Seedling Emergence, Plant Height, and Root Mass of Tall Fescue Grown in Soil Infested with *Cochliobolus sativus*

L. E. TREVATHAN, Professor of Plant Pathology, Department of Plant Pathology and Weed Science, Mississippi State University, Mississippi State 39762

ABSTRACT

Trevathan, L. E. 1992. Seedling emergence, plant height, and root mass of tall fescue grown in soil infested with *Cochliobolus sativus*. Plant Dis. 76:270-273.

Plants were grown in test tube culture with *Cochliobolus sativus* and in the greenhouse in infested fumigated and nonfumigated soil to determine the ability of the fungus to reduce stands in tall fescue (*Festuca arundinacea*). Germinated seeds in pots were inoculated with conidia or were covered with soil mix containing cornmeal and sand inoculum. Fawn, Kenhy, and Kentucky 31 tall fescue seedlings developed root lesions when exposed to the fungus in test tube culture, and root elongation was inhibited up to 50%. Emergence, establishment of seedlings in pot culture, and growth of leaves and shoots were determined weekly for 4 wk. Survival of all seedlings was reduced when inoculum was applied directly to seed or incorporated into fumigated or nonfumigated soil. Plant growth and root dry weights were also reduced. *C. sativus* reduced stand establishment and root and leaf growth of tall fescue in the absence of foliar infection.

Additional keywords: spot blotch

Spot blotch is widespread in Mississippi (L. E. Trevathan, *unpublished*) and is most damaging during the spring and fall when plants are growing most actively. The susceptibility of tall fescue (*Festuca arundinacea* Schreb.) to leaf infection by *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur (anamorph *Bipolaris sorokiniana* (Sacc.) Shoemaker; syn. *Helminthosporium sativum* Pammel, C. M. King & Bakke) has been confirmed by Kreitlow et al (4). However, spot blotch of fescue was infrequent in Georgia (6), and tall fescue foliage was apparently resistant to *C. sativus* (7).

Initial symptoms of this disease include dark brown to black, small, irregular to long and rectangular leaf spots. Later, leaf spots are surrounded by a chlorotic halo. Coalescence of leaf spots and chlorosis cause dieback of heavily infected leaves. However, the effects of the fungus on seedlings have not been determined. The effects of *C. sativus* on yield and quality of tall fescue forage have been documented under controlled conditions (13). Dry matter yields of infected tall fescue were half that of noninfected plants. Some progress has been made in selecting for resistance to foliar disease caused by *C. sativus* in tall fescue and determining the inheritance of resistance (5,12).

Studies on the response of turfgrasses (including *Festuca* spp.) to *C. sativus* have been conducted (4,8). The method of inoculating seed or roots and the condition of roots when inoculated influenced infection of *F. rubra* L. by *C. sativus* (3). Seedling emergence was reduced in *F. rubra* when the fungus was applied to seed or when soil and seed were infested (8,9). These investigations emphasized susceptibility of emerging seedlings and very young plants to *C. sativus*, but only fine-textured species adapted specifically to turf culture were inoculated.

This study was conducted to determine the effects of *C. sativus* on seedling emergence, leaf growth, and root growth of tall fescue following inoculation of either germinated seeds or roots. Results with fine-textured fescue species (3,8,9) suggested a particular need to determine the pathogenicity of *C. sativus* on roots of tall fescue seedlings. The effect of conidial age on virulence of *C. sativus* and comparative responses of tall fescue cultivars to inoculation in fumigated and nonfumigated soil were also determined.

MATERIALS AND METHODS

Inoculum preparation and maintenance. An isolate of *C. sativus* obtained from leaf spots on Kentucky 31 (KY-31) tall fescue plants at Starkville, MS, was used for inoculations. It was maintained under oil on potato-dextrose agar (PDA) slants prepared from white-skinned potatoes (15). To maintain virulence, the fungus was isolated from inoculated tall fescue plants following completion of each experiment in the greenhouse. Reisolated colonies were transferred to PDA slants or to PDA containing antibiotics (Difco PDA amended with 0.10 g of streptomycin sulfate and 0.03 g of oxytetracycline per liter).

