Stimulation of Spore Germination of Thielaviopsis basicola by Fatty Acids from Rhizosphere Soil

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ABSTRACT

No fatty acids (C_{16} to C_{18}) were detected from bean roots after 8 days of growth in aseptic liquid cultures, but plants grown in sand-solution cultures released 0.60, 0.48, and 0.55 μg/plant palmitic acid, stearic acid, and oleic acid, respectively. Total fatty acids were 1 to 2 times higher in extracts of rhizosphere soil than in extracts of nonrhizosphere soil. The total amount of unsaturated fatty acids (linoleic, oleic, palmitoleic) in extracts of soil from the rhizosphere of 6-, 14-, and 21-day-old bean plants exceeded that of extracts from nonrhizosphere soil. Endoconidia and chlamydospores of *Thielaviopsis basicola* did not germinate in nonamended control soil. Considerable germination occurred in bean rhizosphere soil and in nonrhizosphere soil fortified with hexane extracts of rhizosphere soil. Rhizosphere soil extracts from susceptible hosts of *T. basicola* (bean, cotton, tobacco) had more fatty acids than extracts from nonhost plants (corn, wheat, kale); yet corn and wheat rhizosphere soil extracts stimulated chlamydospore germination. Extracts from soil amended with alfalfa hay contained 1 to 2 times more fatty acids than extracts of nonamended soil during the first 4 days after hay incorporation. Extracts from alfalfa-amended soil stimulated spore germination.

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Additional key words: fungistasis, black root rot, exudation.

Soil fungistasis, the inability of fungus spores to germinate when in contact with soil under conditions normally favorable for germination, may be annulled by various nutrients added to soil (11). Complex organic materials such as root exudates, plant extracts, plant residues, molasses, and potato-dextrose broth have been more effective in annulling fungistasis than compounds such as sugars, amino acids, organic acids, or vitamins (11).

Because of soil fungistasis, endoconidia and chlamydospores of *Thielaviopsis basicola* (Berk. & Br.) Ferr., the pathogen that causes black root rot of many economic plants, do not normally germinate in soil (1, 7, 10, 15, 18). Soil fungistasis was not annulled when simple compounds such as sugars, amino acids, inorganic N, and mixtures of sugars and amino acids were added to soil (15, 18). Germination, however, occurred in soil adjacent to germinating bean seeds and in rhizosphere soil (15); in soil amended with alfalfa hay, corn stover, V-8 juice (1, 15, 18); and in soil amended with carrot juice (10). The most unusual compounds that annulled fungistasis were lecithins of plant origin, oil- and fat-soluble vitamin E, and certain higher unsaturated fatty acids (linoleic, linolenic, palmitoleic) and triglycerides (15). Saturated fatty acids (palmitic, stearic) and synthetic lecithin (β-γ-dipalmitoyl-DL-α-lecithin) were unable to annul fungistasis (15). Soybean lecithin is known to contain ca. 68% unsaturated fatty acids (55% linoleic, 9% palmitoleic, and 4% linolenic) (16).

This investigation was performed (i) to determine whether fatty acids of a high molecular weight are exuded by plants and whether they may be found in the rhizosphere or in organic matter decomposing in soil; and (ii) to learn more about the annulment of fungistasis by these long-chain fatty acids.

MATERIALS AND METHODS.—*Soil.*—Galestown-Evesboro loamy sand was used. The soil (pH 5.8) contained 77% sand, 15% silt, and 8% clay, and had a water-holding capacity (WHC) of 30%. The air-dried soil contained 2.94% total C, 0.12% total N, 4 μg/g soil NO_3-N, and 13 μg/g soil NH_4-N. The soil was sieved through a 20-mesh screen and kept before use at ca. 30% of the WHC for at least 2 weeks.

When needed, dry, ground alfalfa hay was added to 250-g soil portions at 0.5% of the oven-dry weight of soil, and the alfalfa-amended soil was kept at ca. 40 to 50% of WHC. Zero, 2, 4, 7, and 21 days after alfalfa addition, soil portions were removed from the containers and extracted for fatty acids. Extracts were also prepared from nonamended soil.

Inoculum.—Isolate Tb3 (bean isolate) and isolate Tb14 (tobacco isolate) of *T. basicola* were used. Endoconidia and chlamydospores were obtained separately as previously described (15). When endoconidia were used, they were added to soil as aqueous suspensions immediately before the initiation of the experiments, so that soil moisture was increased to about 45 to 50% of WHC. Chlamydospores were added to soil as aqueous suspensions, and the soil was maintained at approximately 40 to 50% of WHC for 16 days. The soil was then air-dried, mixed thoroughly for 30 min in a Patterson-Kelley twin shell blender with an intensifier, and kept at 4 to 5 C until needed. This procedure insured the breaking of chlamydospore chains into individual chlamydospores. In experiments with nonextracted rhizosphere soil, aqueous suspensions of chlamydospores were added

to soil immediately before assays.

