Effect of Plant Residues on Chlamydospore Germination of Fusarium solani f. sp. phaseoli and on Fusarium Root Rot of Beans

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ABSTRACT

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The effects of decomposing immature and mature residues of rye, oat, soybean, sorghum, barley, buckwheat, timothy, and corn on Fusarium root rot of bean and on pathogen chlamydospore germination were determined. All mature (high C:N ratio) residues, but only two immature (low C:N ratio) residues, significantly reduced disease. Disease severity was positively correlated with total soil inorganic nitrogen and nitrate, but not with ammonium. Decomposing mature residues were more inhibitory than corresponding immature residues to chlamydospore germination in vitro and in soil, with rye being more effective than corn. Failure of the chlamydospores to germinate in vitro and in soil was related to nutrient deficiency in the soil as well as to formation of an inhibitory material from the decomposing residues. Fungistasis due to nutrient deficiency, but not toxicant production, was overcome in soil in the presence of nutrients.

Additional key words: soil-borne pathogen, organic amendment, fungistasis.

Fusarium solani (Mart.) Appel. & Wr. f. sp. *phaseoli* (Burk.) Snyd. & Hans. causes severe root rot of snapbeans (*Phaseolus vulgaris* L.). Although this disease has caused great crop loss in the bean-growing areas of the United States for many years, application of measures for its control still is in its infancy. Chemical control either is currently not available or is too costly. Cultural control which employs a reduction of soil compaction in conjunction with appropriate irrigation has been successful (5). Unfortunately, the application of biological control which involves plant residue incorporation into soil has met with varied success. In spite of the difficulties inherent in this approach, the stress on the importance of preservation of our environment requires continued exploration into the area of biological control for the suppression of soilborne plant pathogens. For many years, various organic amendments and plant tissues applied to soil were shown to reduce Fusarium root rot of beans in the laboratory and greenhouse (7, 15, 17).

Decomposing plant residues can significantly affect soilborne plant pathogens by alteration of pathogen inoculum level. Of major importance in this respect is the maturity, or carbon:nitrogen (C:N) ratio, of the amendments (1, 16, 19). Population levels, in turn, are affected by the extent of pathogen chlamydospore germination. In soils amended with residues that contain stimulatory nutrients, chlamydospores germinate and can form replacement chlamydospores so that propagule numbers increase (16, 19). If germ tube lysis occurs before replacement chlamydospore formation, the population declines (16, 17). Some amendments of high C:N ratio, such as cellulose or oat straw, allow only slight, or no chlamydospore germination (1, 17). The inhibition of chlamydospore germination due to decomposing mature amendments generally has been attributed to soil fungistasis arising from nutrient deficiency (1, 3, 8, 17, 20).

This report contributes additional information on the effect of decomposing plant residues of various maturities, and the soil nitrogen resulting from the decomposition, on Fusarium root rot of bean. Evidence is also presented that the inhibitory effect of decomposing mature rye and corn residues on chlamydospore germination of the pathogen is caused by the production of a fungitoxicant as well as by nutritional fungistasis. A preliminary report has appeared (11).

MATERIALS AND METHODS

Soil, isolates, and residues.—The soil used was a sandy loam, pH 6.3, which contained 2.3% organic matter and 28 and 25 μg of NH_4-N and NO_3-N/ g of soil, respectively. Two isolates of *F. solani* f. sp. *phaseoli* from diseased snapbeans in Maryland (Fs 2R) and Washington (Fs 16) were studied. Plant residues, composed of leaves, stems, and roots were air-dried and ground in a mill to pass a 0.84-mm (20-mesh) screen. Total carbon and nitrogen contents were determined with a Leco Carbon Analyzer and Coleman 29B nitrogen analyzer (Coleman Instruments Div., Perkin-Elmer Corp., Maywood, IL

60153), respectively. The residues consisted of eight sets of plant tissues; one of each pair being immature (low C:N ratio), and the other being mature (high C:N ratio). Those studied and their respective C:N ratios were: rye (*Secale cereale* L.), 9 and 94; oat (*Avena sativa* L.), 9 and 83; sorghum (*Sorghum vulgare* L.), 13 and 62; buckwheat (*Fagopyrum esculentum* Moench), 11 and 70; corn (*Zea mays* L.), 9 and 81; barley (*Hordeum vulgare* L.), 11 and 70; timothy (*Phleum pratense* L.), 9 and 37; and soybean [*Glycine max.* (L.) Merr.], 12 and 63.