C. sativus from stock slant cultures or from fresh PDA plus antibiotics was transferred to Czapek's salt solution agar (15 g agar per liter Czapek's salt solution) or to cornmeal and sand medium (CMS) (250 ml sand, 15 ml white cornmeal, and 110 ml of Czapek's salt solution per 500-ml flask) (15). All cultures were exposed to a 12-hr diurnal light period at 23 C for 2 wk. Sterile distilled water was added to each culture on Czapek's salt solution agar, conidia and mycelia were dislodged with a sterile transfer loop, and the resultant suspensions were combined and filtered through four layers of cheesecloth. Conidial concentration was adjusted to 500 spores per milliliter, and one drop of Tween-20 was added per liter of inoculum. CMS cultures were mixed in a 1:48 (v:v) dilution with soil medium following the incubation period.

Plant culture. Culture of fescue seedlings was similar for all experiments. Seed of KY-31, Kenhy, and Fawn were surface-disinfested for 10 min in 0.5% NaOCl in a desiccator under vacuum. The NaOCl was poured off the seed and replaced by sterile, distilled water for 10 min. Seed were then rinsed five times with sterile, distilled water and dried overnight in a transfer hood. Dried seed were placed on water agar for 6 days, and germinated seed were then placed on germination paper in tube culture or planted in 10-cm-diameter plastic cups that contained a mixture of 2:2:1 soil: sand:peat moss by volume. Before planting, a portion of the soil medium was fumigated with methyl bromide, and another portion was left untreated. The methyl bromide was allowed to dissipate, and the soil was amended with a 19-3-10 (N-P-K) formulation of fertilizer (Osmocote) at 216 g/m^3. Plants were fertilized weekly with 150 ml of a nitrogen fertilizer at 473 ppm and were watered three to four times a day. Insect pests were controlled with methomyl insecticide as needed.

Pathogenicity on roots of seedlings in test tube culture. To prepare test tube cultures, a single 30- × 250-mm strip of germination paper was rolled and placed inside a 25- × 200-mm glass test tube and autoclaved at 121 C for 20 min.

Published with the approval of the Director, Mississippi Agricultural and Forestry Experiment Station, as Journal Series Paper J-7629.

Accepted for publication 20 September 1991 (submitted for electronic processing).

Autoclaved strips were removed aseptically from test tubes and moistened with sterile, distilled water, and eight germinated fescue seed were placed 25 mm apart on each strip. Before placement on strips, the length of each radicle was measured. One seeded strip was rolled and reinserted to approximately 15 mm from the bottom of each test tube. Sixteen test tubes were prepared for each of the three fescue cultivars. Test tube cultures were placed upright under fluorescent light on a 12-hr diurnal light period and ambient laboratory conditions at 23 C.

When seedling roots extended below the germination paper, 5 ml of conidial suspension of *C. sativus* were pipetted into the bottom of eight test tube cultures of each cultivar. An equal number of cultures of each cultivar received 5 ml of sterile, distilled water and served as controls. Eight days after inoculation, the number of dead seedlings, the presence or absence of lesions, and the root length were determined, and root elongation was calculated. This experiment was repeated twice.

Colony age. Conidial inoculum was prepared as described above. Four germinated seed of the cultivar Fawn were placed on the surface of fumigated soil in plastic cups in the greenhouse. Ten milliliters of conidial inoculum was pipetted over the surface of germinated seeds in each cup. Seed were then covered with 2 cm of soil. Inoculum harvested from colonies grown on Czapek's agar for 1, 2, 3, 4, 5, 6, or 7 wk was tested for virulence. Thirty cups, each containing four germinated seed, and a noninoculated control were included in each treatment; cups were arranged in a randomized complete block design. Emergence was recorded after 4 wk, at which time plant height was measured from the soil line to the tip of the uppermost outstretched leaf. The experiment was repeated twice.

