Predisposition to Thielaviopsis Root Rot of Cotton by Phytotoxins from Decomposing Barley Residues

R. G. Linderman and T. A. Toussoun

Department of Plant Pathology, University of California, Berkeley 94720. Present address of R. G. Linderman: Crops Research Division, ARS, USDA, Beltsville, Maryland 20705.

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ABSTRACT

Phytotoxic ether extracts of barley residues decomposing in soil and four phytotoxic components of such extracts (benzoic, phenylacetic, 3-phenylpropionic (hydrocinnamic), and 4-phenylbutyric acids) did not stimulate *Thielaviopsis basicola* chlamydospore germination in nonsterile soil. Disease caused by this fungus was more severe, however, when cotton seedling roots treated with the barley extract, phenylpropionic acid, or benzoic acid were inoculated with chlamydospore-amended nonsterile soil. Roots treated with phenylpropionic acid or the barley extract were also more susceptible to a nearly avirulent clone of the fungus. Chlamydospore germination on the root surface and germ tube penetration of cells was higher on toxin-treated roots than on the controls. Microscopic examination of noninoculated, toxin-treated roots showed no injury. The increased activity of the fungus on toxin-treated roots may be due to changes in root exudation caused by the toxins.

When crop residues decompose in cold, wet soils, products of that decomposition liberated into the surrounding soil can be highly toxic to successive crop plants (2, 7, 10, 11). In addition, phytotoxic extracts from decomposing residues may increase the incidence and severity of root rots (3, 4, 8, 9, 13, 14). More specifically, *Thielaviopsis* root rot of bean (14) and tobacco (8) was increased by treating host roots with diluted phytotoxic extracts prior to inoculation. Patrick and Koch (9) also showed that resistance to black root rot of tobacco could be overcome by treating tobacco roots with phytotoxic extracts prior to inoculation. The phytotoxic extracts probably were acting directly on the host issue and altering the cell permeability, thereby increasing the exudation of materials favorable to the fungus (9, 14). Whether the extracts also had a direct effect on the fungus in natural soil was not determined. Patrick et al. (12) reported that crude water extracts of decomposing plant residues stimulated *Thielaviopsis basicola* (Berk. & Br.) Ferr. chlamydospore germination, but this work was not done with the fungus in natural soil. Toussoun et al. (15), however, reported stimulation of *Fusarium solani* f. sp. *phaseoli* chlamydospore germination in soil by such water extracts. The purpose of the present study was to determine the involvement of phytotoxins in increasing *Thielaviopsis* root rot on cotton. This was made possible by the recent identification of benzoic acid, phenylacetic acid, 3-phenylpropionic (hydrocinnamic) acid, and 4-phenylbutyric acid as major phytotoxic components in extracts of barley residues decomposing in soil (16).

MATERIALS AND METHODS.—Clones of *T. basicola* used in these studies were isolated from tobacco (*Nicotiana tabacum* L.) (T), cotton (*Gossypium hirsutum* L.) (C), and cherry (*Prunus* sp.) (CH). All clones were maintained as single conidium cultures on potato-dextrose agar (PDA) slants. Pure preparations of chlamydospores were prepared from these slants as previously described (6). These preparations were added to cotton field soil. After the chlamydospore chains had broken up, the soil was air-dried for several months. These chlamydospore-amended soils were then used in germination and inoculation studies on cotton seedlings (Acala 4-42).

Stimulation of chlamydospore germination by known phytotoxins or by phytotoxic extracts was determined by adding the test solutions (250 ppm) to small amounts of previously wet (6) chlamydospore-amended (clones C and T) soil in porcelain cavity plates. Germination counts were made 20 hr later in soil smears. Distilled water and a 10% (by volume) solution of carrot juice were used as controls.

The predisposing capacity of the phytotoxins or of phytotoxic extracts of decomposing residues was determined on cotton seedlings by treating the seedling tap roots with the solutions prior to inoculation. Seedlings were grown in the greenhouse at a constant 32 C for 3-4 days in U.C. Mix (1). They were removed from the mix before lateral roots appeared. The seedlings were gently washed to remove loose soil particles, immersed in the test solutions for 2 hr, washed again, and transplanted into hinged plastic inoculation chambers. The seedling tap roots were inoculated in two places with small units of chlamydospore-amended field soil that had been kept wet for several days prior to use. Water was used as a control treatment, and noninoculated treated seedlings were used to check toxicity of the test solutions. Toxicity was indicated by discoloration or decreased turgidity (as indicated by flexibility when sliced with a scalpel) of the root tissue. Known solutions were adjusted to concentrations where no toxicity was apparent. Concentrations of phytotoxins in unknown extracts were diluted to 1/50 the concentration of the original ether extract (16) and, as with the known solutions, only used once. Clones C (virulent) and CH (nearly avirulent) (5) were used in these studies. Clone CH was used to determine if its virulence could be increased by treatment of the host tissue with phytotoxins. Disease severity ratings were made at intervals up to 4 days, after which differences in lesion severity between treated and untreated seedlings were less obvious, especially when virulent clone

C was used. Severity ratings (0-10 scale) were subjectively based on lesion size and degree of discoloration, numbers of chlamydospores whose germ tubes had penetrated host cells, and the degree of hyphal proliferation on the roots. The last two criteria were not used in all trials, but when used were based on microscopic examination of longitudinal hand sections of inoculated root tissue that had been washed thoroughly in a vigorous stream of water and stained with 0.02% acid fuchsin in lactic acid. These sections were made 24-48 hr after inoculation.