Available NH_4-N and NO_3-N in soil were determined by steam distillation with the magnesium oxide-Devarda alloy method (2). The equivalent of 10 g dry weight of each of the treatment replicate soils were analyzed prior to planting.

Bean root rot.—Soil was infested with *F. solani* f. sp. *phaseoli* (isolate Fs 2R) by adding washed conidia to soil and keeping it moist for 5 weeks in order to convert conidia to chlamydospores (13). Two consecutive crops of beans were planted in the infested soil to increase the inoculum level. One-kg portions of air-dried soil were amended with residues at a rate of 1.0% (w/w) and moistened to 50-60% of their moisture-holding capacity. Three weeks later, soils were placed in 11.3-cm-diameter plastic pots and planted with 10 Topcrop bean seeds per pot. After a 4-wk growing period at 22-27 C, plants were

removed, examined for root-rot symptoms, and rated on a 0-4 scale according to the extent of damage caused by the pathogen. A disease severity index (DSI) was calculated by averaging the values of six replicates.

Chlamydospore germination.—Chlamydospore preparations for germination assays were prepared by the method of Adams et al. (1). Three methods of assay were used. The first was a modification of Jackson's agar-disk technique (10). A 20-g aliquot of amended or nonamended soil was mixed with 10 ml of warm (50 C) 0.5% agar with or without 0.1% potato dextrose broth (PDB) powder. The soil-agar mixture was placed in petri plates and after it had set, three disks (1 × 10 mm) of 2% agar, with or without 0.1% PDB powder, were placed on the soil-agar surface. The assembly was kept at 5 C for 48 hr, then a drop of diluted chlamydospore suspension (50,000/ml) was placed on each disk. After incubation of the assembly at 26 C for 18 hr, disks were transferred to microscope slides, stained with lactofuchsin, and examined.

The second method was the propagule assay method of Papavizas (14) used to detect chlamydospore germination and subsequent germling behavior in soil. Briefly, amended or nonamended soils, heavily infested with chlamydospores of *F. solani* f. sp. *phaseoli*, were suspended in 0.5% carboxymethylcellulose solution and comminuted in a blender. One-ml aliquots were uniformly spread on the surface of modified peptone-
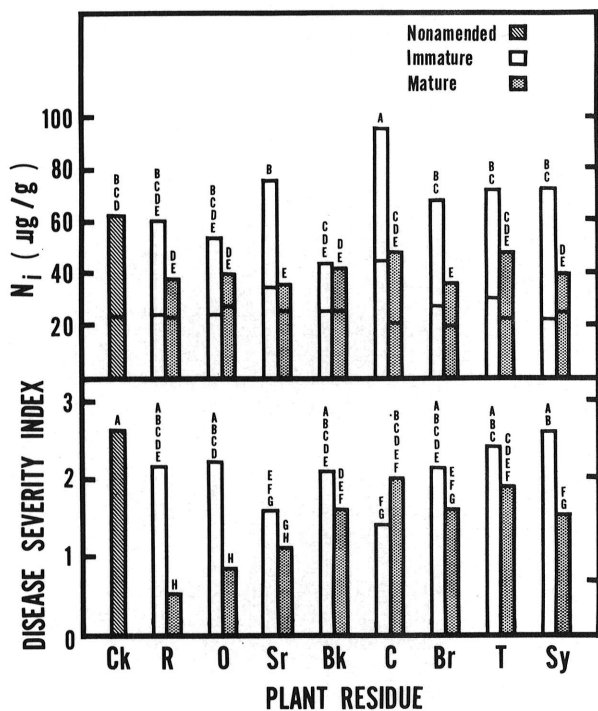

Fig. 1. Effect of decomposing immature and mature plant residues on root rot of bean caused by *Fusarium solani* f. sp. *phaseoli* and total inorganic nitrogen (N_i) content of soil at time of planting. Residues of rye (R), oat (O), sorghum (Sr), buckwheat (Bk), corn (C), barley (Br), timothy (T), and soybean (Sy) added at rate of 1.0% (w/w). Lower portion of bar representing N_i is NH_4-N and upper portion is NO_3-N. Bars with same letter are not significantly different from each other, $P = 0.05$.
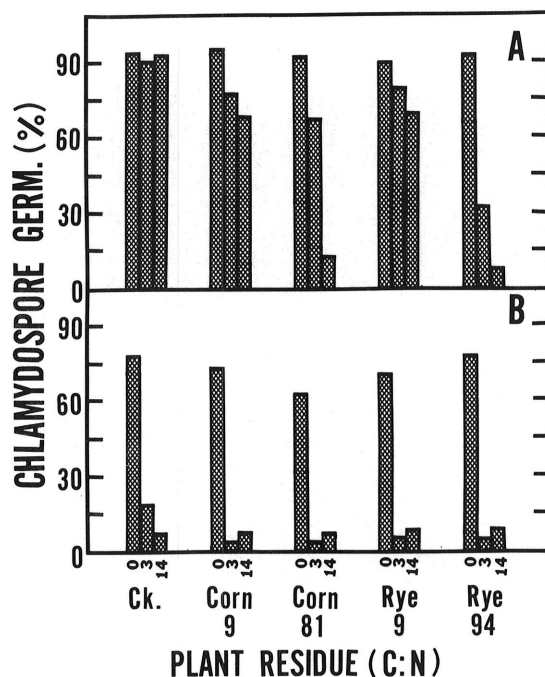

