms were ruled out.

The use of herbicides in production of field crops, including soybeans [*Glycine max* (L.) Merr.], has increased in recent years, and is an essential component of mechanized farming. However, increasing numbers of plant diseases are reported to occur more frequently or more severely after the application of herbicides (5). The most important diseases of soybeans in Michigan are various root rots, including that caused by *Thielaviopsis basicola* (Berk. & Br.) Ferr. (9).

The purposes of this research were to identify which, if any, of the herbicides currently used or potentially useful in soybean culture could increase the severity of root rot caused by *T. basicola*; to determine the effect of soybean cultivars, isolates of the pathogen, and environmental conditions on disease enhancement induced by one of the herbicides (chloramben); and to elucidate the mechanism of disease enhancement by chloramben.

MATERIALS AND METHODS

The pathogen.—*Thielaviopsis basicola* isolate 157, which was isolated from diseased soybeans from southern Michigan, was used unless stated otherwise. Isolates 170, 171, and 172 from other locations in Michigan were used in some experiments. Inoculum for greenhouse and laboratory experiments was grown for 10-50 days at 24 C on potato-dextrose agar (PDA) in petri dishes. For soil infestation in greenhouse experiments, the culture was first blended with 100 ml of water. Inoculum used in field experiments was grown in a soybean-sand medium in 1-liter flasks, each containing 5 g coarsely ground soybean seeds, 250 g sand, and 125 ml of water. The mixture was autoclaved for 30 min.

The herbicides.—The herbicides, with their common names, trade names, chemical names, sources, and concentrations applied, are listed in Table 1. Commercial formulations were used in the greenhouse and field experiments. A stock suspension of 10 mg active chloramben/ml was made by diluting the commercial formulation with distilled water. Technical grade chloramben was used in laboratory experiments, and for application to axenically grown soybeans. It was dissolved in acetone to make stock solutions of 400 and 10,000 μg/ml, which were stored at 4 C in the dark.

Greenhouse tests.—Soybean cultivar Harosoy-63 was used unless stated otherwise. Plants were grown in a mixture of steamed soil, peat, and coarse sand (1:1:3, v/v) in 10.5-cm (4-inch) diameter plastic pots, each containing 1,400 g of soil. Chloramben was mixed with the soil at the rate of 2 mg/kg air-dry soil which approximated the recommended field rate, 3 lb/acre or 3.4 kg/ha. The soil was infested uniformly at the rate of one blended agar culture per four pots. The soil then was potted, and 10-12 seeds were planted 1-2 cm deep. Routine watering was done by filling the saucers beneath the pots. After 3 wk, disease symptoms on roots and hypocotyls were evaluated according to a 0-6 scale: 0, no symptoms; 1,

isolated black flecks; 2, flecks coalescing, but without girdling; 3, coalescent lesions shorter than 1 cm with girdling; 4, girdling lesions longer than 1 cm, and total lesion area less than 50% of the root system; 5, lesion area longer than 50% of the root system; 6, lesion area covering 90-100% of root system. Each pot contained 8-10 plants, and mean ratings per pot were determined. The disease index was the mean of four replicate pot ratings. The fresh weights of plants also were recorded.

Field experiment.—The effect of chloramben and linuron on development of Thielaviopsis root rot was studied in the field. One hundred sixty soybean seeds were sown uniformly by hand in 6-m (20-ft) rows on 5 June 1974. Treatments included chloramben or linuron alone, chloramben or linuron + *T. basicola*, *T. basicola* alone, and an untreated control. The treatment units were single rows replicated six times in a latin square arrangement. Rows to which the pathogen was added were infested with 40 g of a 1-mo-old soybean meal-sand culture of *T. basicola* before covering the seed. Chloramben and linuron were diluted with water and sprayed 1 day after planting with a hand-held CO_2-driven sprayer calibrated to deliver an equivalent of 3.4 kg chloramben/ha (3 lb/acre), or 2.8 kg linuron/ha (2.5 lb/acre). At emergence and at intervals thereafter, all plants from a randomly chosen 0.3-m length of row were removed and examined for symptom development. Stand counts were made at 38 days, and plant height measured 64 days after planting. Plants were harvested in mid-October. The seeds were threshed with a portable thresher and yields were determined.

Spore germination tests.—Endoconidia were removed from PDA cultures with distilled water, then were washed three times with distilled water by centrifugation at 2 C. The conidia (10^4-10^5/ml) were mixed with the diluted stock solution of chloramben to give final concentrations of 2, 10, 30, and 100 μg/ml. Controls were distilled water and acetone at a concentration of 10 μliters/ml. Approximately 60 μliters of each of these spore suspensions were placed into the wells of depression slides, covered with a coverslip, and examined under the microscope for germination. Two hundred spores in each of three replicate wells were counted after 22 hr. All procedures were carried out aseptically.