Plant growth and collection of rhizosphere soil.—Topcrop snapbeans (*Phaseolus vulgaris* L.) were planted in soil in 30 X 50 cm flats. Bean seedlings were carefully removed from the soil after 6, 14, and 21 days. We collected soil closely adhering to the roots by tapping the roots over a clean piece of aluminum foil, then screened soil through a 30-mesh screen to remove root hairs, and divided soil into two portions. One portion was extracted for fatty acids and the other was used for direct spore germination assays.

In one experiment, the following plants were grown in soil in addition to beans: Coker 254 tobacco (*Nicotiana tabacum* L.); Stoneville 7A cotton (*Gossypium hirsutum* L.); Long Standing Green Curled kale (*Brassica oleracea* var. *acephala* DC.); Golden Bantam corn (*Zea mays* L.); and Redcoat wheat (*Triticum aestivum* L.). Bean, cotton, and tobacco are hosts of *T. basicola*; corn, kale, and wheat are not (6, 19). Rhizosphere soil from these plants was collected after 8 and 21 days as described for beans. Half of the rhizosphere soil was extracted for fatty acids and the other half was used for chlamydospore germination bioassays. Nonrhizosphere soil was the control.

Seed sterilization, plant culture, and collection of root exudates.—Bean seeds were surface-sterilized by the method of Ayers & Thornton (3). The seeds were germinated for 3 to 4 days on sterile water agar. For solution cultures, 425 ml of half-strength Hoagland's solution (5) were added to each of several deep storage dishes (8 cm deep, 10 cm diam). The dishes were covered with aluminum foil (0.001 gauge) with five holes. These dishes were placed in growth chambers (petroleum jars 23 cm deep, 12 cm in diam). The jars were covered with 15-cm petri dish lids and autoclaved at 121 C for 1 hr. Air exchange was permitted within the apparatus by supporting the lids with small rubber-tubing pieces placed on the rim of the petroleum jars. Three-day-old seedlings were aseptically placed on the foil with the primary roots through the holes into the nutrient solution. Sterility of the growth medium was checked at the end of growth period by culturing aliquots on nutrient agar, potato-dextrose agar, and maltose yeast extract agar. No condensation water and no guttation fluids were observed on plants during growth. After 7 additional days of growth at 25 C and 1,200 ft-c light, the nutrient solutions from 10 plants grown aseptically were combined and evaporated at 40 C under vacuum to 50 ml.

For sand culture, 75 ml of Hoagland's solution were added to 300 g of washed quartz sand in deep storage dishes, and these were placed uncovered in petroleum jars and autoclaved as before. Four-day-old seedlings free of microbial contamination were transferred to the deep dishes (one seedling/dish). After 7 days of growth, the plants were removed, the sand from each sterile culture was rinsed with 250 ml distilled water, and the combined solutions from 10 sterile dishes were condensed to 50 ml.

Extraction of fatty acids from soil.—Fatty acids of high molecular weight were extracted from soil with a modification of a method described by Wang et al. (20). Four hundred ml of chloroform-methanol solvent (2:1, v/v) were added to 30 g of rhizosphere or nonrhizosphere soil, and the mixture was shaken for 4 hr on a wrist-action shaker. The suspension was filtered through Whatman No. 2 filter paper on a Büchner funnel. The residual soil in the extraction flask was washed twice with 100 ml of chloroform-methanol, and this was combined with the 400-ml portion. The combined filtrate was evaporated to dryness in a rotary evaporator under vacuum at 38 C. The residue was dissolved in 40 ml hexane and centrifuged at 10,000 *g* for 15 min. The hexane extract was divided into two portions. One portion was evaporated to 3 ml and used for germination bioassays. The second portion (20 ml corresponding to 15 g soil) was decanted into a round-bottom boiling flask and concentrated to dryness under vacuum. Three ml of boron trifluoride methanol solution (14% w/v) were added to the residue. This was transferred to a test tube (2 X 15 cm), and the mixture was heated on a water bath at 67 C for 2 min. The heated mixture was washed in a 125-ml separatory funnel with 50 ml petroleum ether to which 20 ml distilled water were added, and the funnel was shaken vigorously. The aqueous methanol layer was drained off and discarded. The petroleum ether layer was drained through filter paper into a 250-ml beaker, and the solvent was evaporated to dryness on a 60-C water bath. The residue was dissolved in 0.2 ml chloroform which was immediately transferred to a small plastic stoppered vial and stored at 0 C until used for gas chromatography.

Extraction of fatty acids from bean culture solution.—The 50 ml of liquid from sand culture rinsings or liquid cultures were adjusted to pH 2.0 with 2 N H_2SO_4 and extracted 3 times with equal volumes of diethyl ether in a separatory funnel. The ether extract was evaporated to dryness at room temperature and the residue dissolved in 10 ml hexane. Further extraction and purification was done as described in the previous section.

