Wheat Seedling Response to Root Infection by *Cochliobolus sativus* and *Fusarium acuminatum*

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ABSTRACT

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Noncontaminated seedling roots of winter wheat cultivars Sandy and CO86 were inoculated with single germinated conidia of *Cochliobolus sativus* and/or macroconidia of *Fusarium acuminatum* to determine if resistance to these fungi could be identified. Four days after inoculation, seedlings were transplanted to soil and placed in a growth chamber at 18 or 29 C with or without water stress. Height, total leaf length, dry shoot weight, dry root weight, and root/crown discoloration were recorded for each plant after 4 wk. At 29 C, infection of CO86 with *C. sativus* resulted in a significant height reduction compared with Sandy. These cultivars are field rated as susceptible and resistant, respectively, to dryland root rot in Colorado. There were no consistent significant differences in other traits between cultivars, whether inoculated with *C. sativus* or not. Inoculation with *F. acuminatum* did not significantly ($P = 0.05$) affect any traits or enhance the effects of *C. sativus* when inoculated on the same seedling. The current experimental procedure, although providing some information, does not seem to be able to consistently select genotypes for increased general resistance to these pathogens.

Additional keywords: foot rot

Foot and root rot occurs in many grain-producing regions of the world (8,16,17) and is often characterized by necrosis of root and crown tissues (2, 9,16). It is usually an inconspicuous disease that reduces seedling vigor and impairs root and crown function. This results in reduced yield and lower grain quality (7,16). The causal agents are widely distributed, unspecialized fungal pathogens capable of infecting several genera of cereals and numerous grasses (1,12,15,16). Disease etiology is often complex and varies regionally. In Colorado and Wyoming (5) and southward through the Texas panhandle (11), the fungi most commonly associated with common root rot of winter wheat are *Cochliobolus sativus* (Ito & Kuribayashi) and *Fusarium acuminatum* Ellis & Everh., along with several *F. roseum* Link:Fr. types. Based on isolations from wheat throughout the growing season in Colorado, *C. sativus* commonly infects winter wheat during the fall (September through December) or early spring (March and April), whereas *F. acuminatum* infection is more prevalent later in the season (5).

C. sativus and *F. acuminatum* are considered weakly virulent pathogens, inflicting the most damage when adverse environmental conditions, such as drought stress, persist (12). The relationship among pathogens of the disease complex is not well understood. Freezing stress and early infection with *C. sativus* may predispose plants to subsequent infection by *F. acuminatum* (3,5).

Horizontal resistance, sensu Vanderplank (14), to common root rot was first reported in 1920 by Stakman (13). Resistance appears to be polygenic (6,10), thereby complicating breeding efforts to incorporate it into agronomically acceptable cultivars. The effects of general resistance can be subtle and not always evident in yield tests. Environment also plays a crucial role in disease development, further complicating breeding efforts by making it difficult to separate yield from environmental or disease effects, or both. The search for general resistance is hindered by lack of a quantitative method sensitive enough to measure disease response that will differentiate small differences in resistence. The major purpose of this study was to determine the effect of inoculating roots of noncontaminated wheat seedlings with single germinated spores of *C. sativus* or *F. acuminatum*, or both, in an effort to develop a sensitive measurement of disease resistance.

MATERIALS AND METHODS

The pathogen isolates used in this study were originally isolated from infected winter wheat (*Triticum aestivum* L.) plants obtained from commercial dryland fields in Colorado. Several isolates of each pathogen were tested and found to be equally virulent under similar experimental conditions. Conidia of *C. sativus* were obtained from dried diseased leaf material. Sporulation was induced in petri dishes containing moist filter paper placed in the dark at room temperature (approximately 21 C). Macroconidia of *F. acuminatum* were obtained from colonies growing on autoclaved carnation leaf pieces in petri dishes containing 1.5% water agar (WA) and stored in the laboratory at room temperature. Single germinated conidia and macroconidia were obtained by placing 1 ml of a spore suspension, diluted so conidia were abundant but separated from one another by at least four times their length, in petri dishes containing WA. Single germinated spores were removed 16 hr later, along with a small piece of agar, for use as inoculum.