The same method was used to determine germination of chlamydospores; germination of one or more cells in a chain was interpreted as germination of that propagule. Germination in chloramben-amended soil was tested with washed spores applied to smoothed soil in petri dishes. The spores were recovered on polystyrene film after incubation and staining (8).

Preparation of chlamydospores.—To obtain single cells or short chains of chlamydospores free of endoconidia, *T. basicola* was streaked on enriched PDA (extract of 200 g potato, 5 g yeast extract, and 30 g dextrose per liter) and incubated for at least 30 days. Most of the endoconidia were removed by washing the surface of the culture with sterile distilled water. The aerial mycelium bearing chlamydospores then was scraped from the agar with a spatula and was transferred to a tissue homogenizer. After 5 min of grinding in an ice bath, the suspension was centrifuged for 25 sec at 800 rpm. The supernatant liquid consisting mainly of endoconidia, was decanted. The sediment was resuspended in 10 ml of water. After eight to ten such centrifugations, the sediment contained nearly 100% of single-celled or short chains of chlamydospores.

Enumeration of soil microorganisms.—The number of

TABLE 1. Herbicides, their sources and rates applied in greenhouse experiments

Common names	Trade names	Chemical names	Sources	Concentration of commercial formulations	Rates applied (kg/ha)[a]
Chloramben	Amiben	3-amino-2,5-dichlorobenzoic acid	Amechem Prod., Inc.	2 lb/gal	3.4
Linuron	Lorox	3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea	E.I. duPont de Nemours & Co.	50% WP	2.8
Alachlor	Lasso	2-chloro-2′,6′-diethyl-N-(methoxy methyl)acetanilide	Monsanto Co.	4 lb/gal	2.8
DNBP	Dinoseb	2-sec-butyl-4, 6-dinitrophenol	Dow Chemical Co.	3 lb/gal	3.4
Trifluralin	Treflan	α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine	Eli Lilly & Co.	4 lb/gal	1.4
Fluorodifen	Preforan	p-nitrophenyl-α,α,α,-trifluoro-2-nitro-p-tolyl ether	CIBA-Geigy Corp.	3 lb/gal	5.0
Metribuzin	Sencor	4-amino-6-*tert*-butyl-3-(methylthio)-*as*-triazin-5(4H)-one	Chemagro Corp.	50% WP	0.56
Bentazon	Basagran	3-isopropyl-2,1,3-benzothiadiazinone-(4)-2,2-dioxide	Badische Anilin- & Soda-Fabrik AG	4 lb/gal	2.2

[a]Active ingredient. For equivalent in lb/acre, multiply kg/ha × 0.89.

propagules of soil fungi, bacteria, and actinomycetes was estimated on soil dilution plates with selective media (4), except that a solution of 0.85% NaCl was used as the diluent in samples for all three population determinations, and Tergitol NPX, a detergent, was added to potato-dextrose agar (1,000 mg/l) to retard fungal growth.

Axenic culture of soybean plants.—Soybean seeds with intact seed coats were surface-sterilized in 1,000 μg/ml sodium hypochlorite for 15 min, then allowed to imbibe sterile water into which an air stream was continuously bubbled for 5 hr. Aeration enhanced uniformity of germination. The seeds again were surface-sterilized for 15 min and placed hilum down on autoclaved filter papers (Whatman No. 1) folded to make parallel grooves, in sterile petri dishes. The filter papers were moistened with 5 ml water or chloramben solution (2 μg/ml). The grooves allowed the radicles to develop straight. The petri dishes were enclosed in plastic bags and incubated at 24 C in the dark for 4-6 days, when the radicles were 3-6 cm long.

The seedlings were transferred to 0.1-strength modified Hoagland's solution (2) containing 2 μg chloramben/ml in 21 × 100 mm test tubes with stainless steel caps. The roots were immersed in the solution and the tubes were incubated under continuous fluorescent light (6.3 klux). Each tube contained four seedlings. Control seedlings were in Hoagland's solution alone and in Hoagland's solution plus acetone (0.5 μg/ml).

Collection and analysis of root exudate.—After incubation for 36 hr, the seedlings were lifted aseptically from the tubes, rinsed five times with 2-ml aliquots of sterile distilled water, and then were incubated for 12 hr in 25 × 180 mm culture tubes, each containing 40 ml of distilled water to collect root exudates. The seedlings were again placed for 36 hr in modified 0.1-strength-Hoagland's solution with chloramben, the roots were washed once more, and the exudate was collected. The incubation and exudation periods were alternately repeated four times. Exudate samples were incubated on PDA to test for contamination.

