Fusarium Blight Symptoms on Seedling and Mature Merion Kentucky Bluegrass Plants Inoculated with Fusarium roseum and Fusarium tricinctum

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ABSTRACT

Complete death of areas of 2-yr-old field-grown Kentucky bluegrass 'Merion' sod in the greenhouse was induced by inoculation with *Fusarium*-infested grass clippings. Conidial suspensions sprayed upon sod killed scattered individual tillers, but large numbers of tillers were not killed within an area unless inoculated with infested grass clippings. These areas were similar to field symptoms of Fusarium blight, except that the serpentine or frog-eye patterns were not present.

Inoculation of seedling and mature plants in growth chambers and in the greenhouse revealed large differences in the effects of temp, grass cultivar, and isolate on blight severity and death of individual plants.

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During the summers of 1959-63, a previously undescribed disease resulted in serious damage to Merion Kentucky bluegrass in Pennsylvania, eastern Ohio, eastern New York, New Jersey, Delaware, Maryland, and the District of Columbia. Couch and Bedford (4) reported that the incitant was *Fusarium roseum* or *F. tricinctum*. Affected areas were elongate streaks, crescents, or circular patches. A circular patch of blighted turfgrass 0.6 to 1.0 m in diameter with a center tuft of apparently unaffected grass called a "frog-eye" was described as characteristic of the disease (4). On seedling plants, the fungus entered the grass blade directly and at the cut tips of the blades. Leaf lesions originated at both the cut tip of the plant and at random over the entire leaf in the form of irregularly shaped dark-green blotches, which eventually became a dull tan. In greenhouse pot culture, with seedling plants grown in sand, Highland bentgrass was most susceptible, Merion Kentucky bluegrass ranked second, and Pennlawn creeping red fescue was most resistant.

Bean (1) observed both crown and leaf spot infection phases for Fusarium blight of mature turf. The characteristic frog-eye symptom was reported only rarely in the Washington, D.C. area. Fusarium blight first appeared in late June, remained active throughout July and August, and, if the temp remained high, into September. Disease severity was correlated with high light intensity and high soil temp, but no correlation was found between disease occurrence and nutrition or disease occurrence and thatch levels.

Bean (2) also reported that Fusarium blight symptoms on Merion Kentucky bluegrass were much more severe on lightly watered turf areas than on heavily irrigated sites. In greenhouse studies he found that sand infested with conidia of *F. roseum* and *F. tricinctum* did not affect Merion seed germination and seedling development.

Greenhouse studies by Cutwright and Harrison (5) showed that Fusarium blight was most severe on seedling Merion Kentucky bluegrass at soil temp of 32 C. Disease severity increased as the soil temp rose from 21 to 32 C. Disease was also positively correlated with inoculum concn. A third positive correlation was made between disease occurrence and nitrogen levels. No correlation was found between calcium levels and disease severity.

Cole et al. (3) found that *Fusarium* isolates from grass seed ranged from highly virulent to avirulent when tested on seedling plants. In general the *F. roseum* isolates were more virulent than the *F. tricinctum* isolates, and among the Kentucky bluegrasses, Merion was most susceptible, followed by Park, and Newport. Disease symptoms were blighted leaves or leaf lesions.

Recently, Vargas and Laughlin (10) reported that *Tylenchorhynchus dubius,* a stylet nematode, may play a dominant role in the development of Fusarium blight. In their experiments reduced top and root weights were used as indices of disease development. No mention was made of foliar blighting symptoms. In no instance did the *Fusarium*-nematode combination result in significantly more severe weight reduction than the nematode alone.

Under natural conditions, Fusarium blight usually appears in late June or early July (1, 4, 5); however, it may appear as late as September. Disease severity has been positively correlated with high soil and air temp, alternating wet and dry soil moisture levels, high intensities of light, extended periods of high atmospheric humidity, and high nitrogen levels.

Isolates of *F. roseum* and *F. tricinctum* have been shown to induce symptoms on seedling plants, but induction of symptoms has not been reported on mature turfgrass plants or turf areas.

The specific objective of these experiments was to induce Fusarium blight field symptoms on mature Merion Kentucky bluegrass plants and sod in the greenhouse.

MATERIALS AND METHODS.—In order to obtain virulent isolates, the pathogenicity of a large collection of *F. roseum* (Lk.) emend. Snyd. & Hans., and *F. tricinctum* (Cda.) emend. Snyd. & Hans. isolates against seedling turfgrass plants was evaluated. The experiment tested 64 isolates on three *P. pratensis* cultivars at three incubation temp. This included 33 isolates of unidentified *F. roseum* cultivars; six of *F. roseum* 'Culmorum'; three of *F. roseum* 'Avenaceum'; three of *F. roseum* 'Graminearum'; three of *F. roseum* 'Gibbosum'; and 16 of *F. tricinctum*.

Thirty-five isolates were from Merion Kentucky bluegrass and the remainder were from bluegrass or bluegrass-fescue mixtures. One-liter pots, 10-cm in diameter, were filled with a pasteurized (100 C, 30 min) mixture of soil:sand:peat (1:1:1, v/v) and seeded with either Merion, Delta, or Fylking Kentucky bluegrass at the rate of 0.011 g of seed/pot. The seed was treated with captan at the rate of 2 g active ingredient/454 g seed. The seed was covered with a 1-cm layer of vermiculite. Five weeks after seedling emergence, plants were inoculated with Fusarium isolates.