Fig. 2-(A, B). Effect of decomposing corn and rye residues on chlamydospore germination of *Fusarium solani* f. sp. *phaseoli* (isolate Fs 2R) determined with the agar disk method. A) Nutrients as potato dextrose broth powder (0.1%) added to disks and soil-agar mixture. B) No nutrients added. Assays performed 0, 3, and 14 wk after addition of residue (1%) to soil. Germination represents percentage of that on agar plates alone with nutrients (76%) and without (82%).

pentachloronitrobenzene agar poured 3-4 days before the assay. After free water was absorbed by the agar (15-20 min), several areas on each plate were stained with lactofuchsin and examined microscopically. Anthrone- and ninhydrin-positive substances were determined colorimetrically (12, 21) after extraction from the soils (18).

The third method of germination assay, which also showed spore behavior in soil, was a modification of Chinn's agar slide technique (6). Drops containing chlamydospores (50,000/ml) were placed on microscope slides along with drops of dilute agar (0.5%) with or without PDB powder (0.1%). After the agar had dried to a film, slides were inserted into moist soils at intervals after residue incorporation. After 24 hr, slides were carefully withdrawn from the soil, rinsed in water, stained with lactophenol-cotton blue, and examined microscopically.

RESULTS

Effect of decomposing plant residues on bean root rot and soil inorganic nitrogen content.—All mature residues reduced disease below the amount in the nonamended control soil, whereas only two immature residues (those of sorghum and corn) were effective in this respect (Fig. 1). Rye and oat residues were the most effective mature amendments to reduce the DSI below a level of 1.0. Similar results were obtained when the experiment was repeated.

At the time of planting, the soil inorganic N content varied between 35 $\mu g/g$ in mature barley-amended soil and 98 $\mu g/g$ in immature corn-amended soil. Soil NH_4-N contents were approximately the same in all amended soils, except in immature corn-amended soil which contained a significantly greater amount (44 $\mu g/g$) than soils amended with other residues. In contrast, soil NO_3-N varied considerably among treatments. There was generally less of this form of N in soils amended with mature than immature residues. Six of the 10 plant residues which reduced disease resulted in significantly less NO_3-N in the soil than the other four; these were mature rye, oat, sorghum, buckwheat, barley, and soybean (Fig. 1). Disease severity was positively correlated with total inorganic $N(r = .34)$ and NO_3-N $(r = .43)$, significant at $P = 0.05$ $(d.f. = 100)$. The correlation between DSI and NH_4-N content was not significant $(r = .01)$.

Effect of decomposing rye and corn residues on chlamydospore germination.—Rye and corn residues were selected for further study because mature rye was the most effective amendment in disease reduction, whereas of the mature residues corn was the least effective.

With the agar-disk method of assay, both mature residues significantly reduced chlamydospore germination of isolate Fs 2R on agar over a 14-wk period (Fig. 2). Immature residues reduced germination approximately 20%. Germination reduction occurred in the presence of nutrients supplied to the assay agars by PDB powder (Fig. 2-A). When these nutrients were not supplied, chlamydospore germination was high at the time the amendments were added, but not during the remainder of the 14-wk test period (Fig. 2-B). Similar results were generally obtained with isolate Fs 16.

An experiment was performed to determine whether inhibition of germination observed with decomposing mature residues was due to a nutrient-deficiency fungistasis in the agar disks after their contact on the soil-agar for 48 hr. Ten such disks were shaken for 24 hr in 100 ml distilled water containing 0.01% $HgCl_2$, after which time the solution was analyzed for anthrone- and ninhydrin-positive substances with colorimetric procedures (12, 21). Disks placed on soil-agar for 48 hr after mature rye and corn residues decomposed for 3 wk contained 157 and 133 μg anthrone-positive substances/disk, respectively, whereas disks from nonamended soil contained 290 μg. There was 3, 15, and 12 μg of ninhydrin-positive substances/disk from nonamended and mature rye- and corn-amended soils, respectively.