The exudate solutions were measured for electrolyte concentration with a conductivity bridge, and then were concentrated to 25% of their original volume under vacuum at 60 C. The concentrated exudates were adjusted to equal volumes and were analyzed quantitatively for sugars, amino acids, fatty acids, and nucleic acids. The anthrone reagent (11) was used to determine the total carbohydrate content, using glucose as a standard. Total amino acids and related compounds were determined with ninhydrin (10) using glycine as the standard. Ferric hydroxymate (13) was used to determine the total fatty acid content. The nucleic acid content of root exudate was determined by ultraviolet absorption at 260 μm.

TABLE 2. The effect of herbicides on Thielaviopsis basicola root rot of soybeans planted in a steamed soil mix and in naturally infested soil in the greenhouse

Herbicide	Rate (kg/ha)[a]	Disease index[b]	
		Greenhouse mix	Naturally infested soil[c]
Chloramben	3.4	6.0 A	4.8 A
Alachlor	2.8	6.0 A	3.3 A
DNBP	3.4	5.9 A	3.6 A
Linuron	2.8	4.3 B	1.0 B
Trifluralin	1.4	5.0 B	1.9 B
Fluorodifen	5.0	5.0 B	1.8 B
Metribuzin	0.56	5.0 B	1.1 B
Bentazon	2.2	5.4 B	2.1 B
Untreated	0	4.5 B	1.1 B

[a]For equivalent in lb/acre, kg/ha × 0.89; for equivalent in mg/kg, kg/ha × 0.6.
[b]Mean of four replicate pots, each containing 8-10 plants. Disease index was based on a scale of increasing symptom severity from 0-6. Means followed by different letters are significantly different, according to Student's paired *t*-test ($P = 0.05$).
[c]The soil was collected from a soybean field in southeastern Michigan, and mixed with one-third volume of coarse sand before use.

TABLE 3. The effect of chloramben on Thielaviopsis basicola root rot of soybean in the greenhouse[a]

Treatment	Disease index[b]		Fresh weight of plants (g)[b]	
	Greenhouse mix	Conover loam	Greenhouse mix	Conover loam
No treatment	0.0 A	0.0 A	26.8 A	27.5 A
Chloramben	0.0 A	0.0 A	23.5 B	20.8 B
T. basicola	5.0 B	2.8 B	24.4 B	20.9 B
Chloramben + *T. basicola*	6.0 C	5.3 C	13.7 C	17.6 C

[a]Chloramben was applied to soil before seeding at the rate of 3.4 kg/ha (=3 lb/acre).
[b]Mean of four replicate pots each containing 8-10 plants. Disease index was based on a scale of increasing symptom severity from 0-6. Student's *t*-test was applied to all data in each column, separately. Means followed by different letters are significantly different ($P = 0.05$).

Spore germination in the rhizosphere.—Soybeans were germinated and grown axenically in petri dishes containing 2 μg chloramben/ml for 7 days. A suspension containing chlamydospores and endoconidia in equal numbers was atomized onto glass slides that were dipped in molten 2% water agar (45 C). Each slide then was transferred to a petri dish, and each of five chloramben-treated and control seedlings was placed on separate slides with their radicles in contact with the agar film. The seedlings and slides were covered with sieved (2 mm), moistened (15-20%) Conover loam. The petri dishes then were covered, and set at a 45-degree angle with the long axis of the slide parallel to the slop to promote root growth in contact with the slide. After 12 hr of incubation, the slides were removed, and the outlines of the radicles were traced with ink on the backs of the slides. After air-drying, most of the soil particles were removed with a camel's-hair brush. The slide was stained with cotton blue lactophenol. Germination of approximately 700 spores of each kind within the marked area was counted on each slide.

All experiments except the field experiment were repeated several times with similar results. Analysis of variance was applied to the data, and differences between means were identified with Student's *t*-test, or by least significance differences.

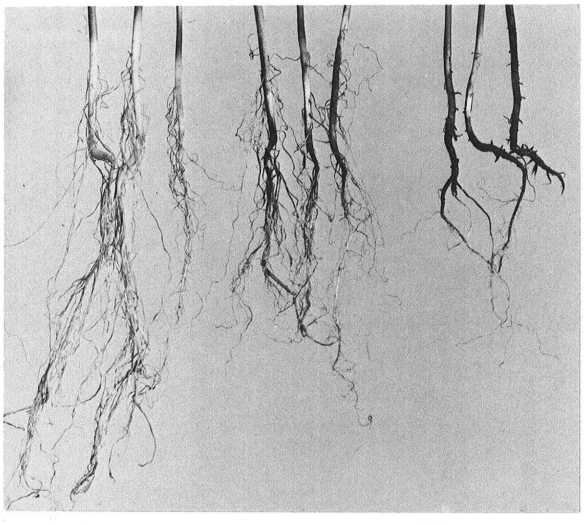

Fig. 1. Effect of chloramben at 3.4 kg/ha (3 lb/acre) on Thielaviopsis basicola root rot of soybean in greenhouse mix. Left, no treatment; center, *T. basicola* alone; right, chloramben + *T. basicola*. Chloramben alone was similar to the no treatment control.

