Relative Pathogenicity of Selected *Fusarium* Species and *Microdochium bolleyi* to Winter Wheat in New York

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ABSTRACT

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Fusarium graminearum, *F. avenaceum*, *F. tricinctum*, and *Microdochium bolleyi* are the most frequently isolated species from winter wheat affected by foot and crown rot in New York. Under greenhouse conditions, *F. graminearum* caused preemergence and postemergence death of winter wheat (cultivar Houser) seedlings and reduced tillering and seed yields of survivors. *F. avenaceum* had similar effects on growth and yield but caused less seedling mortality. Effects of *F. tricinctum* and *M. bolleyi* inoculations were less severe, although reductions in growth and yield were noted at high *F. tricinctum* inoculum levels. Under field conditions, *F. graminearum* caused significant reductions in emergence, stand density, and yield of Houser wheat at two locations. *F. avenaceum* reduced seedling emergence at one location but did not affect yield. *M. bolleyi* and *F. tricinctum* had no significant effects on wheat growth or yield in field studies.

Root and crown rots of Gramineae are diseases of complex etiology. The fungi most often cited as important pathogens are *Bipolaris sorokiniana* (Sacc. ex Sorok.) Shoem., *Fusarium culmorum* (W. G. Smith) Sacc., and *F. graminearum* Schwabe (19). The major components of the root and crown rot complex vary among geographic regions, and other fungi such as *F. avenaceum* (Fr.) Sacc. and *F. acuminatum* Ellis & Everh. are important in some locations (2,7). In addition, a large number of soil-inhabiting fungi are commonly associated with root and crown rots of Gramineae, but these species are generally regarded as saprophytes or weak parasites.

Recent surveys have demonstrated that root and crown rot occurs frequently on winter wheat in New York and that seasonal trends in isolation frequencies of a number of fungi occur (9). Of these, *F. avenaceum*, *F. graminearum*, and *F. tricinctum* (Cda.) Sacc. were more frequently isolated from diseased than from healthy tissue; no other fungi shared this trait. Although *F. graminearum* and *F. avenaceum* have been frequently isolated from cereal root systems, reports of *F. tricinctum* are rare.

Microdochium bolleyi (Sprague) de Hoog & Herman.-Nijhof was recovered at very high frequencies during midseason sampling periods and, in toto, was isolated more often than any other species. *M. bolleyi* has frequently been associated with root systems of Gramineae (11,14,15) but is generally regarded as a saprophyte or weak parasite. However, a few reports (6,16) have suggested that *M. bolleyi* may cause severe symptoms and that its role in root disease complexes may be underestimated (13).

Because of regional variations in predominance and pathogenicity of fungal isolates, studies were undertaken to determine the relative pathogenicity of the predominant *Fusarium* spp. and *M.*

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bolleyi to winter wheat in New York. Along with conventional seedling assays, mature-plant studies were conducted under greenhouse conditions in an attempt to characterize effects of the test fungi on heading, fertility, and seed weight. In addition, pathogenicity tests were conducted at two field locations to observe possible yield-reducing effects of inoculation in a more natural setting.

MATERIALS AND METHODS

The winter wheat cultivar Houser was used in all pathogenicity studies. Houser is a pure-line selection with high yield potential (8) that is widely grown in central and western New York and has no known resistance to common root pathogens.

Fusarium species and *M. bolleyi* isolates used for pathogenicity studies were obtained from winter wheat root systems collected during field surveys. Hyphal-tip or single-spore-derived cultures were identified on wheat leaf agar (WLA = water agar plus γ-irradiated wheat leaves) and half-strength potato-dextrose agar (0.5 PDA) and were stored on WLA at 4 C.

In all greenhouse experiments, the planting medium consisted of a 3:2 (v/v) mixture of field soil/sand. The field soil used was a gravelly loam that was screened to remove rocks and plant debris. The mixture was then steamed for 30 min at 60–70 C, allowed to cool 24 hr, and remixed before use.

Pathogenicity of the test fungi to Houser seedlings was determined by infesting the soil/sand medium with colonized organic substrates before planting. Substrates consisted of autoclaved wheat seed/sand (4:1, v/v) or cornmeal/sand (1:1, v/v) that were infested with two or three isolates of each fungal species. Substrates were incubated at room temperature for several weeks until thoroughly colonized. Colonized substrates were then air-dried and ground to pass a single no. 10 (1.5-mm-diameter openings) screen in a Wiley mill. Measured amounts (2.5–10 cm^3) of this inoculum were added to 500 cm^3 of planting medium, which was then remixed before potting in 10-cm-diameter clay pots. Four seeds were planted per pot at depths of 3.0–3.5 cm.