Gas chromatography.—A gas chromatograph (F & M Scientific Corp. Model 810) equipped with a dual flame ionization detector and stainless steel columns (61 X 0.32 cm) was used for fatty acid determinations. The stationary phase was polyethylene glycolsuccinate 15% on 100/210 mesh "Gas Chrom P" (flux-calcined diatomaceous earth). Injector port and detector temperatures were 300 and 250 C, respectively; helium flow rate, 70 ml/min; hydrogen flow rate, 35 ml/min; and air flow rate, 450 ml/min. Column temperature was maintained at 170 C during analysis. The peak areas on the chromatograms were determined by the triangulation method. Micrograms of fatty acids were calculated from a standard curve. Standard solutions for the fatty acids palmitic, stearic, oleic, linoleic, linolenic, and palmitoleic were prepared in chloroform. Each standard solution consisted of 0.125 μg fatty acid/ml chloroform. One μliter of sample was injected into

the gas chromatograph with a 1-μliter total capacity syringe.

Four replications were used throughout and all experiments were performed twice.

Germination of endoconidia and chlamydospores in rhizosphere soil.—One-half of the soil collected from the rhizosphere of beans, tobacco, cotton, corn, wheat, and kale was divided into 10-g portions, and these were placed in 50-ml beakers. Two ml of an aqueous suspension of chlamydospores or endoconidia were mixed with the soil samples; the beakers were covered with Saran wrap, punctured several times with a transfer needle to facilitate air exchange, and incubated at 20 C for 17 hr. The other half of the collected rhizosphere soil was used for extractions as described in the previous section.

Spore germination was determined by the propagule assay method (14). This method consisted of making a soil suspension (1:5 or 1:10) in 1% carboxymethylcellulose comminuted in a blender at very low speed for 15 sec. One-ml aliquots were spread uniformly on the surface of 5-day-old plates of the V-8 juice dextrose yeast extract agar (13), stained with lacto-fuchsin, covered with a cover glass, and examined immediately with an oil immersion objective (X 90). Germination data obtained with this method were based on four replications of 100 spores each.

Germination of endoconidia and chlamydospores in nonrhizosphere soil fortified with rhizosphere soil extract.—Three ml of hexane extract from 15 g of rhizosphere soil (obtained according to methods described previously) were added to 3 g of nonrhizosphere soil previously infested with chlamydospores in 30-ml beakers, and the hexane was allowed to evaporate. A brief exposure to hexane did not impair germinability of *T. basicola* propagules. After the hexane evaporated, the soil was moistened with 0.9 ml of distilled water/3 g soil and incubated in 60-mm petri dishes for 17 to 18 hr at 20 C. Germinability in the fortified soil was assayed as described in the previous section.

RESULTS.—Several preliminary experiments were performed to improve recovery of fatty acids of high

