Fungi Associated with Root and Foot Rot of Winter Wheat and Populations of *Cochliobolus sativus* in the Texas Panhandle

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ABSTRACT

Specht, L. P., and Rush, C. M. 1988. Fungi associated with root and foot rot of winter wheat and populations of *Cochliobolus sativus* in the Texas Panhandle. Plant Disease 72:959-963.

Cochliobolus sativus was the major pathogen responsible for root and foot rot of winter wheat in the Texas Panhandle. *Fusarium acuminatum* also caused root rot in many fields, but was much less important than *C. sativus* as a cause of foot rot. Fall and winter populations of *C. sativus* in 56 fields ranged from 5.3 to 394 propagules/g of soil, with a mean of 137. Significant correlations occurred between populations of *C. sativus* and both the incidence and severity of lesions on subcrown internodes of wheat seedlings.

Additional keywords: *Cochliobolus sativus*-selective medium, *Fusarium graminearum* Group 1, *Fusarium* spp., inoculum density-disease relationships, *Microdochium bolleyi*, *Rhizoctonia* spp.

Root and foot rots are major diseases of wheat (*Triticum aestivum* L.) worldwide. Typical symptoms include the necrosis of roots, subcrown internodes, crowns, tillers, and lower leaf sheaths (35,36). The most commonly reported pathogens are *Cochliobolus sativus* (Ito & Kurib.) Drech. ex Dastur (anamorph *Bipolaris sorokiniana* (Sacc.) Shoem., syn. *Helminthosporium sativum* Pamm.), *Fusarium culmorum* (Smith) Sacc., *F. graminearum* Schwabe, and *F. avenaceum* (Fr.) Sacc. (35). Common root rot incited by *C. sativus* occurs in many areas of the United States (1,15,30,31) and the Canadian prairie provinces (13,19) and is estimated to cause annual wheat losses of 3–6% throughout most of North America (19,35). Root and foot rots caused by *Fusarium* spp. predominate in the northwestern United States (5,6), California (28), and New York (17), among other areas. *F. culmorum* and *F. graminearum* are responsible for severe foot rots that cause losses of up to 50% in some dryland winter wheat fields in the northwestern states (5,6). Recently, Hill et al (15) reported that *C. sativus* and *F. acuminatum* Ell. & Everh. are both major components of the root-disease complex of winter wheat in Colorado and Wyoming.

There has been no information available to document the major root-disease pathogens of wheat in the Texas Panhandle. Therefore, a study was undertaken to determine this information. The Panhandle accounts for 45% of the wheat grown in Texas, which usually ranks third in the production of hard red winter wheat in the United States. Wheat is also an important crop in neighboring Oklahoma, which has similar climatic conditions. Information gained from this

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Accepted for publication 28 June 1988 (submitted for electronic processing).

study will serve as a basis for future research on root and foot rot diseases of wheat in this region.

MATERIALS AND METHODS

Plant and soil samples were taken from 56 winter wheat fields in 25 counties of the Texas Panhandle from 11 November 1986 to 30 January 1987. Most of the wheat fields visited were nonirrigated, but otherwise were selected at random with no prior knowledge of disease history. In each field, 20–25 wheat seedlings (Feekes scale 3-4) and a general composite soil sample (12–15 cm deep) were collected from an area approximately 100 m^2. An additional 15–20 mature plants (Feekes scale 11.1-11.2) were sampled in 44 of the fields from 1 to 15 June 1987. Soil was taken from within rows of mature plants collected in June and was combined to produce one composite sample per field. The wheat cultivars sampled were not determined for all fields. However, cultivars grown in the area are all of the hard red winter type and have little resistance to root and foot rots.

Disease evaluations. The plant samples were washed thoroughly and processed within 1–2 days after collection. All seedlings from the same field were evaluated as a group and assigned an overall root-rot rating (based on percentage of root area with necrosis) using a three-point scale where 0 = clean to trace (<1%), 1 = slight to moderate (1–5%), and 2 = severe (>5%). Only extensively rotted root tissue was rated as positive for necrosis, and observation of disease-area diagrams (16) aided percentage disease assessment.