RESULTS.—The possibility that toxins might directly stimulate germination of chlamydospores in soil was first investigated. Tests were made with wet chlamydospore-amended field soil (clones C or T) to which an ether extract of decomposing barley or the known phytotoxins at a concentration of 250 ppm were separately added. The results, based on four experiments wherein 100 chlamydospores per clone per treatment were counted, showed that chlamydospore germination did not occur in the presence of the phytotoxic barley ether extract or any of the four phytotoxic acids. Thirty to 65% germination (clones C and T, respectively) occurred, however, in carrot juice controls. No germination occurred in the water controls.

The possible predisposing activity of the phytotoxic acids to *T. basicola* root rot was therefore investigated. Cotton seedling roots treated with test solutions were inoculated with the strongly pathogenic clone C or the weakly pathogenic clone CH by placing small units of chlamydospore-amended soil, kept moist for several days prior to use, on the roots for 4 days, after which the resulting lesions were rated for disease severity. The averaged disease severity ratings from several experiments are shown in Table 1. The data indicate that the barley ether extract, phenylpropionic acid, and to a lesser extent benzoic acid, increased the severity of root rot caused by the virulent clone C. The effects of the barley ether extract and phenylpropionic acid were similar but less striking with the nearly avirulent clone CH. No phytotoxicity was evident at the highest concentrations of the compounds used. Clone C was used to determine the reason for the increase in disease on toxin-treated roots. Longitudinal hand sections of inoculated root tissue were made 24-48 hr after inoculation, stained, and examined microscopically. Chlamydospores which had germinated on the root surface, and whose germ tubes had penetrated the host, were much more numerous on toxin-treated roots than on the water-treated controls (Fig. 1-a, c). The resulting lesions 67 hr after inoculation were more severe on the root treated with phenylpropionic acid than the water-treated root (Fig. 1-b, d). Following penetration, the rate of hyphal proliferation, both on the root surface and within the host tissue, was much greater in toxin-treated than in the water-treated roots.

DISCUSSION.—As chlamydospores of *T. basicola* germinate (6) in soil only in the presence of certain nutrients (such as in carrot juice), the failure of the toxins to stimulate chlamydospore germination would indicate that they are not used by the fungus as nutrients.

TABLE 1. Predisposition of cotton roots to *Thielaviopsis basicola* root rot by some phytotoxins from decomposing plant residues. Disease ratings (0-10 scale) taken within 4 days after inoculation of roots with small units of previously wet chlamydospore-amended soil

Treatment	Concn (ppm)[a]	Disease severity ratings[b] Clone C (virulent)	Clone CH (weakly pathogenic)
Water		268/60[c] = 4.5	26/38 = 0.7
Benzoic acid	100	353/56 = 6.3	20/21 = 1.0
Phenylacetic acid	75	205/40 = 5.1	
Phenylpropionic acid	100	123/13 = 9.5	
Phenylpropionic acid	75	367/43 = 8.5	63/30 = 2.1
Phenylpropionic acid	50	482/56 = 8.6	
Phenylbutyric acid	75	147/34 = 4.3	
Barley ether extract	(1/50 dil)	180/28 = 6.4	55/14 = 3.9

[a] The concentrations used were the highest that gave no toxicity.

[b] Averages of six experiments for clone C, three for clone CH.

[c] Ratio of sum of ratings to the number of inoculations.

However, since certain of the compounds predispose roots to more severe infection by increasing chlamydospore germination on the host surface, it is believed that the mode of action may be similar to that proposed by Toussoun and Patrick (14). They suggested that host exudation is increased, or altered in composition, or both, due to changes brought about in cell permeability following treatment with phytotoxic extracts. This exudation would then be directly responsible for the observed increased chlamydospore germination. It is not known, however, whether the sugars and ninhydrin positive substances detected by Toussoun and Patrick (14) exuding from toxin-treated tissue stimulate *T. basicola* germination in soil. Preliminary studies by Linderman and Toussoun (*unpublished data*) indicate little or no stimulation of chlamydospore germination in soil by several sugars and nitrogen compounds. Regardless of the mechanism involved, however, the presence of toxins at natural inoculum concentrations in field soil could mean the difference between disease and escape. While the occurrence of these toxins in nature in sufficient quantities to affect root rot severity is still to be determined, the tests reported here show such activity at concentrations as low as 50 ppm for phenylpropionic acid; and it is believed that such concentrations probably do occur locally in the field.