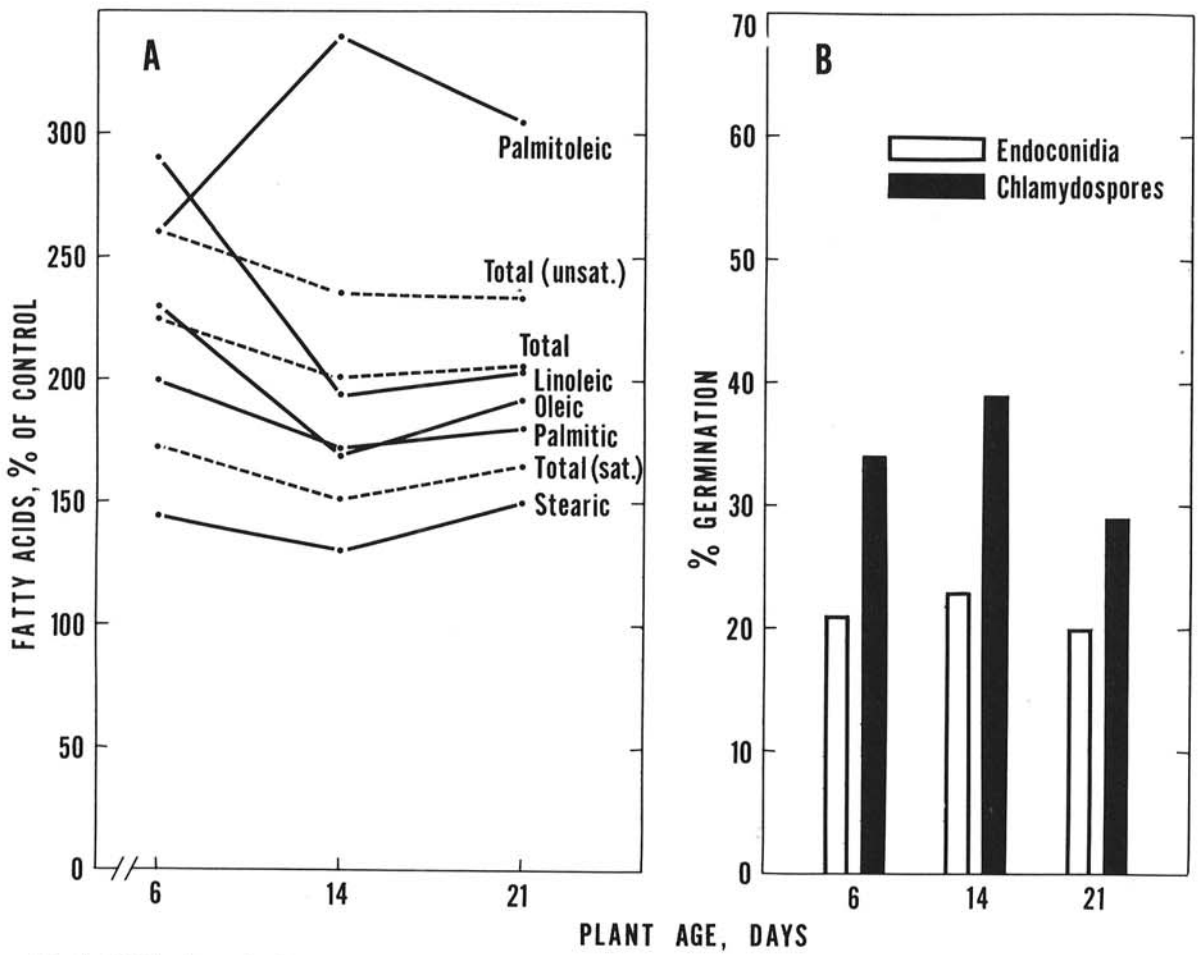

Fig. 1. A) Identity and relative quantity of fatty acids in rhizosphere soil (in relation to nonrhizosphere soil) from 6-, 14-, and 21-day-old bean plants. B) Per cent germination of endoconidia and chlamydospores of *Thielaviopsis basicola* in fungistatic soil fortified with hexane extracts of rhizosphere soil from 6-, 14-, and 21-day-old bean plants.

molecular weight added to natural soil. Methanolic NaOH used as one of the solvents by Wang et al. (20) resulted in an average recovery of only 43% of fatty acids added to soil at 5 μg/g soil in our experiments. The use of chloroform-methanol (2:1, v/v) improved recovery considerably. With this solvent, we obtained 86% recovery of saturated fatty acids (palmitic, stearic) and 65% recovery of unsaturated fatty acids (linoleic, linolenic, oleic, palmitoleic). Pretreatment of soil with HF-HCl (20) was omitted in our experiments because it was felt that HF-HCl was too drastic a treatment for the scope of these experiments.

Exudation of fatty acids by sterile bean plants.—No detectable amounts of fatty acids (C_{16} to C_{18}) were exuded by 50 bean plants grown aseptically for 7 days in solution culture. Bean plants grown aseptically in sand-solution culture released, in 7 days, the following fatty acids (μg/plant): palmitic acid, 0.60; stearic acid, 0.48; and oleic acid, 0.55. No other fatty acids were detected.

Fatty acids in bean rhizosphere soil and their effect on spore germination.—Gas chromatography analyses of extracts from rhizosphere soil of 6-, 14-, and 21-day-old beans grown in natural soil revealed the presence of five fatty acids (C_{16} to C_{18}, as methyl esters). The unsaturated acids linoleic, oleic, and palmitoleic were predominant in rhizosphere soil from plants of all three ages examined (Fig. 1-A). The total content of unsaturated fatty acids exceeded by 150% that of the nonrhizosphere soil at all times. Also, concentrations of the saturated acids palmitic and stearic were higher in rhizosphere soil than in nonrhizosphere soil. The total content of higher fatty acids in rhizosphere soil exceeded that in nonrhizosphere soil by more than 120% at all times. In a repetition of this experiment, the R:S ratios (μg fatty acids per g rhizosphere soil per μg fatty acids per g nonrhizosphere soil) for total, unsaturated, and saturated acids were 2.0, 2.7, and 1.6, respectively, after 14 days of growth. In the same experiment, linoleic acid had a R:S ratio of 3.7.

No germination of endoconidia or chlamydospores occurred in nonrhizosphere soil and in nonrhizosphere soil fortified with hexane only (Fig. 1-B). Approximately 20% of endoconidia germinated in nonrhizosphere soil amended with extracts from bean rhizosphere soil. Chlamydospores responded to extracts better than endoconidia. Approximately 30 to 40% of the chlamydospores germinated after 18 hr in nonrhizosphere soil fortified with rhizosphere soil extract.