RESULTS

Effect of herbicides on Thielaviopsis root rot.—Eight herbicides commercially used or with potential to be used for soybeans were tested in the greenhouse at rates within the recommended ranges, for their effect on Thielaviopsis root rot. The tests were done using an artificially infested potting mix or naturally infested soil collected from a soybean field in southeastern Michigan. Chloramben, alachlor, and DNBP significantly enhanced the symptoms of *T. basicola* on soybeans planted in both soils (Table 2). Linuron had no significant effect on symptom development, but the roots were lighter in color than those of control plants. Trifluralin, fluorodifen, metribuzin, and bentazon did not cause significant changes in disease development in either soil. No disease symptoms were detected on plants from any of the herbicide treatments in noninfested soil.

Chloramben appeared to increase *T. basicola* root rot to a greater extent than did the other herbicides; therefore, it was investigated further. Soybean seeds were sown in the greenhouse in a potting mix or in Conover loam. The treatments were: noninfested soil treated with chloramben, infested soil without chloramben, infested soil with chloramben, or untreated soil. After 3 wk the plants were removed from soil, washed, weighed, and disease symptoms evaluated. Again, soybeans grown in chloramben-treated soils were more severely diseased than those in soils without chloramben (Table 3, Fig. 1). Noninoculated plants showed no symptoms. *Thielaviopsis basicola* or chloramben alone reduced the fresh weights by 9-24%, but chloramben + *T. basicola* reduced fresh weights by 36-49% as compared with untreated controls.

Effect of chloramben and linuron on Thielaviopsis root rot of soybeans in the field.—Disease severity of soybeans

TABLE 4. Effect of chloramben on Thielaviopsis basicola root rot of soybean in the field[a]

Treatment	Disease index[b]	Stand count[c]	Plant height[d] (cm)	Seed yield[e] (g)
No treatment	0.5 A	140 A	47.0 A	1472 A
Chloramben	0.5 A	138 A	43.7 A	1503 A
T. basicola	2.2 B	110 B	42.9 A	1314 B
Chloramben + *T. basicola*	3.8 C	94 C	35.6 B	1283 C

[a]Student's *t*-test was applied to each column of data separately, and means followed by different letters are significantly different ($P = 0.05$).

[b]Disease symptoms are evaluated 23 days after seedling. Each value is the mean of six replications of 40 to 50 plants from a randomly chosen 0.3-m length of row. Symptoms were rated on a scale of increasing symptom severity from 0-6.

[c]Stand count was made 38 days after seeding. Data are mean numbers of plants in six replicated rows each 6 m long.

[d]Plant height was measured 64 days after seeding. Data are means of six replications each of 10 plants randomly chosen from a 6-m-long row. The height was measured from the soil line to the node of youngest leaf.

[e]Mean of six replications, each 6 m long, except for 3, 0.6 m longths removed due to previous sampling.