Seed of winter wheat cultivars Sandy and CO86 and durum (*T. turgidum* L. var. *durum*) cultivars Vic and Calvin were surface-disinfested by immersion in a sequential series of solutions containing 70% ethanol, 20% NaOCl, 0.1% $HgCl_2$, and autoclaved distilled water (4). Disinfested seed were placed in petri dishes (five per dish) containing potato-dextrose agar and stored in the dark at room temperature (20–23 C). After 5 days, noncontaminated seedlings were transferred aseptically to test tube slants containing WA. A single germinated conidium of *C. sativus* or macroconidium of *F. acuminatum*, or both, was placed on the largest root of each plant. Inoculated seedlings were grown at room temperature under 18 hr of incandescent and fluorescent light (38 $\mu E \cdot m^{-2} \cdot s^{-1}$) per day. After 4 days, seedlings were transplanted to 10.2 × 10.2 × 8.0 cm pots (one per pot) containing a soil mixture (1:1, v/v, Weld silt loam and sand) treated with aerated steam for 0.5 hr, and pots were moved to a greenhouse bench with no supplemental light.

Four weeks later, roots were rinsed for 1 hr under tap water and plant height (from seed to tip of longest leaf), length of longest leaf, and total length of all leaves were recorded. Plants were air-dried for 30 min, the extent of root and crown tissue discoloration was recorded, and plants were placed in an oven at 70 C. Dry shoot and root weights were recorded 24 hr later.

Treatments included seedling roots inoculated with single geminated conidia of either *C. sativus* or *F. acuminatum* and their uninoculated controls. There were 15 replications per treatment and the experiment was repeated once. Traits were compared between inoculated and

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uninoculated treatments of each cultivar with a paired t test.

Subsequent experiments utilized growth chambers to reduce environmental variation over time in the greenhouse and to evaluate the effects of temperature and moisture stress on response to infection. Seedlings were inoculated with single germinated conidia and transplanted using the procedure described previously. Plants were grown in a growth chamber at 18 or 29 ± 1 C with a 12-hr day length (65–75 $\mu E \cdot m^{-2} \cdot s^{-1}$). Moisture treatments were stressed (75% available moisture, approximately −2 bars) or field capacity (approximately −1/3 bar). Moisture levels were maintained by weight. Pots were weighed and the appropriate amount of water added every other day. There were 10 replications per treatment and the experiment was repeated once. After 3 wk in the growth chamber, plant height, total length of all leaves, dry shoot weight, and dry root weight were recorded. The analysis of variance for the experiment was a $2 \times 2 \times 2$ factorial in a completely random design. The factors were temperature, inoculation, and moisture stress, with means separated by Duncan's multiple range test at $P = 0.05$. There were no significant differences in variance between replicated experiments, so data were pooled for analysis.

The effect of inoculation with both pathogens was determined by inoculating roots of noncontaminated seedlings with single germinated conidia (as previously described) of *C. sativus* or *F. acuminatum*, or both, on adjacent roots. Inoculated plants were placed in a growth chamber at 18 or 29 ± 1 C with a 12-hr day length (65–75 $\mu E \cdot m^{-2} \cdot s^{-1}$). Soil was maintained at field capacity by weight. Pots were weighed and watered daily. There were 10 replications per treatment and the experiment was repeated once. Plant response was recorded as described previously.

A final experiment was designed to determine if differences in lesion size and sporulation capacity existed between Sandy and CO86 after inoculation of the subcrown internode (SCI) with single germinated conidia of *C. sativus*. Surface-disinfected seed were planted in pots containing the steam-treated soil mixture and covered with 15 cm of autoclaved vermiculite. After 2 wk, the vermiculite was removed, uniform seedlings with a SCI of at least 1.5 cm were selected, and the coleoptile sheathing was removed. SCIs were inoculated with single germinated conidia of *C. sativus* and transferred to a dew chamber at 22 C. After 17 hr, the vermiculite was replaced and pots were transferred to a growth chamber at 24 ± 1 C with a 12-hr day length (65–75 $\mu E \cdot m^{-2} \cdot s^{-1}$). There were 15 replications per treatment and the experiment was repeated once.

After 7 days, lesions were measured and the SCI trimmed to 1.5 cm, with the lesion centered on the section. Sporulation was induced by placing the infected SCIs in petri dishes containing moist filter paper. After 7 days at room temperature in the dark, conidia were removed by placing the SCI in a test tube containing 15 ml of distilled water and agitating for 20 sec. Conidia were counted and recorded as number of spores per lesion.