Subcrown internodes of seedlings from each field were evaluated for disease incidence (percentage of internodes with lesions) and disease severity. Disease severity on individual subcrown internodes was measured using a four-point scale (19), where clean, slight, moderate, and severe ratings were 0, 1–10, 11–50, and 51–100% of the internode covered with lesions, respectively. An overall subcrown internode disease-severity rating (0–100 scale) for plants from each field was calculated using the formula: $[(SL \times 1) + (MO \times 2) + (SE \times 4)]/4$, where SL, MO, and SE were the percentages of internodes placed in the slight, moderate, and severe categories, respectively.

To assess the incidence of foot rot, 75–100 tillers were selected at random from the 15–20 mature plants sampled from each field in June. The outer-leaf sheaths of tillers were pulled away and the percentage of tillers with lesions (mild to severe), and also the percentage with severe lesions, were determined. Lesions on tillers were rated as severe if they circumscribed the stem and were dark brown to black in color.

Isolation and identification. Representative necrotic roots, subcrown internodes, and tillers were cut into 1-cm-long segments and rinsed vigorously in running tap water for 20 min. For each field, five to 10 root, one to 10 subcrown internode, and 10 to 15 tiller segments were plated out onto petri dishes containing 2% water agar amended with 100–200 mg of streptomycin/L. Colonies of fungi that emerged within 2 days were transferred to half-strength Difco potato-dextrose agar (0.5 PDA), and were subsequently stored at 5 C.

The fungal isolates were identified using published keys (9,20,36). *Fusarium* spp. were hyphal tipped and identified on carnation-leaf agar using the taxonomic system of Nelson et al (20). The nuclear condition of *Rhizoctonia* spp. was determined with the HCl-Giemsa staining procedure (14). Multinucleate isolates were paired with isolates of *Rhizoctonia solani* Kühn belonging to hyphal anastomosis groups (AG) 1–8 (22), provided by Earl G. Ruppel and David M. Weller. The identity of representative isolates of some fungi were confirmed by Paul E. Nelson and Carol E. Windels (*Fusarium* spp.), Randy T. Kane (*Microdochium bolleyi* (Sprague) de Hoog & Herm.-Nijhof), and David S. Marshall (*Gaeumannomyces graminis* (Sacc.) v. Arx & Oliver var. *tritici* Walker).

Pathogenicity studies. The fungi were tested for pathogenicity to seedlings of the hard red winter wheat cultivar Tam 105 planted in test tubes containing presterilized vermiculite (15). Five-mm^3 agar plugs of the fungi (grown on PDA) were placed adjacent to surface-disinfested seeds (10 min in 0.5% NaOCl) at planting. The severity of lesions that developed on shoots and roots of seedlings after growth for 14 days (at a temperature of 25 C and a 12-hr/day light intensity of 100 $\mu E \cdot m^{-2} \cdot s^{-1}$) were evaluated on a 0–4 scale where: 0 = clean, 1 = slight, 2 = moderate, 3 = severe, and 4 = shoot/root dead or almost dead. Mean shoot- and root-virulence ratings for each fungal isolate were determined by averaging the disease ratings on three replicate seedlings (one seedling per test tube).

Soil assays. Each composite soil sample was sieved (3.35-mm mesh), mixed thoroughly, and assayed for propagules (mainly conidia) (3) of *C. sativus* using the dilution-plate technique. The homemade potato-sucrose agar medium for the plate counts, a modification of previously reported media (8,24), was prepared by boiling 35 g of sliced, unpeeled white potato for 40 min in 500 ml of distilled water, straining the liquid through cheesecloth, and bringing the volume up to 1 L. Five grams of sucrose and 20 g of agar were then added before autoclaving. The following chemicals were added per liter of cooled (48 C) molten medium: 25 mg of benomyl (added as 50 mg of Benlate, 50 WP, dissolved in 10 ml of a 50/50 acetone-methanol mixture), 4 mg of dicloran (added as 5.3 mg of Botran, 75 WP), 35 mg of rose bengal, 300 mg of streptomycin, 300 mg of neomycin, and 2 mg of chlortetracycline. The pH of the molten medium was adjusted to 6.0 with 0.1 N H_2SO_4. The medium was stored in the dark at 5 C until used. Soils collected

Table 1. Fungi isolated from necrotic tissues of winter wheat in the Texas Panhandle in 1986–1987