Seedling emergence and growth. The experimental design consisted of a split plot with treatments applied randomly to plants in fumigated or nonfumigated soil as main plots. Cultivar subplots were replicated four times within the soil main plots. Each subplot was represented by a cup containing four plants as subsubplots. The experiment was repeated four times.

Germinated seed of KY-31, Kenhy, and Fawn were placed on the surface of fumigated or nonfumigated soil in plastic cups. Inoculum was either incorporated into the soil mixture from CMS cultures or delivered as conidial suspensions as described above. Controls included treated soil, cups containing only cornmeal and sand incorporated into the soil mixture, or cups containing germinated seed treated with 10 ml of sterile, distilled water. Plants were maintained with daily watering in the greenhouse under natural conditions of light and a mean temperature of 25 C. Emergence data and seedling growth were recorded over a 4 wk period. At the end of the growth period, plants and soil were dislodged from cups, and roots were washed free of soil. Roots were dried to a constant moisture, and root dry weights were recorded. Data were subjected to analysis of variance and linear regression procedures.

RESULTS

Pathogenicity on roots of seedlings in test tube culture. Roots of each cultivar tested became infected with *C. sativus* and developed dark brown to black lesions during the 8-day incubation period. Lesions were typically 2–5 mm long and caused root distortion. Inoculated roots became horizontally oriented in test tubes in contrast to the vertical orientation of control roots. Similar effects were recorded in all three experiments and average seedling mortality was 8, 12, and 10% for KY-31, Kenhy, and Fawn, respectively. Root elongation was inhibited 21% in both KY-31 and Kenhy and 50% in Fawn.

Colony age. Emergence of Fawn seedlings in fumigated soil was reduced by conidial inoculum ranging in age from 1 to 7 wk (Table 1). As early as 1 wk after planting, conidial inoculum of all ages tested suppressed emergence to less than 80%. After 4 wk, 2- and 3-wk-old inoculum had suppressed emergence to 52 and 58%, respectively. Suppressions caused by 2- or 3-wk-old inoculum were as great or significantly greater than those occurring in other treatments.

Plant growth was suppressed in fumigated soil by 1- to 3-wk-old inoculum, and 1- or 2-wk-old inoculum caused the greatest reductions. Inoculum older than 3 wk had no significant effect on plant height. Similar results were obtained for all experiments.

Seedling emergence and growth. Seedling stand, plant height, and root dry weight were all influenced by inoculation with *C. sativus*, whether plants were grown in fumigated or nonfumigated soil and regardless of the manner in which inoculum was introduced (Table 2). In fumigated soil, the incorporation of CMS inoculum into the soil mixture or the application of conidial inoculum directly to germinated seed resulted in significant reductions in seedling stand, plant height, and root weight. For each variable measured, treatment means were significantly different from respective controls. This was also the case for seedling stand in nonfumigated soil, but plant growth and root weight were dependent upon the inoculation method. Only when CMS inoculum was incorporated into the nonfumigated soil mixture

Table 1. Percent Fawn fescue seedling emergence in response to conidial inoculum of *Cochliobolus sativus* applied to germinated seed in the greenhouse

Inoculum age[x] (wk)	Plant stand[y] (%)	Plant height (mm)
Control	93 a[z]	113 ab
1	69 cd	95 d
2	52 e	81 d
3	58 de	107 c
4	78 bc	126 b
5	85 ab	143 a
6	65 cde	128 b
7	79 bc	147 a

[x]Ten milliliters of inoculum, containing 500 conidia per milliliter, was pipetted over the surface of four germinated seed per pot at planting.
[y]Percentage of germinated seed planted.
[z]Average of 30 replicates. Means within columns not sharing a common letter differ significantly ($P \leq 0.05$) according to Student-Newman-Keul's multiple range test.