RESULTS

Greenhouse. The visual effect of root inoculation with *C. sativus* was discoloration (brown to black) of the lower leaf sheath, crown, and roots. Height, length, and weight of durum cultivars Calvin and Vic were, in general, reduced by infection, but no differentiation between Vic and Calvin, field rated as resisitant and susceptible to root rot, respectively, was expressed.

Seedling plant height of winter wheat cultivar CO86 was significantly reduced, from 21.1 to 18.3 cm, by infection with *C. sativus*; however, the reduction of seedling plant height from 17.1 to 15.5 cm in cv. Sandy was not significant. Other traits of Sandy and CO86 varied among and between experiments, with no consistent differences between inoculated and uninoculated seedlings.

No visual symptoms or differences in height, length, and weight were found after root inoculation of any cultivar with *F. acuminatum*. Both pathogens could be isolated from root tissue of inoculated seedlings but not of uninoculated seedlings.

Growth chamber. Only Sandy and CO86, which demonstrated consistent differential seedling height response to infection, were used in these experiments (Tables 1 and 2).

Seedling height and leaf length were significantly greater and shoot weight and root weight were significantly less at 29 C than at 18 C for both uninoculated cultivars. All parameters of both cultivars were significantly less than those of uninoculated plants under both temperatures when inoculated, except

Table 1. Average height, leaf length, dry shoot weight, and dry root weight of seedlings of winter wheat cultivar Sandy uninoculated or root-inoculated with single germinated conidia of *Cochliobolus sativus* and incubated at 18 or 29 C with or without moisture stress

Temperature (C)	Treatment[w]	Height[x] (cm)	Leaf length[y] (cm)	Shoot weight (mg)	Root weight (mg)
18	U,N	31.3 bc[z]	58.9 c	84 a	56 a
	I,N	29.5 c	49.8 de	63 b	44 b
	U,S	27.4 d	44.1 de	53 cd	40 b
	I,S	26.6 d	42.1 e	47 de	34 c
29	U,N	34.4 a	80.6 a	57 bc	28 d
	I,N	32.0 b	71.0 b	49 ef	19 e
	U,S	29.7 c	56.6 cd	40 ef	27 d
	I,S	27.3 d	53.1 cd	36 f	20 e

[w] Average of 20 plants obtained by pooling two experiments with 10 plants per treatment. U = uninoculated, I = root-inoculated, S = with moisture stress, N = without moisture stress.
[x] Average length from soil level to tip of highest leaf of 20 plants.
[y] Average of the total length of all leaves of 20 plants.
[z] Numbers in a column followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 2. Average height, leaf length, dry shoot weight, and dry root weight of seedlings of winter wheat cultivar CO86 uninoculated or root-inoculated with single germinated conidia of *Cochliobolus sativus* and incubated at 18 or 29 C with or without moisture stress

Temperature (C)	Treatment[w]	Height[x] (cm)	Leaf length[y] (cm)	Shoot weight (mg)	Root weight (mg)
18	U,N	31.9 b[z]	51.5 c	72 a	59 a
	I,N	29.1 c	43.4 d	56 b	43 bc
	U,S	26.7 d	39.4 de	49 c	47 b
	I,S	26.7 d	37.1 e	44 c	38 c
29	U,N	33.5 a	66.6 a	51 bc	30 d
	I,N	29.3 c	60.6 b	43 d	22 e
	U,S	29.4 c	54.2 c	39 d	29 d
	I,S	24.6 e	44.5 d	28 c	18 e

[w] Average of 20 plants obtained by pooling two experiments with 10 plants per treatment. U = uninoculated, I = root-inoculated, S = with moisture stress, N = without moisture stress.
[x] Average length from soil level to tip of highest leaf of 20 plants.
[y] Average of the total length of all leaves of 20 plants.
[z] Numbers in a column followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 3. Average lesion size and lesion sporulation on subcrown internodes of seedlings of winter wheat cultivars Sandy and CO86 inoculated with single germinated conidia of *Cochliobolus sativus*

Parameter	Replications	Cultivar	
		Sandy	CO86
Lesion size[x] (mm)	1[y]	60	90
	2	60	50
Sporulation[z]	1	2,850	3,800
	2	2,450	2,550

[x] Average of 15 measurements.
[y] No significant differences ($P = 0.05$) in lesion size or sporulation between cultivars according to unpaired t tests.
[z] One lesion per 1.5-cm section of subcrown internode.

for height of Sandy under the lower temperature.