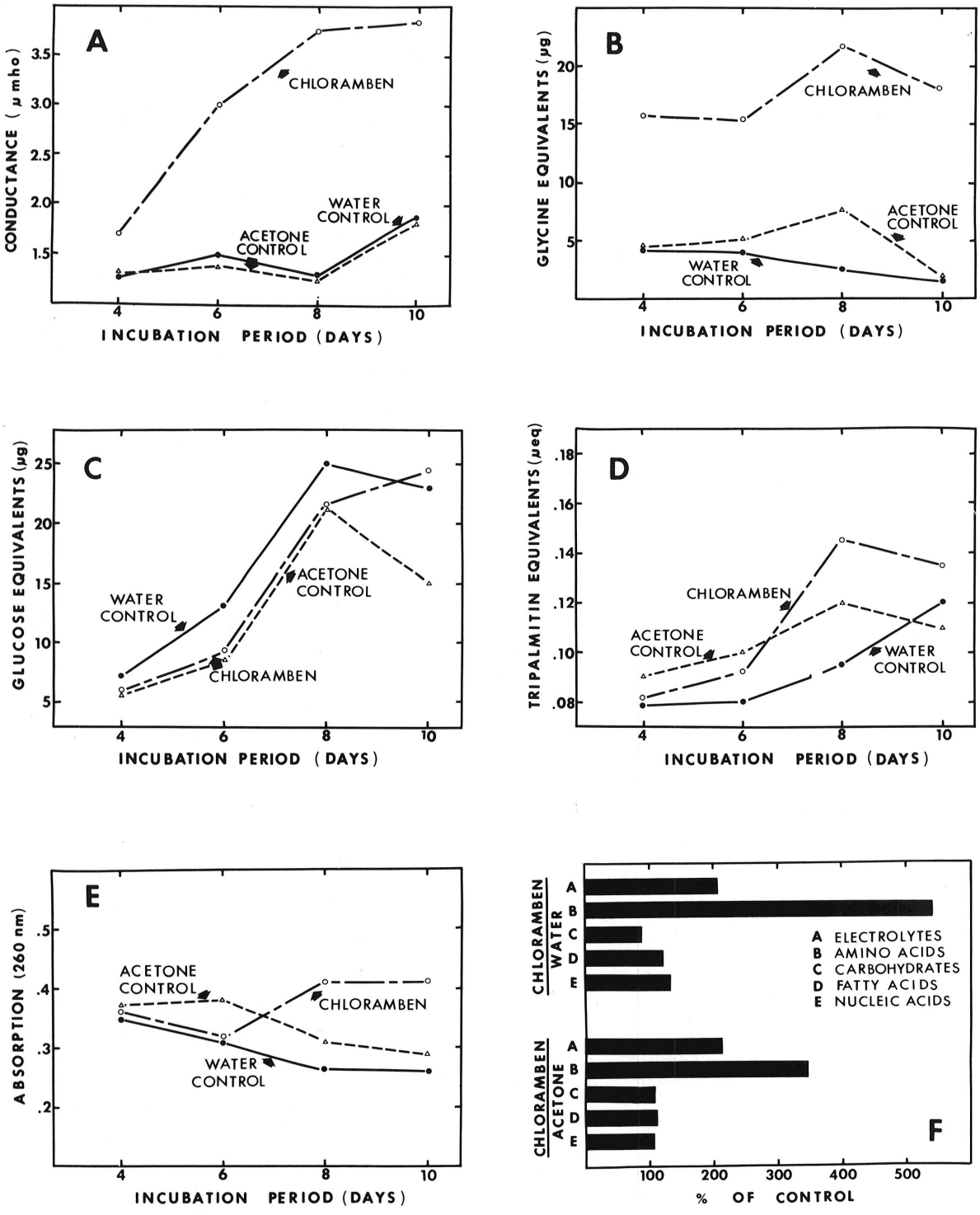

Fig. 2-(A to F). Effect of chloramben on quantities of electrolytes, amino acids, carbohydrates, fatty acids, and nucleic acids exuded by roots of soybean seedlings collected during four, 12-hr periods. Values are means of 64 seedlings. Soybeans were grown axenically in a dilute salt solution containing 2 μg chloramben/ml, acetone in an amount equivalent to that in the chloramben treatment (0.5 μl/ml), or in the salt solution alone (water control). **A)** Electrolytes, **B)** Amino acids, **C)** Carbohydrates, **D)** Fatty acids, **E)** Nucleic acids, **F)** Total amounts exuded in relation to acetone and water controls.

grown in the field in chloramben-treated soil was increased over that of soybeans grown without chloramben. Plants in nontreated soil or in soil treated with chloramben but without the pathogen had very mild symptoms of the disease, presumably from indigenous *T. basicola* (Table 4). Linuron had no significant effect on disease. Plant population 38 days after planting was reduced 21% by *T. basicola* and 33% by *T. basicola* + chloramben, as compared with untreated controls. These values differed significantly ($P = 0.05$). Chloramben alone did not reduce stand counts. *Thielaviopsis basicola* often was observed to colonize and sporulate on seeds and on non-emerged seedlings. The pathogen may cause pre-emergent damage to soybeans in the field, in addition to its pathogenesis to established plants (9). Treatment with chloramben + *T. basicola* decreased heights of soybean plants as compared with those that received other treatments. Seed yield of plants in soil infested with *T. basicola* was reduced by 11%, and that of plants in soil with *T. basicola* + chloramben by 13%, compared with the untreated controls. These values differed significantly ($P = 0.05$).

Effect of soybean cultivar, pathogen isolate, and spore type on chloramben-enhanced root rot.—Disease severity in the soybean cultivars Corsoy, Hark, and Harosoy-63 was compared in chloramben-treated soil and in nontreated soil in the greenhouse. Symptom expression in all three cultivars was enhanced to a similar extent by application of chloramben ($P = 0.05$).

Disease induced by four isolates of *T. basicola* from different localities was increased similarly in soil treated with chloramben ($P = 0.05$).