Fatty acids in rhizosphere soil from host and nonhost plants and their effect on spore germination.—Linoleic, oleic, palmitic, and stearic acids were found in detectable amounts in rhizosphere soil from 8-day-old bean, cotton, tobacco, corn, wheat, and kale plants (Table 1). Palmitoleic acid was detected in appreciable amounts in bean rhizosphere soil only. The three known host plants of *T. basicola* (bean, cotton, tobacco) had larger quantities of fatty acids than the nonhost plants (corn, wheat, kale). The lowest quantities of fatty acids were detected in nonrhizosphere soil and in kale rhizosphere soil.

Many chlamydospores of isolate Tb3 germinated in control soil fortified with hexane extracts of rhizosphere soil from bean, cotton, and tobacco plants (Table 2). Since results with isolate Tb14 were similar to those of Tb3, only the latter isolate was included in the table. Chlamydospores did not germinate in nonrhizosphere soil and in nonrhizosphere soil fortified with extracts from kale rhizosphere soil. Although wheat and corn are not known to be hosts of *T. basicola*, extracts from their rhizosphere soil stimulated some chlamydospore germination.

Fatty acids in alfalfa hay-amended soil and their effect on spore germination.—Analyses of extracts from soil amended with alfalfa hay revealed the presence of stearic, palmitic, oleic, and linoleic acids in detectable amounts (Fig. 2-A). The amounts of fatty acids in the alfalfa hay-amended soil were ca. 1- to 2-fold higher than those detected in nonamended soil. The highest amounts of fatty acids were detected 0 and 2 days from amendment addition. Amounts fell off sharply by the 4th day, and by the 7th day the total amount of fatty acids was only about 20% higher than that in control soil.

Assays with chlamydospores of isolate Tb3 and

TABLE 1. Identity and quantity of fatty acids (as methyl esters) detected in extracts of rhizosphere soil from 8-day-old bean, cotton, tobacco, corn, wheat, and kale plants

Rhizosphere soil from plant	Quantity of fatty acids (μg/g soil)					
	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Palmitoleic acid	Total
Bean	2.24	1.20	0.96	1.05	1.50	6.95
Cotton	2.27	1.51	1.16	1.61	Tr[a]	6.55
Tobacco	1.76	1.16	1.14	1.20	Tr	5.26
Corn	1.60	0.76	1.48	0.84	Tr	4.68
Wheat	1.36	0.64	0.68	0.64	Tr	3.32
Kale	0.88	0.44	0.40	0.36	Tr	2.08
Control soil	1.00	0.60	0.70	0.48	Tr	2.78

[a] Tr = <0.01 μg/g soil.

with endoconidia and chlamydospores of isolate Tb14 in nonamended soil fortified with hexane extracts from the alfalfa hay-amended soil showed a similar downtrend of germination with length of time of alfalfa incubation (Fig. 2-B). There was considerable spore germination in soil fortified with extracts from alfalfa hay-amended soil after 0, 2, and 4 days of incubation. Very little or no germination occurred when the hexane extract came from soil amended with alfalfa hay for 7 days.

Effect of an artificial "rhizosphere mixture" of fatty acids on spore germination.—The effect of an artificial bean "rhizosphere mixture" of fatty acids on spore germination was also studied. The mixture was composed of palmitic, stearic, oleic, linoleic, and palmitoleic acids added to hexane in proportions equivalent to those detected in extracts of rhizosphere soil from 8-day-old beans (Table 1). The fatty acids were added to 3 g of soil containing endoconidia or chlamydospores of *T. basicola* at concentrations of 0, 5, 10^1, 10^2, 10^3, and 10^4 μg/g soil.

No germination of endoconidia or chlamydospores occurred in soil amended only with hexane, and very little germination occurred when fatty acids were added at 5 μg/g soil (Fig. 3). Considerable germination occurred with all other concentrations used. The concentration 10^1 μg/g soil in Fig. 3 corresponds approximately to that of the bean rhizosphere soil because the total amount per g rhizosphere soil in Table 1 (6.95 μg/g) represents only ca. 70 to 75% of the actual amount in bean rhizosphere soil. The method of recovery used was only 70 to 75% effective.

DISCUSSION.—The results reported here indicate that no detectable C_{16} to C_{18} fatty acids were released by bean roots growing undisturbed in a solution culture for 7 days. With plants growing in sand-solution culture, only three fatty acids were detected and these at less than 1 μg/plant. The