Height and shoot weight of the water-stressed plants were significantly less than those of the nonstressed inoculated plants at 18 C for both cultivars. At 29 C, height and leaf length of Sandy and leaf length of CO86 for the water-stressed uninoculated plants were significantly less than those of the nonstressed inoculated plants. Root weight of both water-stressed cultivars was significantly greater than that of the nonstressed inoculated plants but not of the nonstressed uninoculated plants.

Height, length, and weight of inoculated, water-stressed CO86 seedlings were significantly less than those of the uninoculated for all traits under both temperatures except for shoot weight at 29 C. For Sandy, leaf length at 18 C and shoot and root weight at 29 C did not differ significantly between inoculated, water-stressed plants and uninoculated plants.

Root weight at 18 C and all traits at 29 C were significantly less for inoculated, water-stressed CO86 seedlings than for those only water-stressed. For Sandy, only height and root weight at 29 C of inoculated, water-stressed seedlings were significantly less than for those only water-stressed. Uninoculated, water-stressed seedlings were significantly shorter than inoculated seedlings at the lower temperature. At 29 C, both inoculation and water stress were necessary to significantly decrease height from that of either treatment separately.

Height, leaf length, shoot weight, and root weight of Sandy and CO86 were compared for the nonstressed, uninoculated treatment using t tests ($P = 0.05$). Traits with no significant difference between cultivars were further tested by comparing treatment effects between cultivars. Significance was found only for plant height at 29 C, where inoculated and inoculated, water-stressed seedlings of CO86 were significantly shorter than those of Sandy.

The effect of dual inoculation with *C. sativus* and *F. acuminatum* was not significantly different from the effect of inoculation with *C. sativus* alone for either cultivar. Both pathogens were recovered in all isolation attempts from roots of both cultivars inoculated with both isolates.

There were no significant differences between cultivars for lesion size and sporulation (Table 3).

DISCUSSION

The single germinated spore inoculation technique eliminated variation in response to infection by mass inoculation. All inoculations resulted in infection. Disinfested seed and use of noncontaminated seedlings eliminated possible confounding effects by bacteria or by other fungi. Transferring the experiments to a controlled environment reduced variation in disease response and increased sensitivity. Of the four cultivars, only Sandy and CO86, field rated as resistant and susceptible, respectively, to dryland root rot, demonstrated response differences to infection consistent with their field ratings. All cultivars were equally susceptible (100%) to infection by both pathogens with this method. In addition, there were no cultivar differences in lesion size and sporulation capacity. Differences in field reaction may not be due to differences in resistance to infection but possibly to differential reactions of infected plants to environmental stresses later in the season.

In general, there were no significant differences in response to infection and stress between cultivars for any trait except height. Water-stressing inoculated plants significantly reduced most traits of both cultivars at both temperatures. However, the reaction of Sandy under these conditions was variable, indicating that the water stress and the higher temperature were not always additive in effect. Most of the CO86 reductions were significantly lower for inoculation plus water stress than for water stress alone, which indicates a greater additive response than with Sandy. This is consistent with the field ratings of the cultivars and a measurable differential response to stress. The method can differentiate cultivar differences in response to infection if differences are large enough. The method is not practical for screening large numbers of genotypes for general resistance to root rot because of low sensitivity and the intensive labor involved.

F. acuminatum is confirmed as a weakly virulent pathogen capable of causing damage to wheat plants only in conjunction with subsequent stresses. These experiments demonstrated no effect of infection on seedlings, even though the pathogen could be readily isolated from inoculated plants. It is also interesting to note that under these experimental conditions, dual infection did not significantly differ in reaction from infection with only *C. sativus*. The effects of infection with *F. acuminatum* under these conditions on height, length, and weight were not significant, even in seedlings coinfected with *C. sativus*. Additional experiments with longer incubation periods, perhaps to maturity, may reveal information on host, pathogen, and environmental interactions of this disease complex of wheat useful in selection for increased resistance.

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