Table 2. Seedling emergence, plant height, and root dry weight of fescue from fumigated and nonfumigated soil infested with *Cochliobolus sativus*

Treatment[x]	Plant stand[y] (%)	Plant height (mm)	Root dry weight (g)
Fumigated soil			
Untreated control	91 a[z]	198 a	0.09 b
Sterile cornmeal/sand	95 a	182 a	0.12 a
Sterile distilled water	88 a	199 a	0.08 b
Cornmeal/sand inoculum	74 b	153 b	0.06 c
Conidial inoculum suspension	63 c	145 b	0.05 c
Nonfumigated soil			
Untreated control	93 a	197 a	0.11 a
Sterile cornmeal/sand	92 a	193 a	0.09 b
Sterile distilled water	94 a	185 ab	0.09 b
Cornmeal/sand inoculum	78 b	159 c	0.07 c
Conidial inoculum suspension	85 b	179 b	0.09 b

[x]Treatments with distilled water or conidial inoculum suspension received 10 ml of sterile, distilled water or 10 ml of inoculum containing 500 conidia per milliliter pipetted over the surface of germinated seed at planting. Cornmeal/sand treatments mixed in a 1:48 (v:v) dilution with soil mixture.
[y]Percentage of germinated seed planted.
[z]Means not sharing a letter in common differ significantly ($P \leq 0.05$) according to least significant difference.

were significant differences recorded in plant height and root weight compared to the respective controls.

No significant differences occurred in final seedling stand of the three cultivars in fumigated or nonfumigated soil (Table 3). The cultivar Fawn produced the tallest plants and greatest root mass, followed by KY-31 and Kenhy. This result was the same whether plants were grown in fumigated or nonfumigated soil.

A comparison of parameter estimates of linear regression equations for treatment effects on fescue seedling emergence was made (Table 4). When slopes were compared, seedling emergence increased over time for all treatments except CMS inoculum incorporated into the soil mixture. This treatment reduced seedling number through 4 wk after planting. In a similar comparison of the effects of soil fumigation, the rate of seedling emergence was significantly greater in fumigated soil compared to nonfumigated soil (Table 5).

DISCUSSION

C. sativus was pathogenic to three major cultivars of *F. arundinacea* under the conditions described for this study. The fungus incited root lesions, increased seedling mortality, and inhibited plant growth and root dry weights of Fawn, Kenhy, and KY-31 tall fescue cultivars cultured in test tubes or in pots in the greenhouse. KY-31 is the most widespread cultivar in Mississippi and is productive across a range of soil types and temperatures. Kenhy, which was derived from a ryegrass-tall fescue hybrid, is adapted to a wide range of environmental and cultural conditions. Weight gains by cattle are higher on Kenhy than other fescue cultivars because of improved palatability and digestibility. Fawn has high digestibility, crude protein content, vigor, and seed production relative to other fescue cultivars.

Responses of seedlings to the fungus were similar to those reported for other cool season grasses and small grains (1–3). Tall fescue leaf tissue is susceptible to *C. sativus* (4–7,12,13), and these results provide evidence that other plant tissues are susceptible as well. Symptoms of root rot caused by *C. sativus* confirm the ability of the fungus to directly infect fescue root tissues. However, the relative susceptibility of various tall fescue plant tissues or tissues of different ages have not been determined. In other winter crops, the responses of seedlings to this fungus were not correlated with reactions of adult plants (10,14).

Two- or 3-wk-old inoculum reduced seedling emergence as much as or more than inoculum of any other age up to 7 wk. Seedling growth was least when 1- or 2-wk-old inoculum was used. With increasing inoculum age, there was less decrease in growth of tall fescue exposed to the fungus. Thus, plantings that avoid periods of early inoculum development may be a means of escape of the effects of the fungus on seedling emergence and development.

Wheat plants grown in soil infested with *C. sativus* have been reported to produce less growth than wheat grown in autoclaved soil (1). Initial measurements were made 11 days after planting, and the effect became more pronounced as plants aged. Similar responses in plant growth and root dry weight occurred when the tall fescue cultivars Fawn, Kenhy, and KY-31 were cultured for 4 wk in soil infested with the fungus. However, this response apparently was affected by other organisms. Reduced seedling growth occurred in fumigated soil regardless of the manner in which inoculum was applied and in nonfumigated soil only when inoculum was incorporated with cornmeal and sand.