Seedling pathogenicity assays were conducted in a 24 C greenhouse with supplemental light provided by 15W cool-white fluorescent bulbs. Pots were watered until the soil reached field capacity, then were allowed to dry down to near the wilting point potential before rewetting. All treatments were fertilized with 2.35 mg/cm^2 of a 20 N/20 P_2O_5/20 K_2O fertilizer 2 wk after planting.

Seedling assays were harvested 6–8 wk after planting, when most plants had three to five tillers. Growth parameters measured at harvest included number of shoots per plant and oven-dry weight of top growth (foliage biomass). Also, the extent of colonization and symptom development was measured with a subjective root and crown rot index (RCRI) of 1–5 based on the progressive development of symptoms on scutellar nodes, seminal roots, subcrown internodes, and crowns, where 1 = no symptoms; 2 = discoloration of seminal roots and scutellar node; 3 = discoloration more extensive including lesions on the subcrown internode; 4 = necrosis of scutellar node and subcrown internode, with discoloration extending to the crown; and 5 = extensive necrosis of the crown and stem base resulting in plant death.

Because Houser wheat is of the winter type, artificial vernalization of seedlings for mature-plant studies was necessary to stimulate heading in the greenhouse. The primary vernalization method employed was as follows. Houser seeds were planted in flats containing an autoclaved medium that consisted of 1:1:1 peat/sand/soil. Flats were held in a 24 C greenhouse until shoots were 3–5 cm long, then transferred to a lighted 4 C chamber for 6–8 wk. After vernalization, plants were rinsed thoroughly under running tap water to remove adhering debris and potted immediately, one plant per 12.5-cm-diameter clay pot. Vernalized plants were inoculated as described previously for seedling assays. At harvest, yield parameters measured for mature plant studies included number of fertile heads produced, seed weight and number (per head or per plant), and 1,000-kernel weight.

Two locations with different soil types in Tompkins County, New York, were chosen for field pathogenicity assays. The Tailby Farm site has a Chenango very gravelly loam (loamy, skeletal, mixed, mesic Typic Dystrochrept), a medium textured soil of low pH that tends toward droughtiness. The Helfer Farm site has a mixture of Rhinebeck (fine, illitic, mesic Aeric Ochraqualf) and Madalin (fine, illitic, mesic Mollic Ochraqualf) silt loams, which are characterized by somewhat poor to poor drainage and alkaline pH. Both fields received yearly applications of 220 kg/ha of a 10 N/20 P_2O_5/20 K_2O fertilizer before planting.

The inoculation technique differed from that reported for greenhouse experiments. Surface-disinfested Houser seeds were submerged 3–5 min in *Fusarium* spp. or *M. bolleyi* spore suspensions (25–100 $\times$ 10^3/ml) and allowed to air-dry before planting. To supplement the seed inoculation, a small amount of colonized wheat seed/sand inoculum (25–50 g/12-m row) was spread in the furrow of the center two rows of each six-row plot after planting but before emergence. Each experiment was a randomized complete block design with five observations per treatment. Individual plots were 3 m long $\times$ 1.5 m wide. The planting rate was 125 kg seed/ha (about 2 bu/acre). Plots were planted 28–30 September 1983 and were harvested in mid-July 1984.

Plants were removed from field plots periodically and examined for symptom development and colonization. Twenty to 30 plants from the four outermost rows of each plot were removed, adhering soil was rinsed away from root systems, and symptoms were rated as described previously. Eight to 10 tissue samples per plot were surface-disinfested and cultured on 0.5 PDA to confirm the presence of the inoculant fungi. Plants were sampled at early tillering (10 November), stem elongation (3 May), and early dough (6 July) stages. Plant growth and yield parameters measured included emergence, number of heads per meter row, and grain yield (g/plot). To measure these parameters, the two center rows of each six-row plot were examined.

For all greenhouse and field studies, statistical analysis of data was as follows. Continuous data were subjected to analysis of variance (AOV) procedures followed by Waller k-ratio t tests (multiple-comparisons approach) or by linear contrasts between means of interest. Analyses of frequency distributions of attribute data (e.g., RCRI ratings) consisted of chi-square (χ^2) goodness of fit tests (10).