Endoconidia or chlamydospores of isolate 157 of *T. basicola* were used as inocula in chloramben-treated or untreated soil in the greenhouse. Endoconidia were applied at 4×10^4/g soil and chlamydospores at 2×10^3/g soil. Soybeans grown in chloramben-treated soil developed more severe disease than controls with either form of inoculum ($P = 0.05$).

Effect of concentration of chloramben and application method on disease development.—Chloramben applied at half the usual rate, 1.5 mg/pot (≅ 1.7 kg/ha, or 1.5 lb/acre), did not result in enhanced disease development, whereas the disease index for plants treated with 3.0 mg chloramben/pot in the presence of *T. basicola* was significantly greater than that for *T. basicola* alone ($P = 0.05$).

Chloramben was either incorporated into soil as in previous greenhouse experiments, or an equal amount was sprayed with an atomizer onto the soil surface after sowing to simulate field practice. The chloramben used for surface application was diluted to 5 ml per pot so that the volume of liquid received per unit surface area approximated that of a field application. One hundred ml of water was added to the saucer under each pot daily, and the same amount of water was sprinkled onto the soil surface to simulate natural rainfall once every 6 days. Surface application of chloramben enhanced disease to a level comparable to that obtained by incorporation into soil (disease indices = 5.2 and 5.5, respectively). *Thielaviopsis basicola* alone gave a disease index of 3.3, which was significantly less than the indices of the other treatments ($P = 0.05$).

Effect of soil temperature on the development of Thielaviopsis root rot of soybeans in chloramben-treated soil.—Containers of soybeans in soil treated with chloramben and/or *T. basicola*, were grown in water baths at 14, 18, 22, and 26 C in the greenhouse. Disease indices for *T. basicola* without chloramben were 5.3, 4.8, 4.2, and 1.5 C, respectively. Chloramben significantly ($P = 0.05$) enhanced disease severity at all temperatures; disease indices were 6.0, 6.0, 5.3, and 2.5 C, respectively.

Possibility of direct stimulation of germination and growth of Thielaviopsis basicola by chloramben.—Endoconidia germinated less than 1% and chlamydospores about 10% in water or on soil containing chloramben at concentrations of 0, 2, 10, 30, and 100 μg/ml. There were no significant differences with either spore type. Germination was 70% on sterilized soil. Growth of *T. basicola* was measured in plates of 0.1-strength PDA containing 0, 1, 3, 10, 30, or 100 μg/ml chloramben, and incubated at 24 C. Colony diameters measured daily from the 3rd to the 10th day showed no significant differences among treatments.

Dry weight and sporulation were measured in colonies of *T. basicola* growing in modified potato-dextrose broth (per liter: extract of 200 g potato, 30 g dextrose and 5 g yeast extract) to which technical chloramben was added at 2 μg/ml. Control flasks contained no chloramben. Five flasks per treatment, each containing 50 ml broth, were inoculated with approximately 1,000 endoconidia and the cultures were incubated at 24 C for 21 days. The mycelium in each flask was fragmented in a Waring Blendor, and four 2-ml samples were dried at 70 C overnight in pre-weighed pans. Sporulation was determined by counting the endoconidia in samples of the fragmented tissue with a haemocytometer. No significant differences in sporulation or dry weight were detected between the chloramben treatments and controls when paired *t*-tests were applied to the data.

Possibility of increased virulence of Thielaviopsis basicola due to chloramben.—The effect of chloramben on virulence of *T. basicola* was tested by growing the pathogen for 21 days in modified potato-dextrose broth containing chloramben at 2 μg/ml. The washed and fragmented mycelium was mixed (3 ml/pot) with vermiculite into which soybean seeds were sown. Pots were replicated three times and kept in a greenhouse for 4 wk. Disease index was the same (4.4) for plants grown in vermiculite infested with *T. basicola* cultured in chloramben-amended media as with the fungus grown without chloramben.

Effect of chloramben on microbial populations in soil.—The possibility was investigated that chloramben enhanced disease development by *T. basicola* indirectly by altering the microbial population. Conover loam (approximately 1,500 g) at 12% moisture was supplemented with 2 mg chloramben/kg (3 lb/acre) in the presence or absence of glucose at 0.1%. Control soil contained no chloramben. The soils were incubated in closed plastic bags at 24 C. Populations of fungi, bacteria, and actinomycetes were determined 1 and 3 wk after treatment. Total fungal and actinomycete numbers were not affected by chloramben. Changes in the bacterial populations were not consistent from experiment to experiment, and thus were inconclusive. Glucose increased the population of all three microbial groups, but no differential effects due to chloramben were

revealed by the methods used.