To determine whether chlamydospores could germinate with this nutrient concentration, agar disks were prepared to contain 3 μg ninhydrin-positive and 130 μg anthrone-positive substances/disk. Since there was more than 80% germination with these nutrients in the absence of soil, germination reduction on disks (containing comparable amounts) over amended soils was not due to nutrient deficiency.

Chlamydospore germination in Fs 2R was also assayed by the propagule assay method 1 wk after residue addition to soil. In this method, chlamydospores were present in soil when amendments are added. Similar results to those using the agar-disk method were obtained. Chlamydospores did not germinate in nonamended soil, and poorly in soils containing decomposing mature residues (Table 1). Substantial

TABLE 1. Effect of decomposing residues on chlamydospore germination of *Fusarium solani* f. sp. *phaseoli* (isolate Fs 2R) in soil determined with the propagule assay method, and presence of anthrone- and ninhydrin-positive substances in soil

Plant residue[a]	Carbon:nitrogen ratio (C:N)	Chlamydospore germination (%)[b]	Anthrone-positive substances (mg/g soil)	Ninhydrin-positive substances (mg/g soil)
None (control)		0 b[c]	0.2 b	0.4 b
Rye	9	55 a	1.5 a	1.5 a
Rye	94	6 b	0.4 b	0.3 b
Corn	9	56 a	1.4 a	1.6 a
Corn	81	11 b	0.5 b	0.4 b

[a]Added to soil at rate of 1%.
[b]Assayed 1 wk after amendment addition.
[c]Numbers in each column followed by same letter are not significantly different from each other, $P = 0.05$.

germination occurred in soils with decomposing immature residues which was significantly correlated with the presence of ninhydrin-positive (r = .81) and anthrone-positive (r = .95) substances at $P = 0.05$ (d.f. = 18). There was little evidence of germination or the presence of ninhydrin- and anthrone-positive substances in any soil 3 or more weeks after the amendments were added.

With the buried-slide method, poor chlamydospore germination (5 and 3%) occurred on slides coated with agar containing nutrients when retrieved after 24 hr of incubation in soil amended for 3 wk with mature rye and corn residues, respectively. On the contrary, 68, 63, and 70% of the chlamydospores germinated on slides from nonamended and immature rye and corn residue-amended soils, respectively. There was less than 5% germination in any treatment without additional nutrients supplied to the slides. This observation indicated that added nutrients could not overcome the inhibitory effects of decomposing mature residues. There was a similar pattern of chlamydospore germination 7 and 14 wk after amendment addition.

Effect of residue extracts on chlamydospore germination.—Extracts of residues decomposing in soil were prepared by extracting 1-kg portions of amended soil (2%) with chloroform:methanol (1:1, v/v) for 24 hr, after which the extracts were concentrated under vacuum to a brown, viscid material. Nonamended soil, soil amended with immature rye and corn, and with mature rye and corn yielded 160, 360, 400, 580, and 490 mg of extract/kg of soil. Extracts were homogenized with water in a blender so that an amount equivalent to that found in 2% nonextracted residue was added to soil containing chlamydospores. Glucose and NH_4Cl were added to soil to stimulate germination. Chlamydospore germination in Fs 2R was determined by the propagule assay method 16 hr after the extract was added to soil. Extracts from decomposing mature rye and corn residues inhibited

chlamydospore germination in soil even in the presence of nutrients (Table 2). Significantly greater inhibition occurred with mature rye than with mature corn. Decomposing immature residues did not inhibit germination. A similar, but not as definitive, pattern occurred with Fs 16. Extracts prepared after 6 wk of decomposition were as effective as extracts prepared after 3 wk of decomposition. Many spores eventually germinated when left on the assay agar.