RESULTS

Greenhouse studies. Soil infested with *F. graminearum* at the higher inoculum level caused significant preemergence or postemergence mortality to seedlings (Table 1). Symptoms were severe on surviving plants and included rotting of scutellar tissues, subcrown internodes, outer leaf sheaths, and crowns. Root necrosis was usually limited to regions adjacent to the node. Discoloration of the stem often extended above the soil surface, and inner leaf sheaths were visibly affected at harvest (about 6 wk). This severe symptom expression is reflected by RCRI ratings in the 3–4 range for about 70% of the survivors. *F. graminearum* also caused significant reductions in shoot weights and tillering (Table 1).

Symptom development after soil infestation with *F. avenaceum* was nearly as severe as with *F. graminearum*. However, soil infestations did not cause preemergence or postemergence seedling death at the inoculation rates tested. *F. avenaceum* caused reduced shoot weights and tillering as well as RCRI ratings of 3–4 on 40% of the plants (Table 1).

Seedlings inoculated with *F. tricinctum* showed less extensive symptom development. Tissue discoloration was limited to seminal roots, scutellar node tissues, and lower portions of subcrown internodes (RCRI = 3). Significant effects on shoot weights and tillering were observed

(Table 1), and these effects, for the most part, occurred at the high inoculum level only.

Seedling inoculations with *M. bolleyi* resulted in fewer symptoms than were observed for *F. tricinctum*. Tissue discoloration was limited to the scutellar node-epithelium region and to the upper portions of seminal roots. Small (<2 mm) lesions were also occasionally observed on subcrown internodes. Some of the discoloration observed was due to formation (by *M. bolleyi*) of "sclerotialike" aggregates in epidermal and cortical cells. These aggregates were composed of clumps of dark, thick-walled chlamydospores, which are characteristic resting structures of this fungus. Chlamydospores were not observed in vascular tissues. No other significant effects on seedling growth were observed (Table 1).

In mature-plant assays, vernalized seedlings inoculated with higher inoculum levels of *F. graminearum* and *F. avenaceum* consistently reduced the number and weight of seeds harvested per plant (Table 2). The highest inoculum level of *F. graminearum* also reduced the 1,000-kernel weight. Symptoms induced by *F. graminearum* and *F. avenaceum* included crown and basal stem rots that were often observed on individual tillers of infected plants. Sterile (white) heads were observed on some *F. graminearum*-infected plants.

F. tricinctum reduced seed numbers and total weight of seeds per plant at the highest inoculum level, although no severe symptoms were observed. Numbers of heads produced and 1,000 kernel weights were not significantly affected (Table 2). *M. bolleyi* had no significant effects on yield parameters measured in mature-plant assays.

Field studies. Highly variable results were obtained from field experiments, consequently the large error (s^2) terms limited the number of significant treatment effects detected by AOV procedures (Table 3). At the Tailby Farm, *F. graminearum* was the only species that caused significant reductions in emergence (at 50,000/ml only), number of heads per meter of row, and yield.

At the Helfer Farm, reduced emergence followed *F. avenaceum* as well as *F. graminearum* treatments. However, by season's end, only the high inoculum level of *F. graminearum* caused significant reductions in heads per meter of row, and the observed reduction in yield was not statistically different from the control. No differences in 1,000-kernel weight were observed for *F. graminearum* or *F. avenaceum* treatments, and no significant effects were detected at either location after *F. tricinctum* or *M. bolleyi* treatments.

RCRI ratings from samples collected at the Tailby location appear in Table 4. On 10 November, all *Fusarium* treatments induced significant symptom expression on Houser seedlings. However, by 3 May, symptoms associated with *F. tricinctum* inoculations were no longer significantly greater than for controls. *M. bolleyi* inoculation did not cause significant symptom expression at any time. The increase in 2+ RCRI ratings for control plots were presumably due to colonization by soilborne fungi present at this location. *F. graminearum* and *F. avenaceum* inoculations consistently caused more severe symptoms, although relatively few plants received RCRI ratings of 3 or greater. Also, it was apparent that plants with RCRI ratings of 3 or more had fewer tillers, regardless of treatment.