The influence of other microorganisms was by-passed by testing whether chloramben would increase disease in axenically-grown plants. Soybean seedlings grown for 8 days in the presence or absence of chloramben were transferred to autoclaved soil (15 g) in each of 32 test tubes (16 × 120 mm). Two ml of washed endoconidial suspension (5×10^4 spores/ml) were transferred into each test tube. Thirty micrograms of chloramben (2 μg/g soil) from a stock solution in acetone were added to half of the tubes. The control tubes received water. All procedures were done aseptically. The tubes were incubated for 14 days at 24 C under continuous fluorescent light (6.3 klux). After incubation, portions of the soil in each tube were plated on PDA; only *T. basicola* was observed after incubation for 2 and 5 days. The average disease index for chloramben-treated plants was 5.7, which differed significantly ($P = 0.05$) from that of control plants, 3.8.

Predisposition of soybeans to Thielaviopsis root rot by chloramben.—Soybeans were grown in chloramben-treated potting mix (2 mg/kg soil) for 7 days, washed and transplanted into *T. basicola*-infested soil with no chloramben. The roots were examined 2 wk later. The disease index of such plants was significantly greater (4.0) than that of plants transplanted from soil without chloramben (2.8) ($P = 0.05$). This experiment indicated that soybeans were predisposed to Thielaviopsis root rot by chloramben.

To determine whether or not chloramben caused a decrease in host resistance, 5 μliters of a suspension of endoconidia containing 300 spores/μliter were injected, with a sterilized microsyringe, into the hypocotyls of 9-day-old soybeans growing in pots in a soil mix containing 2 mg chloramben/kg soil. The inoculated soybean plants were grown for 2 more weeks with the pots in a water bath at 18 C. The mean lesion lengths for 41 chloramben-treated and 37 untreated soybeans was 1.9 and 2.0 cm, respectively. Untreated plants and plants injected with sterile water showed no symptoms. The experiment indicated that chloramben apparently did not alter host resistance.

Spore germination in soybean rhizospheres.—An average of 39% of chlamydospores and 17% of endoconidia germinated in the rhizospheres of chloramben-treated plants, whereas 14% of chlamydospores and 4% of endoconidia germinated in rhizospheres of untreated soybeans. The results differed significantly ($P = 0.05$) for both kinds of spores. The experiment indicated that chloramben had caused quantitative or qualitative changes in the nutrient level of the rhizosphere. A study of root exudates followed.

Root exudation.—The chemical nature of the root exudates of plants grown aseptically in the presence or absence of chloramben (2 μg/ml) was investigated. The data were based on four replications, each of which was the pooled contents of four test tubes. Chloramben-treated seedlings exuded significantly ($P = 0.05$) more electrolytes and amino acids than did seedlings exposed to either acetone solution (0.5 μliters/ml) or water, in measurements made on the 4th, 6th, 8th, and 10th days (Fig. 2-A,B). Chloramben had no significant effect on the exudation of carbohydrates and nucleic acids, but the amount of fatty acids in exudates collected on the 8th day was significantly higher than that in the controls (Fig. 2-C,D,E). The total material exuded by plants cultured with chloramben, as percentages of that exuded by plants cultured in dilute salt solution alone, were as follows: 540% for amino acids, 205% for electrolytes, 80% for carbohydrates, 123% for fatty acids, and 132% for nucleic acids (Fig. 2-F). Exudates from chloramben-treated soybeans, as percentages of acetone solution controls were 213% for electrolytes, 368% for amino acids, 108% for carbohydrates, 110% for fatty acids, and 106% for nucleic acids.

Germination of endoconidia in root exudates of chloramben-treated soybeans.—Washed endoconidia were suspended in sterile distilled water and 50 μliters of this suspension was placed in the wells of sterilized depression slides. Concentrated (×4) root exudates were collected from soybeans cultured aseptically in the presence or absence of chloramben, and passed through 0.22-μm Millipore filters. Ten μliters of each of the filtrates was added aseptically to each of six wells. Sterilized coverslips were applied and the slides were incubated in a moist petri dish for 22 hr at 24 C. Two hundred spores per well were then examined for germination. The exudate from chloramben-treated soybeans supported a mean of 20% germination, whereas the exudate from water and acetone controls supported 5% germination. None of the spores germinated in water alone.

Effect of root exudates from chloramben-treated soybeans on lesion development.—Concentrated (4×) root exudates from soybeans grown aseptically with or without chloramben solution (2 μg/ml), and acetone of the same concentration as in the chloramben solution, were mixed with equal volumes of a washed endoconidial suspension. Five μliters of these mixtures were applied separately to the hypocotyls of each of 20 axenically grown 6-day-old soybean seedlings, kept moist in petri dishes. The inoculated plants were incubated at 24 C under fluorescent light (6.3 klux; 12 hr/day) for 7 days. The mean length of lesions was 20.8 mm for seedlings receiving exudates from chloramben-treated soybeans. By contrast, hypocotyls receiving exudates from untreated or acetone-treated soybeans, or water alone, had lesion lengths of 8.1, 8.4, and 4.2 mm, respectively.