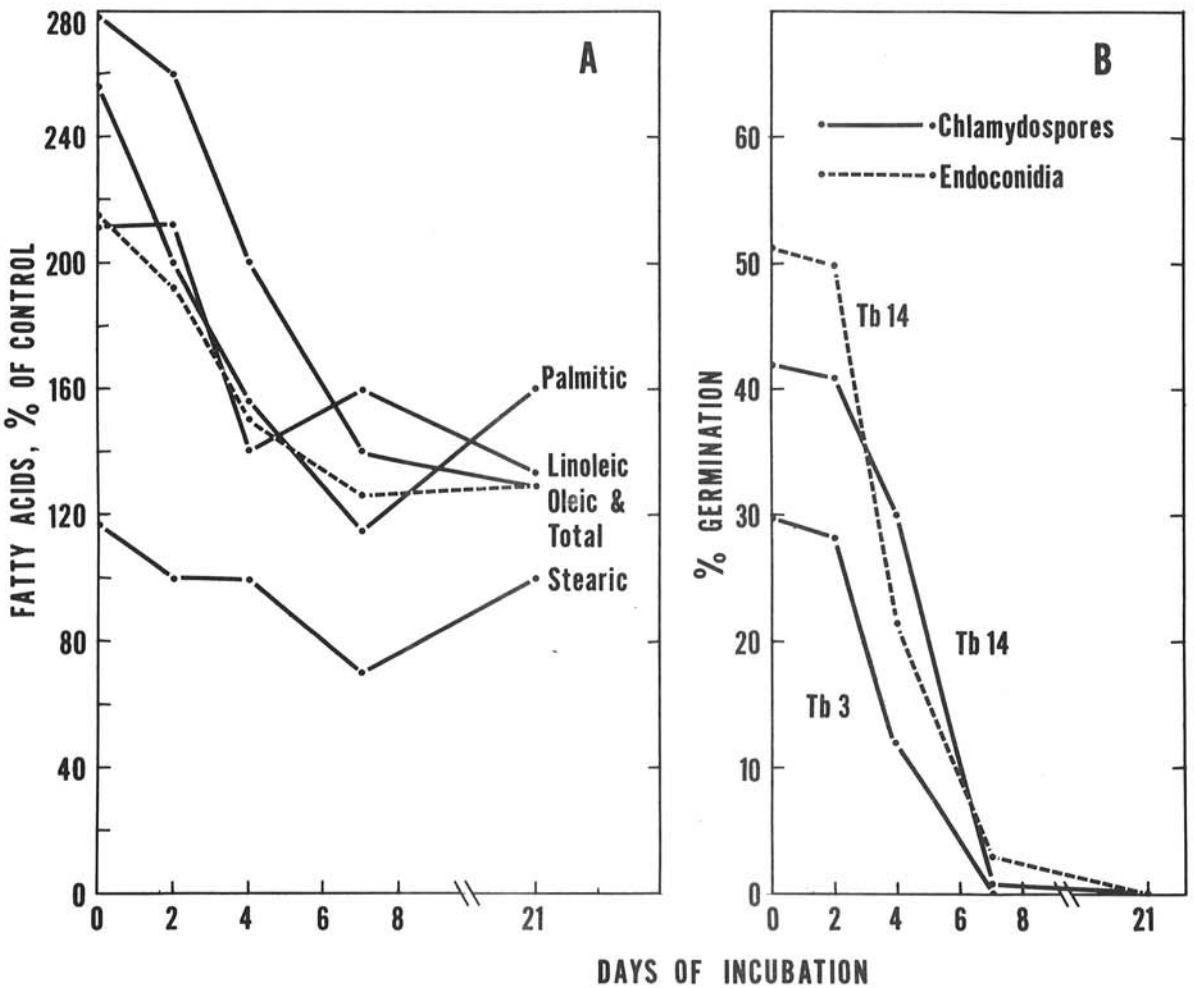

Fig. 2. A) Identity and relative quantity of fatty acids extracted from alfalfa hay-amended soil (in relation to nonamended soil) at intervals after amendment addition. B) Per cent germination of endoconidia and chlamydospores of *Thielaviopsis basicola* in fungistatic soil fortified with hexane extracts of alfalfa hay-amended soil (days of incubation = days of incubation at time of extraction).

TABLE 2. Germination of chlamydospores of *Thielaviopsis basicola* (Tb3) in nonextracted rhizosphere soil collected from 8- and 21-day-old plants and in control soil amended with hexane extracts of rhizosphere soil from the same plants

	Per cent chlamydospore germination after 18 hr in soil			
	Rhizosphere soil from		Nonrhizosphere soil[a] amended with hexane extract of rhizosphere soil from	
Plant	8-day-old plants	21-day-old plants	8-day-old plants	21-day-old plants
Bean	47	56	20	27
Cotton	48	53	29	36
Tobacco	51	49	24	33
Corn	14	6	14	12
Wheat	10	8	12	6
Kale	1	0	0	0

[a] Germination in nonrhizosphere soil was 0%.

observation on lack of release of higher fatty acids in solution culture and the greater yields of fatty acids in the exudates of sand-grown versus those grown in solution culture are in line with the observations of Ayers & Thornton (3) that abrasion of root hairs and tips of wheat and pea seedlings in sand may result in greater amounts of "exuded" soluble nitrogenous materials. Roots of wheat and peas experimentally damaged by Ayers & Thornton (3) in 1 hr released amino N of from 73 to 120% that released by "normal" exudation over a 2-week period. If longer periods of growth were used in our experiments, we might have obtained higher yields of higher fatty acids in the sand-grown plants. Higher amounts, however, might have been attributed to the action of autolytic enzymes on accumulated debris and not to actual release.