Isolations from healthy as well as diseased seedlings yielded relatively high frequencies of recovery (40–60%) of all test fungi. *F. avenaceum* was the most readily recovered species; autumn isolations yielded 75–90% recovery,

Table 1. Pathogenicity of *Microdochium bolleyi* and *Fusarium* spp. to winter wheat (cultivar Houser) seedlings

	Inoc. (cm^3)[w]	Shoot wt (mg)[x]	No. shoots/plant[y]				RCRI class[y]				No. plants surviving
			3+	2	1	χ^2	1	2	3+	χ^2	
Control	···	320 a	14	4	2	···	19	1	0	···	20
M. bolleyi	5.0	291 ab	10	8	2	ns[z]	7	13	0	*	20
	10.0	324 a	9	8	3	ns	8	12	0	*	20
F. tricinctum	5.0	240 bc	10	7	3	ns	15	5	0	ns	20
	10.0	244 bc	7	9	4	*	8	11	1	*	20
F. avenaceum	2.5	254 bc	6	3	11	**	0	12	8	**	20
	5.0	230 c	6	4	8	*	0	11	7	**	18
F. graminearum	2.5	211 c	4	3	9	**	0	4	12	**	16
	5.0	225 c	3	3	6	**	1	3	8	**	12*

[w] Number of cubic centimeters of colonized-grain inoculum added to steamed soil before planting surface-disinfested seeds.
[x] Mean oven-dry foliage weights for individual plants; means followed by the same letter are not significantly different ($P = 0.05$) as determined by AOV followed by linear contrasts.
[y] Numbers in columns are counts of plants with the appropriate attribute; e.g., number of shoots or root and crown rot index class on a scale of 1–5, where 1 = no symptoms; 2 = discoloration of seminal roots and scutellar node; 3 = discoloration more extensive including lesions on the subcrown internode; 4 = necrosis of scutellar node and subcrown internode, with discoloration extending to the crown; and 5 = plant death.
[z] Symbols indicate significant difference from control at * = $P = 0.05$ or ** = $P = 0.01$ after chi-square (χ^2) analysis; ns = no significant difference.

Table 2. Influence of *Microdochium bolleyi* and *Fusarium* spp. on yield components of mature winter wheat (cultivar Houser)

	Inoc. (cm^3)[x]	No. fertile heads[y]	Total no. seeds/plant	Total wt of seeds (g)	1,000-Kernel wt (g)
Control	···	3.5 a[z]	106.6 a	3.81 a	35.7 a
M. bolleyi	5.0	3.6 a	100.5 ab	3.57 a	35.6 a
	10.0	3.5 a	109.7 a	3.75 a	34.2 ab
F. tricinctum	5.0	3.2 a	97.1 ab	3.36 ab	34.6 ab
	10.0	3.1 a	82.1 b	2.79 bc	34.0 ab
F. avenaceum	5.0	2.9 a	100.4 ab	3.63 a	36.2 a
	10.0	3.2 a	77.5 b	2.78 bc	35.9 a
F. graminearum	2.5	2.8 a	82.8 b	3.22 ab	38.5 a
	5.0	2.6 a	81.7 b	2.34 c	28.6 b

[x] Cubic centimeters of colonized-grain inoculum added to steamed soil before planting vernalized seedlings.
[y] Number of mature heads that contained 10 or more kernels at harvest.
[z] Means in columns followed by the same letter are not significantly different ($P = 0.10$); data analyzed by AOV followed by linear contrasts.

Table 3. Pathogenicity of *Microdochium bolleyi* and *Fusarium* spp. in field tests conducted during 1983–1984 at two locations

		Tailby farm			Helfer farm		
	Conidia/ml[v] × 1,000	No. emerged[w]	No. heads/ 1-m row[x]	Yield[y] (g)	No. emerged	No. heads/ 1-m row	Yield (g)
Control	···	95 a[z]	58 a	352 a	101 ab	73 a	469 a
M. bolleyi	100	95 a	54 ab	309 abc	104 a	70 a	439 a
F. tricinctum	25	93 a	55 ab	335 a	102 a	71 a	479 a
	50	95 a	55 ab	307 abc	100 ab	70 a	461 a
F. avenaceum	25	99 a	56 ab	338 a	101 ab	72 a	475 a
	50	97 a	54 ab	325 ab	89 b	73 a	452 a
F. graminearum	25	93 a	50 bc	281 bc	88 b	69 ab	475 a
	50	73 b	45 c	268 c	72 c	65 b	403 a

[v] Approximate number of conidia per milliliter in suspensions used to inoculate seed before planting.
[w] Number of seedlings emerged per 2 m of row; counts were made in the two center rows of each six-row plot.
[x] Number of mature heads per 1 m of row; counts were made in the two center rows of each six-row plot and averaged.
[y] Yield in grams from the two center rows of each 3-m-long plot.
[z] Means in columns followed by the same letter are not significantly different ($P = 0.05$) by AOV followed by linear contrasts.