To verify that amino acids were the component of the root exudate mainly responsible for the increased severity of symptoms, a mixture of amino acids (Difco Vitamin-Free Casamino Acids) was added to sterilized soil in test tubes in which axenic soybean seedlings were growing. The tubes contained 12 g soil each, infested with 4×10^3 endoconidia/g, and were incubated at 24 C for 21 days. The quantity of amino acids exuded during four 12-hr periods over 10 days by a soybean seedling cultured with chloramben was approximately 71 μg. Therefore, the amount of Casamino acids used, 200 μg per tube, was considered a reasonable estimate of the amount exuded during 21 days. The mean disease index for 18 plants in tubes supplemented with Casamino acids was 2.7 and that of the inoculated controls without Casamino acids was 2.0. The difference was significant at $P = 0.05$. Plants grown at the same time in chloramben-amended soil (2 mg/kg soil) infested with *T. basicola* also had an average disease index of 2.7, whereas controls without chloramben had a disease index of 2.0.

DISCUSSION

Soybeans grown in soil containing the herbicide chloramben at 3.3 kg/ha (3 lb/acre) had more severe symptoms of Thielaviopsis root rot than those grown in soil without chloramben. Chloramben-induced enhancement of Thielaviopsis root rot was nonspecific with respect to different soybean cultivars, various isolates of the pathogen, type of inoculum, or soil temperatures from 14 to 26 C. The possible economic importance of this effect was shown in a field experiment in which *T. basicola* + chloramben gave lower plant stands, and lower seed yields than plants grown in the presence of the pathogen alone. Chloramben was used on 32% of the treated acreage of soybeans in states of the north central region in 1973 (3). The wide host range and geographical distribution, and long survival time of the pathogen in soil suggest that chloramben be avoided where there is evidence for the presence of *T. basicola* in soybean fields.

Recently, Katan and Eshel (5) identified four possible mechanisms whereby disease severity could be increased by herbicides: (i) direct stimulation of the pathogen, (ii) increased virulence of the pathogen, (iii) increased host susceptibility, and (iv) suppression of microorganisms antagonistic to the pathogen. With the methods used, we found no evidence that host resistance per se was reduced by chloramben, but germination of chlamydospores and endoconidia near the roots of chloramben-treated soybean seedlings was increased due to increased root exudation, particularly of amino acids. Increased symptom severity resulted when roots were treated with exudate from chloramben-treated plants, or with Casamino acids. We also found no evidence that disease enhancement was due to direct stimulation of the pathogen, to increased virulence, or to population changes of other soil microorganisms.

Wyse et al. (16, 17) found that EPTC and chloramben increased exudation from hypocotyls and roots of navy bean, and attributed increased Fusarium root rot in bean fields treated with EPTC to this effect. Other examples of the association of herbicide-induced enhanced root exudation with increased root disease are Rhizoctonia damping-off of sugar beet seedlings in soil treated with Pyramin and Ro-Neet (1), and Pythium and Rhizoctonia seedling root rot of corn treated with picloram (6).

Toussoun and Patrick (15) reported that ether extracts of decomposing plant materials, later found to contain benzoic acid, increased the exudation of ninhydrin-positive substances from bean stems, and increased pathogenesis by *T. basicola*, *Fusarium solani* f. sp. *phaseoli*, and *Rhizoctonia solani*. Ether extracts of decomposing barley stimulated the germination of *T. basicola* chlamydospores on root surfaces of cotton seedlings (7). Chloramben [3-amino-2,5-dichlorobenzoic acid] is a substituted benzoic acid; its enhancement of Thielaviopsis root rot may involve the same mechanism as its analogue, benzoic acid.

The role of amino acids in increasing the inoculum potential of *T. basicola* toward soybean plants exposed to chloramben is consistent with findings that the carbohydrate, amino acid, organic acid, and lipid constituents of alfalfa hay all supported germination of *T. basicola* (14). Papavizas and Kovacs (12) also found germination of *T. basicola* to be stimulated in soil amended with unsaturated fatty acids and soybean lecithin. Although total fatty acids exuded by soybeans treated with chloramben were 23% higher than those exuded by control plants, in the present work the differences were statistically significant only on the 8th day. Possibly, during that short period of increased fatty acid exudation, the inoculum potential was further increased. Organic acids were not evaluated in the present work. If their exudation was increased by chloramben, they also could have contributed to the increased inoculum potential.

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