There are few reports in the literature on the annulment of soil fungistasis by materials other than sugars and simple N compounds. Coley-Smith & King (4) showed that volatile materials such as alkyl sulphides and others liberated by roots of *Allium* spp. may stimulate germination of sclerotia of *Sclerotium cepivorum* in natural fungistatic soil. Volatile components of alfalfa hay stimulated germination and growth of sclerotia of *S. rolfsii* (9). Papavizas & Adams (15) showed earlier that addition to soil of natural lecithins, unsaturated fatty acids, and unsaturated triglycerides annulled soil fungistasis and allowed *T. basicola* spores to germinate in soil. The data in this paper substantiate our previous observations (15) that long-chain unsaturated fatty acids may be responsible for induction of germination of *T. basicola* propagules in the rhizosphere of plants and in soil. The fact that addition of fatty acids to control soil in amounts equivalent to those presumably present in bean rhizosphere soil stimulated spore germination (Fig. 3) further substantiates the contention that fungistasis to *T. basicola* spores in soil can be annulled by the fatty acids present in plant rhizosphere.

Our data also show that higher amounts of fatty acids may be found in rhizosphere soil than in control soil. It is possible that the fatty acids found in rhizosphere soil of beans and other plants may be there as a result of microbial processes and interactions as well as release by plants. It is also possible that some of the detected fatty acids may have been the result of the breakdown of microbial cells during the extraction procedures. Undoubtedly, it is important to know the origin of the fatty acids in rhizosphere soil; but this does not minimize their importance in the rhizosphere.

Our observation that higher fatty acids were released from nonhost plants (corn, wheat) and that some stimulation of *T. basicola* spore germination occurred in rhizosphere soil from nonhost plants is not surprising or unusual. Armstrong & Armstrong (2) found that many nonsusceptible plants may serve as carriers of wilt fusaria. Others observed that soil populations of *Verticillium dahliae* increased in rhizosphere of susceptible, resistant, and immune mint plants (*Mentha piperita*) (8, 12). *V. dahliae* may penetrate and form sclerotia in roots of plants that are immune to the disease activities of the pathogen and to development of the pathogen in the vascular system, as suggested by Martinson & Horner (12). Further studies are needed to ascertain whether the observed differences in fatty acid content among host and nonhost plants of *T. basicola* play any role in the fluctuations of inoculum density of *T. basicola* in soil.

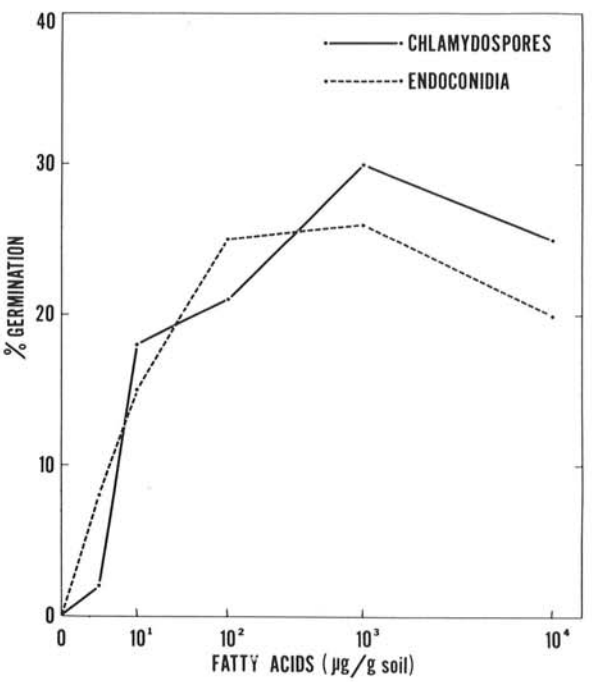

Fig. 3. Per cent germination of endoconidia and chlamydospores of *Thielaviopsis basicola* in fungistatic soil fortified with hexane solutions of fatty acids added to soil in concentrations from 0 to 10^4 µg/g soil.

Adams & Papavizas (1) showed that the addition of alfalfa hay to natural fungistatic soil resulted in a decrease in the fungistatic level of the soil to near zero; then, in an increase to a level greater than that of the nonamended soil. In those experiments, the factor(s) in alfalfa hay which stimulated germination was lost from soil within 4 to 7 days. Our present data (Fig. 2-A, B), in addition to substantiating the observation made earlier (1), provide evidence that unsaturated fatty acids may be responsible, at least partly, for the ability of alfalfa hay to annul fungistasis during the first few days following addition of the amendment to soil. In addition to fatty acids, alfalfa hay may also contain vitamin E acetate (α-tocopheryl acetate) (17), which also was found to stimulate germination of *T. basicola* propagules (15).