Fungus	N[b]	No. isolates per plant part[a]			No. fields[c]
		Root	SCI	Tiller	
Cochliobolus sativus	448	52	213	183	49
Fusarium equiseti	370	237	73	60	51
F. acuminatum	96	69	8	19	37
Microdochium bolleyi	64	25	8	31	28
F. scirpi var. *compactum*	38	25	6	7	13
Rhizoctonia solani	37	17	11	9	22
F. graminearum Group 1	33	3	0	30	6
F. sambucinum	31	27	1	3	12
Binucleate *Rhizoctonia* spp.	13	8	1	5	7
F. oxysporum	10	7	3	0	8
Gaeumannomyces graminis var. *tritici*	8	7	0	1	3
SWB[d]	8	3	0	5	3
F. solani	5	4	1	0	3
Curvularia spp.	4	3	0	1	4
F. avenaceum	3	1	0	2	2
F. moniliforme	2	1	0	0	1
Bipolaris spicifera	2	2	0	0	1

[a] Number of isolates obtained from roots, subcrown internodes (SCI), and tillers. Isolates from roots and SCI were from plants sampled 11 November 1986 to 30 January 1987, and isolates from tillers were from plants sampled 1–15 June 1987.

[b] Total number of isolates of each species or genus. The total number of isolates of all fungi listed is 1,172.

[c] Total number of fields where each fungus was isolated one or more times.

[d] Unidentified sterile white basidiomycete with clamp connections.

during the fall/winter (November–January) and in June were assayed using 1:50 and 1:100 dilutions, respectively, and 10 dilution plates were prepared per sample. The plates were incubated at 22 ± 2 C for 7–9 days under low daytime light intensities (4–8 $\mu E \cdot m^{-2} \cdot s^{-1}$). Colonies of *C. sativus* on the plates were observed macroscopically and their identity was confirmed by microscopic examination. The efficiency of recovery of conidia of *C. sativus* on this medium is close to 100% with 1:50 or higher soil dilutions (*unpublished data*). Soils collected in the fall/winter also were analyzed for soil pH in 0.01 M $CaCl_2$ (23) and for soil texture (21).

RESULTS

Disease assessment. Rotted roots and/or necrosis of subcrown internodes of wheat seedlings were observed in 51 of 56 fields. Of the 56 fields, 28, 23, and five had seedlings with zero-to-trace, slight-to-moderate, and severe levels of root rot, respectively. The percentages of seedlings having lesions on the subcrown internode were 0, 1–32, 33–66, and 67–100% in 10, seven, 17, and 17 fields, respectively. The overall subcrown internode disease-severity ratings were 0, 1–32, 33–66, and 67–100 in 10, 23, 12, and six fields, respectively. No subcrown internodes were present on seedlings sampled from five fields, presumably because of shallow seeding.

Foot rot occurred in all of the fields revisited in June. The percentages of tillers with lesions were 0, 1–32, 33–66, and 67–100% in zero, seven, 17, and 20 fields, respectively. The percentages of tillers with severe lesions were 0, 1–32, 33–66, and 67–100% in three, 27, 14, and zero fields, respectively. Lesions were generally restricted to the lower 3–5 cm of tillers, and usually did not progress beyond the first node above the crown.

Identification and virulence of fungi. Most of the fungi isolated were identified (Table 1). *C. sativus*, *Fusarium equiseti* (Corda) Sacc., *F. acuminatum*, and *M. bolleyi* made up 448, 370, 96, and 64 of the isolates, respectively. These four species were obtained from 49, 51, 37, and 28 fields, respectively (Table 1). Most isolates of *C. sativus* were from subcrown internodes of seedlings and tillers of mature plants, while most isolates of *F. equiseti* and *F. acuminatum* were from roots of seedlings (Table 1). Thirty-three isolates of *F. graminearum* Group 1 ecotype, sensu Francis and Burgess (11), also were obtained, and most of these were from tillers. However, this fungus was found in only six fields.

Of the above fungi, only isolates of *F. graminearum*, *C. sativus*, and *F. acuminatum* generally caused at least moderate lesions on shoots and roots of 2-wk-old wheat seedlings. Mean virulence ratings for these three fungi were 3.60, 2.80, and 1.73, respectively, for lesions on shoots (Table 2), and 2.99, 2.09, and 1.47 for lesions on roots (*not shown in tables*). These data were analyzed by ANOVA, using variation among isolates as experimental error. Two separate analyses were carried out, one for the shoot-disease data and a second for the root-disease data. Weighted analyses, where weight = $1/\sigma^2$ (27,32), were used because various transformations of the data failed to equalize the error variances. The virulence ratings for *F. graminearum*, *C. sativus*, and *F. acuminatum* all were significantly ($P < 0.01$) different from each other, according to Tukey's HSD multiple range test (27,32).