Table 4. Development of root and crown rot symptoms in 1983–1984 Tailby field plots

		RCRI class[a]											
		10 November[b]				3 May				6 July			
	Conidia/ml × 1,000	1	2	3+	χ^2	1	2	3+	χ^2	1	2	3+	χ^2
Control	···	62	10	3	···	47	26	2	···	52	22	1	···
M. bolleyi	100	59	15	1	ns[c]	39	36	0	ns	49	21	5	ns
F. tricinctum	25	31	43	1	**	35	38	2	ns	43	30	2	ns
	50	51	23	1	*	40	35	0	ns	43	31	1	ns
F. avenaceum	25	28	47	0	**	28	32	15	**	30	38	7	**
	50	17	54	4	**	10	53	12	**	28	40	7	**
F. graminearum	25	31	40	4	**	31	37	7	**	36	33	6	*
	50	24	43	8	**	25	43	7	**	29	36	10	**

[a] Data represent number of plants of 75 with symptoms falling into each root and crown rot class on a scale of 1–5, where 1 = no symptoms; 2 = discoloration of seminal roots and scutellar node; 3 = discoloration more extensive including lesions on the subcrown internode; 4 = necrosis of scutellar node and subcrown internode, with discoloration extending to the crown; and 5 = plant death.
[b] Growth stages of Houser wheat at each of the sampling dates: 10 November = early tillering, 3 May = stem elongation, and 6 July = early dough.
[c] Symbols indicate significant difference from control at * = $P = 0.05$ and ** = $P = 0.01$ after chi-square (χ^2) analysis; ns = no significant difference.

whereas late-season isolations (during grain fill) yielded 30–40% *F. avenaceum*. Isolations from control plants with RCRI ratings of 2 or more yielded *F. acuminatum*, *F. culmorum*, *F. equiseti*, *F. oxysporum*, and *M. bolleyi*. Low background levels (2–5% recovery) of *F. graminearum* and *F. avenaceum* were observed in control plots at both locations.

DISCUSSION

In greenhouse pathogenicity studies, inoculations with *F. graminearum* and *F. avenaceum* resulted in typical root and crown rot symptoms on Houser wheat, including crown and basal stem rot of mature plants. Significant reduction in plant growth and yield were also measured in these experiments, which indicates that the symptoms observed can be correlated with adverse effects on plant development and productivity. *F. graminearum* appeared to be more pathogenic than *F. avenaceum* on germinating seedlings, because *F. graminearum* infections resulted in greater preemergence and postemergence blighting. However, there was little difference in pathogenicity between these species once plants had developed beyond early stages.

F. tricinctum and *M. bolleyi* were only weakly pathogenic in greenhouse tests despite the high inoculum levels applied. Symptom development was limited to scutellar-embryo regions and adjacent portions of seminal roots and subcrown internodes. Despite the lack of severe symptoms, *F. tricinctum* reduced growth and yield parameters in some experiments.

In field studies, only *F. graminearum* inoculations led to significant reductions in yield parameters, even though *F. avenaceum* treatments caused similar symptoms on surviving plants throughout the season. High variability inherent in field studies of this type (5,13) may have led to the lack of significant effects on yield of *F. avenaceum* and some *F. graminearum* treatments. Another possibility is that less affected plants in inoculated plots may have been able to "compensate" (12) for early infections and stand reduction by increasing growth and tillering.

The results reported here concur with previous investigations concerning the pathogenicity of the species examined. *F. graminearum* and *F. avenaceum* have been reported as root pathogens of small grains in many areas of the world (3). In general, *F. graminearum* predominates in warmer climates, whereas *F. avenaceum* occurs more frequently in cooler climates. Although *F. avenaceum* is often regarded as less pathogenic than *F. graminearum*, it has been found to cause serious losses (2,17) and appears to be an important component of the root and crown rot complex in New York.

Early reports of *F. tricinctum* in association with Gramineae are considered unreliable because of oversimplification of the taxonomic system and confusion with other species such as *F. poae* and *F. sporotrichoides* (1,4). Similarly, *M. bolleyi* has not been extensively studied due to problems with taxonomy and identification (4,11). More recent studies have reported significant effects of *F. tricinctum* on growth and yield of small grains (17,18) similar to those reported here. Also, *M. bolleyi* has been associated with more severe symptoms (6,16) than were observed in this study. Therefore, *F. tricinctum* and *M. bolleyi* should be regarded as potentially important minor components of the disease complex on winter wheat in New York.

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