The increased disease development when the weakly pathogenic clone CH followed the root treatment with phenylpropionic acid or the barley ether extract suggests that a plant could be rendered susceptible to a fungus that normally did not affect that plant and perhaps even to microorganisms normally innocuous. This phenomenon recalls the work of Patrick and Koch (9)

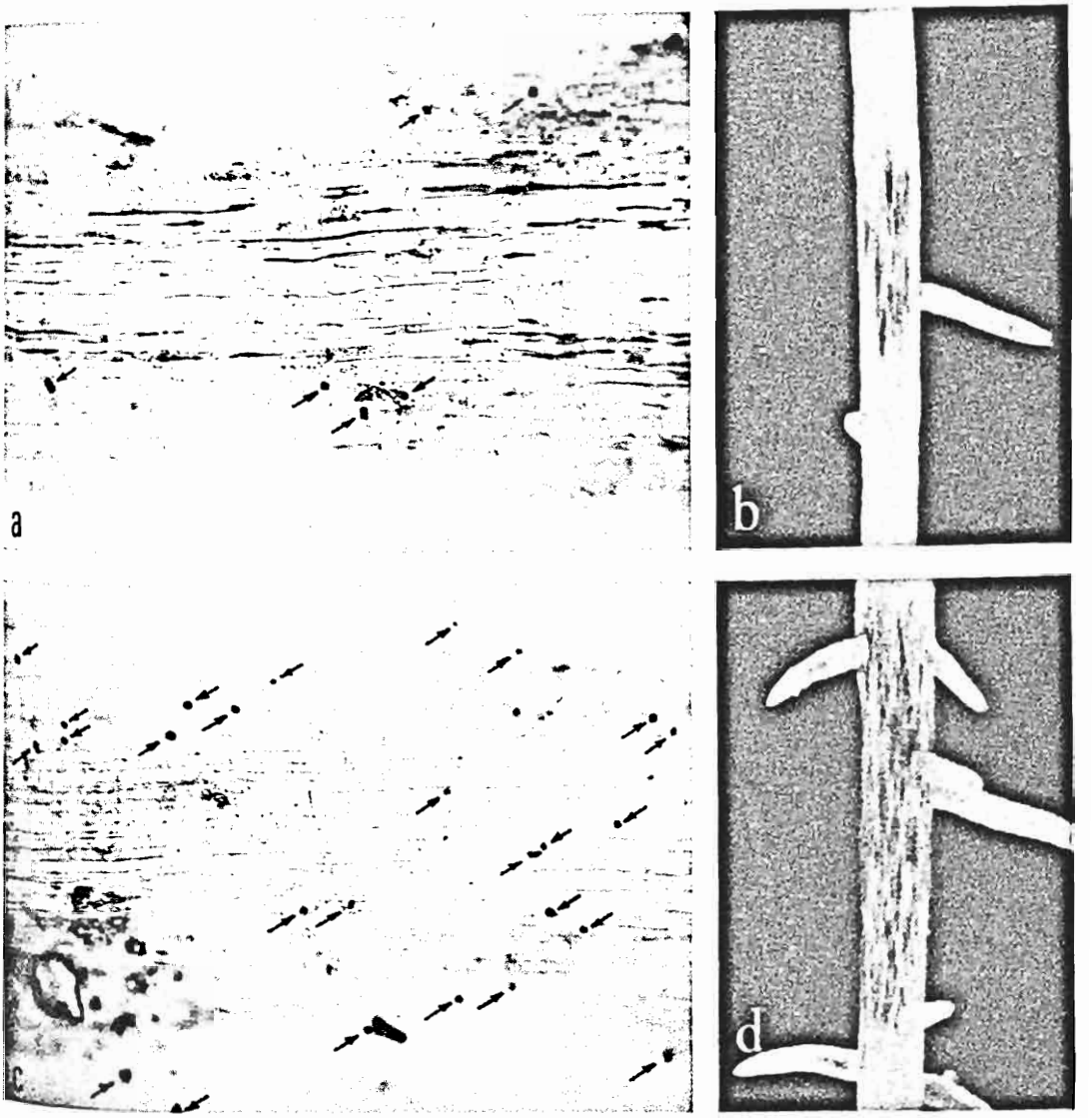

Fig. 1. Predisposition of cotton roots to *Thielaviopsis basicola*. Roots were inoculated with virulent clone C, chlamydospore-amended soil. **a)** Longitudinal hand section of inoculated root tissue (each arrow indicates a chlamydospore which has germinated and its germ tube penetrated) treated 2 hr with water prior to inoculation. **b)** Lesion resulting 67 hr after inoculating the water-treated root. **c)** Longitudinal hand section of root treated 2 hr with phenylpropionic (hydrocinnamic) acid at 50-100 ppm. **d)** Lesion resulting 67 hr after inoculating phenylpropionic acid-treated roots. The large lesion shown in d) is the result of the coalescence of a greater number of small lesions than in b) as well as the more extensive fungus proliferation.

where susceptibility to *T. basicola* was altered by treatment with phytotoxic water extracts from decomposing residues prior to inoculation. It is not known whether the aromatic acids discussed here are responsible for this breakdown of resistance, for the roles of decomposing residues in the etiology of root rots are yet to be fully understood. Indeed, the present work is apparently the first report of the predisposing capacity of these compounds.

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