Several theories may be advanced to interpret the annulment of fungistasis by unsaturated fatty acids found in the rhizosphere of host plants or in alfalfa hay-amended soil. None is more intriguing, however, than the possible indirect effect through stimulation of fatty acid-metabolizing microorganisms near endoconidia or chlamydospores. This type of microflora may break down fatty acids to simple compounds with stimulatory activity on germination; or the microflora may be responsible for synthesizing different compounds that induce germination of *T. basicola* propagules. It is also possible that the fatty acid-decomposing microorganisms may alter the permeability of cell walls of *T. basicola* propagules. More research is needed to illucidate the mechanism of annulment of fungistasis by fatty acids.

LITERATURE CITED

1. ADAMS, P. B., & G. C. PAPAVIZAS. 1969. Survival of root-infecting fungi in soil. X. Sensitivity of propagules of Thielaviopsis basicola to soil fungistasis in natural and alfalfa-amended soil. Phytopathology 59:135-138.
2. ARMSTRONG, G. M., & JOANNE K. ARMSTRONG. 1948. Nonsusceptible hosts as carriers of wilt fusaria. Phytopathology 38:808-826.
3. AYERS, W. A., & R. H. THORNTON. 1968. Exudation of amino acids by intact and damaged roots of wheat and peas. Plant Soil 28:193-207.
4. COLEY-SMITH, J. R., & J. E. KING. 1969. The production by species of Allium of alkyl sulphides and their effect on germination of sclerotia of Sclerotium cepivorum Berk. Ann. Appl. Biol. 64:289-301.
5. HOAGLAND, D. R., & D. I. ARNON. 1950. The water-culture method for growing plants without soil. California Agr. Exp. Sta. Circ. 347. 32 p.
6. JOHNSON, J. 1916. Host plants of Thielavia basicola. J. Agr. Res. 7:289-300.
7. KO, WEN-HSIUNG, & J. L. LOCKWOOD. 1967. Soil fungistasis: Relation to fungal spore nutrition. Phytopathology 57:894-901.
8. LACY, M. L., & C. E. HORNER. 1966. Behavior of Verticillium dahliae in the rhizosphere and on roots of plants susceptible, resistant, and immune to wilt. Phytopathology 56:427-430.
9. LINDERMAN, R. G., & R. G. GILBERT. 1969. Stimulation of Sclerotium rolfsii in soil by volatile components of alfalfa hay. Phytopathology 59:1366-1372.
10. LINDERMAN, R. G., & T. A. TOUSSOUN. 1967. Behavior of chlamydospores and endoconidia of Thielaviopsis basicola in nonsterilized soil. Phytopathology 57:729-731.
11. LOCKWOOD, J. L. 1964. Soil fungistasis. Annu. Rev. Phytopathol. 2:341-362.
12. MARTINSON, C. A., & C. E. HORNER. 1962. Importance of nonhosts in maintaining the inoculum potential of Verticillium. Phytopathology 52:742 (Abstr.).
13. PAPAVIZAS, G. C. 1964. New medium for the isolation of Thielaviopsis basicola on dilution plates from soil and rhizosphere. Phytopathology 54:1475-1481.
14. PAPAVIZAS, G. C. 1967. Survival of root-infecting fungi in soil. I. A quantitative propagule assay method of observation. Phytopathology 57:1242-1246.
15. PAPAVIZAS, G. C., & P. B. ADAMS. 1969. Survival of root-infecting fungi in soil. XII. Germination and survival of endoconidia and chlamydospores of Thielaviopsis basicola in fallow soil and in soil adjacent to germinating bean seed. Phytopathology 59:371-378.
16. STANLEY, J. 1951. Production and utilization of lecithin, p. 593-647. *In* K. S. Markley [ed.]. Soybeans and soybean products. Vol. II. Intersci. Publ., Inc., New York. 1145 p.
17. STECHER, P. G. [ed.]. 1968. The Merck index. An encyclopedia of chemicals and drugs [8th ed.]. Rahway, New Jersey. 1713 p.
18. TSAO, P. H., & B. T. HAWTHORNE. 1970. Soil fungistasis, soil amendments, lysis, and biological control of Thielaviopsis basicola, p. 534-535. 7th Intern. Congress Plant Protection Proc. 21-25 September 1970. Paris.
19. UNITED STATES DEPARTMENT OF AGRICULTURE. 1960. Index of plant diseases in the United States. U.S. Dep. Agr. Handbook No. 165. U.S. Government Printing Office, Washington. 531 p.
20. WANG, T. S. C., YU-CHENG LIANG, & WEI-CHIANG SHEN. 1969. Method of extraction and analysis of higher fatty acids and triglycerides in soils. Soil Sci. 107:181-187.