Based on the results of this study, *C. sativus* is apparently capable of causing significant mortality of tall fescue seedlings. In fumigated soil, where competition with other organisms was minimal, reductions of 19–30% in seedling stand occurred. These reductions were greatest when inoculum was introduced as a conidial suspension. In nonfumigated soil, the range in percentage reduction was 9–16%, with the greatest reduction occurring when inoculum was incorporated in the upper soil profile in cornmeal and sand. In the case of the cultivar Fawn, emergence and root dry weights were reduced most when inoculum was incorporated as cornmeal and sand instead of as a conidial suspension. This was consistent with results from test tube culture where root growth of Fawn was inhibited by 50%, whereas that of Kenhy and KY-31 were inhibited by 21%. Despite this fact, Fawn produced the tallest plants and greatest root mass when grown under greenhouse conditions. Since the primary root of grasses persists only a short time following germination, elongation of this structure may not be an appropriate measure of root susceptibility to *C. sativus*. Additionally, retardation of the primary root could stimulate secondary root growth from nodes of the young culm. Field performance data over a 3-yr period are consistent with greenhouse data presented

Table 3. Seedling emergence, plant height, and root dry weight of fescue cultivars from fumigated and nonfumigated soil infested with *Cochliobolus sativus*

Cultivar	Plant stand[y] (%)	Plant height (mm)	Root dry weight (g)
Fumigated soil			
Fawn	84 a[z]	200 a	0.09 a
Kentucky 31	84 a	166 b	0.08 ab
Kenhy	79 a	161 b	0.07 b
Nonfumigated soil			
Fawn	91 a	207 a	0.11 a
Kentucky 31	89 a	172 b	0.08 b
Kenhy	86 a	170 b	0.08 b

[y] Percentage of germinated seed planted.
[z] Means not sharing a letter in common differ significantly ($P \leq 0.05$) according to least significant difference.

Table 4. Comparison of parameter estimates of linear regression equations for effect of inoculation with *Cochliobolus sativus* on fescue seedling emergence

Treatment	Slopes (b_1)	Plant stand (%)
Untreated control	1.146 a[y]	91.8 a[z]
Sterile cornmeal/sand	1.438 a	92.2 a
Sterile distilled water	2.480 a	89.3 a
Cornmeal/sand inoculum	−1.728 b	78.8 b
Conidial inoculum suspension	1.770 a	73.4 c

[y] Slopes not sharing a letter in common differ significantly based on F test for homogeneity ($P \leq 0.05$).
[z] Means not sharing a letter in common differ significantly based on least significant difference ($P \leq 0.05$).

Table 5. Comparison of parameter estimates of linear regression equations for effect of soil fumigation on fescue seedling emergence

Treatment	Slopes (b_1)	Plant stand (%)
Fumigated soil	1.600 a[y]	81.3 b[z]
Nonfumigated soil	0.442 b	88.9 a

[y] Slopes not sharing a letter in common differ significantly based on F test for homogeneity ($P \leq 0.05$).
[z] Means not sharing a letter in common differ significantly based on least significant difference ($P \leq 0.05$).

here and show that Fawn produces higher dry matter yields than Kenhy or KY-31 (16). These cultivars grown in fumigated or nonfumigated soil infested with *C. sativus* maintained the same relationship.

Based on the results of previous studies with red fescue (3), preemergence damping-off may be a general response of fescue species to *C. sativus*. Additionally, the manner in which inoculations are made with respect to seed or roots and the condition of roots at the time of inoculation may affect the success of infection. When inoculum was added in cornmeal and sand, seedling emergence at 4 wk was lower than or equal to that in the first week. Thus, plants were killed by both preemergence and postemergence damping-off when inoculum was incorporated into the upper soil profile. By contrast, the use of a conidial inoculum suspension resulted in an emergence percentage during the first week that was lower than final seedling emergence. Therefore, preemergence damping-off accounted for most plant mortality when inoculum was placed in proximity to the germinated seed. This was the case whether plants were grown in fumigated or nonfumigated soil.