Thirty-seven isolates of *R. solani* and 13 of binucleate *Rhizoctonia* spp. were obtained from necrotic wheat tissues (Table 1). Mean virulence ratings for these two fungi (based on lesions on shoots) were 2.09 and 0.91, respectively (Table 2). One of the isolates of *R. solani* was AG-2, nine were AG-3, 23 were AG-4, and four did not anastomose with any of the tester isolates. Mean virulence ratings (based on lesions on shoots) for isolates belonging to AG-2, AG-3, and AG-4 were 2.00, 1.26, and 2.72, respectively. The isolates of AG-4 were significantly ($P < 0.05$) more virulent on shoots of wheat seedlings than either the AG-3 or the binucleate isolates. Isolates of *R. solani* and binucleate *Rhizoctonia* spp. only caused slight lesions on roots of 2-wk-old wheat seedlings in these tests. For all isolates of fungi, except *R. solani*, the virulence ratings on roots and shoots of wheat seedlings were generally similar.

***C. sativus* populations.** Fall/winter populations of *C. sativus* in the 56 fields ranged from 5.3 to 394 propagules/g of oven-dry (105 C) soil, with mean and median values of 137 and 123, respectively. Forty-three fields contained fewer than 200 propagules/g (Table 3). Ninety-five percent confidence intervals (experimental

Table 2. Virulence of fungi isolated from winter wheat in the Texas Panhandle on shoots of 2-wk-old wheat seedlings of cultivar Tam 105

Fungus	N[b]	Percentage of isolates in disease-rating category[a]					Mean ± 95% CI[c]
		NP	SL	MO	SE	DEAD	
Fusarium graminearum Group 1	33	0	0	0	33	67	3.60 ± 0.13
Cochliobolus sativus	100	1	1	25	61	12	2.80 ± 0.13
F. avenaceum	3	0	0	67	33	0	2.22 ± 1.72
Rhizoctonia solani	37	24	8	24	19	24	2.09 ± 0.49
SWB[d]	8	0	50	25	12	12	1.96 ± 1.00
Gaeumannomyces graminis var. *tritici*	8	0	0	88	12	0	1.94 ± 0.30
F. oxysporum	10	0	30	50	20	0	1.90 ± 0.49
F. acuminatum	96	6	30	46	17	1	1.73 ± 0.16
F. moniliforme	2	0	100	0	0	0	1.00
Binucleate *Rhizoctonia* spp.	13	54	8	30	8	0	0.91 ± 0.65
Curvularia spp.	4	50	50	0	0	0	0.67 ± 0.97
F. sambucinum	31	45	55	0	0	0	0.53 ± 0.14
Microdochium bolleyi	64	48	48	3	0	0	0.51 ± 0.11
F. solani	5	60	40	0	0	0	0.40 ± 0.68
Bipolaris spicifera	2	50	50	0	0	0	0.33
F. scirpi var. *compactum*	38	79	21	0	0	0	0.21 ± 0.11
F. equiseti	100	88	12	0	0	0	0.13 ± 0.05

[a] Percentage of isolates in each of five disease-rating categories: NP = nonpathogenic or insignificantly pathogenic on shoots, SL = slight lesions on shoots, MO = moderate lesions on shoots, SE = severe lesions on shoots, and DEAD = shoots dead or near dead. Disease-rating categories of NP, SL, MO, SE, and DEAD represent virulence ratings of 0–0.49, 0.5–1.49, 1.5–2.49, 2.5 –3.49, and 3.5–4.0, respectively.

[b] Total number of isolates tested.

[c] Mean rating (on shoots) of all isolates with a 95% confidence interval (CI). Experimental error was based on variation among isolates. Confidence intervals were not calculated for species with less than three isolates.

[d] Unidentified sterile white basidiomycete with clamp connections.

error based on assay variation) for samples with 1–50, 51–100, and greater than 100 propagules/g averaged ±21, ±33, and ±57 propagules/g, respectively. Populations of *C. sativus* in fields sampled in June ranged from 27 to 2,907 propagules/g of soil, with mean and median values of 347 and 251, respectively.