DISCUSSION

The importance of high C:N ratio materials such as mature barley, corn, and sorghum residues in the suppression of Fusarium root rot of bean was shown experimentally as early as 1959 (7). However, relatively little work has been done to unravel possible mechanisms of control by these amendments. Nitrogen immobilization by soil microbial competitors to *F. solani* f. sp. *phaseoli* was thought to be the mechanism of action of high C:N ratio residues (1, 16, 17). We also show in this paper that low root-rot severity brought about by high C:N ratio residues was positively correlated with low total inorganic-N and low NO_3-N. Disease severity was previously shown to be positively correlated with NO_3-N, but not NH_4-N, content of soil (9). However, since several mature residues (rye, oat, sorghum) were more effective than others (buckwheat, corn, barley), soil N content alone could not account for disease suppression or increase. Decomposing mature rye residue (C:N 94), for example, resulted in a soil inorganic N content of 37 $\mu g/g$ and a DSI of 0.5; corn (C:N 81) resulted in 49 $\mu g N/g$ and a DSI of 2.0 (Fig. 1). Burke (4) also suggested that the N status of soil may not be the major determinant in amendment effectiveness for suppression of Fusarium root rot.

Since N content of soil could not explain why some amendments were more effective than others, attempts were made to determine whether amendment decomposition in soil had any adverse or beneficial effects on chlamydospore germination. With the agar-disk method, the lack of germination as a result of amendment decomposition was due to fungistasis arising from nutrient deficiency (Fig. 2-B). Both rye and corn residues, regardless of maturity, resulted in a fungistatic soil. This agrees with earlier observations (1, 3, 8, 17) that decomposing cellulose and other organic materials prevented chlamydospore germination in soil. In the present study, when nutrients provided by PDB powder were added to both agar disks and to the soil-agar mixture after 14 wk of amendment decomposition, there was more than 60% chlamydospore germination in all treatments except those containing decomposing mature rye and corn residues (Fig. 2-A). In some instances, then, nutrients overcame fungistasis so that chlamydospores could germinate. In an earlier report (17), glucose added to cellulose-amended soil nullified the cellulose effect and negated fungistasis, so that approximately 65% of the chlamydospores germinated in soil. In the present study, however, adequate nutrients for germination were present in agar disks over mature rye- and corn-amended soils. Despite this, germination was poor with these treatments. This poor germination must have been due to some other phenomenon.

TABLE 2. Effect of chloroform:methanol extracts of decomposing rye and corn residues on *Fusarium solani* f. sp. *phaseoli* chlamydospore germination in soil as determined by the propagule assay method[v]

Plant residue extract[w]	Nutrients[x]	Germination (%) of indicated isolate	
		Fs 2 R	Fs 16
None[y]		0 d[z]	0 d[z]
None	+	89 a	86 a
Control	+	87 a	78 ab
Rye (C:N 9)	+	87 a	67 b
Rye (C:N 94)	+	22 c	30 c
Corn (C:N 9)	+	78 a	63 b
Corn (C:N 81)	+	52 b	30 c

[v]Residues extracted 3 wk after addition to soil.

[w]Added at a rate equivalent to that found in 2% soil amendment.

[x]Glucose and NH_4Cl each added at 150 $\mu g/g$ of soil.

[y]"None" indicates no extract from nonamended or amended soil was added to chlamydospore-infested soil. "Control" indicates extract from nonamended soil was added.

[z]Numbers in each column followed by same letter are not significantly different from each other, $P = 0.05$.

Similar observations were noted for chlamydospore germination in soil. With the propagule assay method, chlamydospores germinated more than 50% in immature rye- and corn-amended soils because there were enough nutrients present (anthrone- and ninhydrin-positive materials) to allow germination. In the absence of nutrients in mature rye- and corn-amended soils, germination did not occur. The buried-slide method also demonstrated that nutrients, in this instance provided on the slide, were necessary for germination. However, although the nutrients reversed the inhibition due to immature amendment decomposition, they did not nullify the inhibitory effect of decomposing mature corn and rye residue. These observations, both in vitro and in the soil, suggest that fungistasis, due to nutrient deficiency, cannot entirely explain the inhibition of chlamydospore germination as a result of decomposing mature residues.

The possibility of formation of a toxic principle was investigated. Previous evidence for production of an extractable toxicant against soilborne plant pathogens has been minimal (20). Evidence, based on our data with agar-disk and buried slide methods, suggests that a factor toxic to chlamydospore germination was associated with mature amendment decomposition in soil. We further obtained direct evidence for a toxicant by extracting a crude preparation of the toxic material from decomposing mature rye and corn residues and demonstrating its inhibitory effect on chlamydospore germination with the propagule assay method (Table 2). Inhibitory materials were not extracted from decomposing immature residues. The greater inhibitory effect observed with decomposing rye, than with corn, could explain why rye residue reduced bean root rot more than corn residue (Fig. 1). This is one of the few reports in which an active fungitoxicant has been extracted and demonstrated.

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