The inoculum threshold of *C. sativus* on winter crops in the field is not known (11). In this study, both conidial suspensions and cornmeal and sand cultures reduced emergence and growth of tall fescue seedlings under controlled conditions. Therefore, evaluation of both conidial and mycelial inoculum of this fungus will be necessary in the presence or absence of an exogeneous energy source to determine whether similar reductions in emergence and growth of tall fescue occur in the field.

LITERATURE CITED

1. Christensen, J. J. 1922. Studies on the parasitism of *Helminthosporium sativum*. Minn. Agric. Exp. Stn. Tech. Bull. 11.
2. Hayes, H. K., Stakman, E. C., Griffee, F., and Christensen, J. J. 1923. Reaction of barley varieties to *Helminthosporium sativum*. Minn. Agric. Exp. Stn. Tech. Bull. 21.
3. Hodges, C. F., and Watschke, G. A. 1975. Pathogenicity of soilborne *Bipolaris sorokiniana* on seed and roots of three perennial grasses. Phytopathology 65:398-400.
4. Kreitlow, K. W., Sherwin, H., and Lefebvre, C. L. 1950. Susceptibility of tall and meadow fescues to *Helminthosporium* infection. Plant Dis. Rep. 34:189-190.
5. Linscombe, S. D., Watson, C. E., Jr., and Trevathan, L. E. 1983. Inheritance of resistance to infection by *Drechslera sorokiniana* in tall fescue. Crop Sci. 23:1085-1087.
6. Luttrell, E. S. 1951. Diseases of tall fescue grass in Georgia. Plant Dis. Rep. 35:83-85.
7. Luttrell, E. S. 1953. Spot blotch of brome grasses, tall fescue grass, and barley. Plant Dis. Rep. 37:150-151.
8. Madsen, J. P., and Hodges, C. F. 1980. Pathogenicity of some select soilborne dematiaceous hyphomycetes on germinating seed of *Festuca rubra*. Phytopathology 70:21-25.
9. Madsen, J. P., and Hodges, C. F. 1980. Nitrogen effects on the pathogenicity of *Drechslera sorokiniana* and *Curvularia geniculata* on germinating seed of *Festuca rubra*. Phytopathology 70:1033-1036.
10. Nutter, F. W., Jr., and Pederson, V. D. 1984. Barley seedling disease reaction not a good predictor of adult plant reaction to spot blotch (*Helminthosporium sativum*). (Abstr.) Phytopathology 74:1270.
11. Reis, E. M., and Wunsche, W. A. 1984. Sporulation of *Cochliobolus sativus* on residues of winter crops and its relationship to the increase of inoculum density in soil. Plant Dis. 68:411-412.
12. Smith, M. A., Watson, C. E., Jr., Linscombe, S. D., and Trevathan, L. E. 1986. Selection for resistance to *Cochliobolus sativus* in tall fescue (*Festuca arundinacea* Schreb.). Theor. Appl. Genet. 72:807-810.
13. Smith, M. A., Watson, C. E., Jr., Watson, V. H., and Trevathan, L. E. 1986. Effects of *Drechslera sorokiniana* infection on the yield and quality of tall fescue forage. Anim. Feed Sci. Technol. 5:41-46.
14. Stack, R. W. 1981. Seedling disease response not a good indication of adult plant susceptibility to Helminthosporium root rot. (Abstr.) Phytopathology 71:257.
15. Tuite, J. 1969. Plant Pathological Methods. Burgess Publishing, Minneapolis, MN.
16. Watson, C. E., Jr. 1983. Tall fescue variety performance, 1980-83. Miss. Agric. For. Exp. Stn. Res. Rep. Vol. 8, No. 12.