Relationship of diseases to populations of *C. sativus*. The incidence of subcrown internodes of wheat seedlings with lesions was 60% or higher in nine of 12 fields where fall/winter populations of *C. sativus* were greater than 200 propagules/g of soil, but was 60% or higher in only four of 23 fields with less than 100 propagules/g; seven of 10 fields with no lesions on subcrown internodes of seedlings had fewer than 40 propagules/g. Linear-correlation analyses indicated significant relationships between fall/winter populations of *C. sativus* and both the incidence of subcrown internodes with lesions ($r = 0.37$, $P < 0.01$, $N = 51$) and overall disease-severity ratings on subcrown internodes ($r = 0.34$, $P < 0.05$, $N = 51$). Rank correlations (r_s) of these same parameters were higher than linear correlations, and were 0.47 ($P < 0.001$) and 0.44 ($P < 0.001$), respectively.

There were no significant relationships between fall/winter populations of *C. sativus* and the severity of root rot on wheat seedlings ($r = 0.09$, *NS*, $N = 56$), the incidence of tillers with lesions ($r = 0.13$, *NS*, $N = 44$), or the incidence of tillers with severe lesions ($r = 0.16$, *NS*, $N = 44$). However, there were moderately significant relationships between June populations of *C. sativus* and the incidence of tillers with lesions ($r = 0.30$, $P < 0.10$, $N = 44$) and also severe lesions ($r = 0.34$, $P < 0.05$, $N = 44$). The rank correlations were 0.35 ($P < 0.05$) and 0.39 ($P < 0.05$), respectively. Log transformations of *C. sativus* populations did not significantly improve any of the correlations. Soil pH ranged from 5.43 to 7.53, with a mean value of 6.83. Soil texture in most fields was silty clay or clay. There were no significant correlations between the pH or texture (% sand, silt, or clay) of soils and either populations of *C. sativus* or disease incidence/severity.

Table 3. Fall/winter populations of *Cochliobolus sativus* in 56 winter-wheat fields in the Texas Panhandle[a]

Propagules/g soil	No. fields[b]
0	0
1–50	12
51–100	13
101–200	18
201–300	7
301–400	6

[a] Soils were collected 11 November 1986 to 30 January 1987.
[b] Number of fields containing populations of *C. sativus* within indicated range.

DISCUSSION

Root and foot rot was prevalent on winter wheat in the Texas Panhandle. Obvious symptoms of foot rot were usually restricted to the bases of tillers, and whiteheads (sterile heads) were rarely observed. Many different fungi were associated with the necrotic tissues, but *C. sativus* was isolated most frequently. This fungus comprised 38% of all fungi isolated and identified, while *F. acuminatum* and *F. graminearum* represented 8 and 3%, respectively. *C. sativus* was obtained in very high frequencies from both subcrown internodes of seedlings and tillers of mature plants, and was the predominant fungus associated with plants having foot rot. *F. acuminatum*, an important cause of root rot of wheat along with *C. sativus*, was isolated primarily from roots, while *F. graminearum* was isolated primarily from tillers. *F. avenaceum* was isolated infrequently (0.3% of all isolates), and *F. culmorum* was not found. The latter two fungi tend to occur in climates with cool and intermediate temperatures, while *F. graminearum* occurs commonly in warmer areas (35), which agrees with our findings. *F. graminearum* was associated with the few fields where plants had foot rot with whiteheads and necrosis of tillers two to three internodes above the crown.

C. sativus is frequently associated with root and foot rot of wheat in the Great Plains region of the United States (6). *F. acuminatum* also probably occurs fairly commonly as a pathogen of wheat in this region (at least in the central and southern areas), because our findings are supported by those of Hill et al (15) for the root and foot rot diseases of winter wheat in Colorado and Wyoming. However, their studies did not determine the relative importances of *C. sativus* and *F. acuminatum* as pathogens of wheat roots versus tillers. Cook (6) indicated that root and foot rots of wheat are distinctly different diseases not necessarily caused by the same pathogens or correlated in occurrence. For example, a root rot caused by *Fusarium* spp. may sometimes lead to foot rot, but the succession is not automatic and is dependent upon extremely low plant water potentials during heading and plant maturation, such as can occur in dryland winter wheat fields in the northwestern United States (6).

Both *R. solani* and binucleate *Rhizoctonia* spp. were isolated from necrotic roots, subcrown internodes, and tillers of wheat plants. *R. solani* AG-4 was the most common and the most virulent of all the Rhizoctonialike fungi encountered. However, it was not conclusively determined that *Rhizoctonia* spp. were a major problem in any of the fields sampled. Another important root pathogen of wheat, *G. graminis* var. *tritici*, was isolated infrequently. Typical symptoms of take-all of wheat, which include black root lesions (35), were observed in only one field. Additional studies indicated that pathogenic *Pythium* spp. also occur on wheat in the Texas Panhandle, but that they are not a major problem in this area (*unpublished data*). Most of the fields sampled were nonirrigated, and *Pythium* diseases of wheat are generally not severe under dry soil conditions (35).

F. equiseti, which was generally nonpathogenic on 2-wk-old wheat seedlings, comprised 48% of all isolates from roots (Table 1). This fungus is a common soil and root inhabitant, and its frequent occurrence supports the conclusions of others that it is a vigorous saprobe and secondary colonizer of senescent/necrotic wheat tissues (2). *M. bolleyi*, which was generally nonpathogenic or weakly pathogenic to seedlings, also was commonly isolated. This fungus is often associated with wheat tissue, but is usually considered to be a minor pathogen (26,29).

The correlations between fall/winter populations of *C. sativus* in wheat fields and the incidence ($r = 0.37$) and severity ($r = 0.34$) of lesions on subcrown internodes of seedlings were significant but low. Many factors, such as different environmental conditions and cultural practices among the fields, could have affected disease development and thus affected these relations. However, soil pH and soil texture were two environmental factors that had no relationship to root and foot rot development in the fields. Multiple infections of subcrown internodes of wheat by *C. sativus* occur in the field (33). Therefore, the correlation between populations of *C. sativus* and the incidence of subcrown internodes with lesions ($r = 0.37$) should be improved by the multiple-infection transformation $y = ln[1/(1-x)]$, where y and x are the estimated and observed proportions of disease, respectively (34). However, when the above transformation was tested, and an observed-disease proportion of 0.99 assigned (for analysis purposes) whenever all internodes had lesions (three fields), there was no improvement in the correlation ($r = 0.36$).

The 153% higher mean population of *C. sativus* in soils collected in June (347 propagules/g), as compared with soils collected the previous fall and winter (137 propagules/g), was apparently due to sporulation of the pathogen on diseased wheat tissues and release of the conidia into the soil. The significant correlations between June populations of *C. sativus* and the incidence of tillers of mature plants with lesions ($r = 0.30$), and also severe lesions ($r = 0.34$), indicates that pathogen reproduction was related to the occurrence of foot rot. However, there were no significant relationships between fall/winter populations of *C. sativus* and subsequent development of foot rot in

June. Similar results have been reported by Chinn et al (3) and Ledingham (18) in Canada. Ledingham found that 20–50 conidia/g of soil caused up to 60% infection of subcrown internodes of wheat seedlings, and concluded that beyond some modest inoculum concentration further increases in inoculum do not substantially increase disease development. Other factors such as climatic conditions and cultural practices appear to play at least equal or even more important roles (4,18,19).

Almost all of the wheat grown in the Texas Panhandle is planted early for grazing purposes. Climatic conditions at this time are relatively warm and dry. Average soil temperatures (10 cm deep) at Bushland, TX (near the center of the Panhandle) during the planting months of August and September are 26.6 and 23.4 C, respectively. Average soil temperatures during the plant-maturation months of May and June are 19.4 and 25.1 C, respectively (10). Many fields are nonirrigated and total annual precipitation at Bushland averages 47.1 cm. Our findings support those of others that plant stress associated with warm, dry soil conditions favors root and foot rots of wheat caused by *C. sativus* and *Fusarium* spp. (4,7,12,25,35).

ACKNOWLEDGMENTS

This research was supported by the Texas Agricultural Experiment Station, Texas A&M University. We thank E. G. Ruppel and D. M. Weller for providing tester isolates of *R. solani*, and P. E. Nelson, C. E. Windels, R. T. Kane, and D. S. Marshall for assisting in identification of fungi. We also thank B. Coufal and S. Jackson for technical assistance, the Department of Plant Pathology, Washington State University, for manuscript preparation, and R. J. Cook for reviewing the manuscript.

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