Inoculum was prepared from cultures initiated by the single-spore culture method described by Toussoun and Nelson (9) grown on 2% potato-dextrose agar slants incubated at 25 C under alternating 12-h periods of light and darkness. All isolates used in the experiment came from Pennsylvania turfgrass samples and were stored in vials of a mixture of sterile soil:peat:perlite (1:1:1, v/v).

Inoculum was harvested from 12- to 14-day-old cultures by washing the spores from the slants with distilled water. The concn of the spore suspension used was ca. 450,000 spores/ml. Twenty-four hr prior to inoculation, all grass was clipped to a height of 5-cm and the soil was saturated so that it would be near container capacity at the time of inoculation. The grass in each pot was inoculated by atomizing the spore suspension onto the leaf and crown areas of the plants until the point of runoff. Each pot was then covered with an individual polyethylene cover large enough to allow approximately 12 cm of air space above the foliage surface and placed at one of three ambient temp: 9 ± 1 C, 21 ± 5 C, or 29 ± 1 C. The 21 ± 5 C temp was in a greenhouse under partially shaded conditions; (normal day-length at the time the experiment was conducted was ca. 12 h) the two remaining temp were achieved in controlled environment chambers with alternating 12-h periods of light and darkness. In general, temp beneath the plastic covers ranged higher, especially in the greenhouse, than ambient temp. Based on periodic thermometer checks, the temp are reported as 12, 26, and 33 C in all subsequent sections of this paper. After 7 days, the covers were removed and after 14 days the plants were examined for leaf and crown symptoms of Fusarium blight. Evaluations were made according to the following scale: 1 = no disease symptoms; 2 = mild foliar lesions occurring on less than 10% of the plants; 3 = moderately severe foliar symptoms occurring on 10 to 40% of the plants; 4 = severe foliar blighting and crown decay symptoms occurring on 40 to 70% of the plants; and 5 = severe blighting, crown decay, and death occurring on more than 70% of the plants.

A second series of experiments was carried out with the eight most virulent isolates from the first experiment. These were R1137, *F. roseum* 'Avenaceum'; R1302, R1495, R1497, *F. roseum* 'Culmorum'; R1146, R1684, *F. roseum,* unidentified cultivars; and T120, *F. tricinctum*. Inoculation and incubation procedures were the same as the first experiment. Mature 1-yr-old plants of the previously described cultivars grown in pots in steam-treated (100 C for 30 min) soil were used as test plants. Three replicates of each treatment were made. Reisolation from inoculated plants was made from plant parts surface-sterilized in 10% Clorox and plated on 2% PDA.

Laboratory.—This experiment was designed to compare growth rates of eight isolates of *F. roseum* and *F. tricinctum* at various temp to provide a basis for interpretation of the temp-isolate-pathogenicity interactions. A single conidium of an isolate was placed in the center of a polystyrene petri dish containing 20 ml of 2% potato-dextrose agar. This procedure was replicated three times for each isolate for every temp. Dishes were incubated at temp from 12 to 36 C in 3-C increments. Measurements of radial growth of the mycelium were taken after 48, 72, and 96 h. All measurements were converted to a radial growth rate in mm per day.

Based on data from the preceding experiments, field-grown sod was inoculated. A greenhouse bench was divided into 24 sections measuring 30-cm × 45-cm, separated by 25-cm-high dividers. A layer of steam-treated Hagerstown silty clay loam, 15-cm deep, was placed in each section over a 1-cm layer of coarse gravel. A heating cable under the gravel kept the soil temp at 29 ± 3 C. Air temp beneath the cover was in the same range based on thermometer spot checks. A 30-cm × 45-cm rectangle of 2-yr-old field-grown Merion Kentucky bluegrass sod was placed in each section. The grass was maintained at a height of 2.5-cm. Just prior to inoculation, soil moisture content was found to average 45% on an oven-dry wt basis. The sod was not watered following inoculation during the course of each experiment. The treatments were: 1 = uninoculated check; 2 = inoculated with *Fusarium*-infected Merion clippings; 3 = inoculated with *Fusarium* conidial suspension. Eight replicate randomized complete block designs with three treatments were employed.

Clipping inoculum was prepared from Merion Kentucky bluegrass clippings which were collected and placed in 0.5-liter glass jars. The clippings were treated with propylene oxide (1.0 ml/jar/24 h) and then conidia from each of the previously mentioned eight isolates were placed on clippings in separate glass jars. Within 5 days the section of sod received 16 g of infested clippings consisting of 2 g of each isolate blended together.

Conidial suspensions of the eight isolates were prepared as described in the first series of experiments. Aliquots (20-ml) of conidial suspensions from each of the eight isolates were bulked together in one flask, and the mixture was atomized onto the turf in the appropriate sections to the point of runoff. The entire bench was then covered with a polyethylene tarp supported 30-cm above the foliage surface. After 6 days, the plastic was removed; and after 14 days, each section was visually inspected, and disease severity evaluated by the system used in the first experiments.

All data obtained from growth chamber, greenhouse, and laboratory experiments were subjected to analyses of variance (ANOV) (8) and Duncan's Modified Least Significant Difference tests (DMLST) (6, 11, 12).

RESULTS.—*Greenhouse experiments.*—Symptoms on infected seedlings appeared as rapidly enlarging, dark-green, water-soaked areas or gray-green lesions on the foliage. Often complete leaves were involved. On some leaves a reddish margin was present; a few infected areas were pink or red. After removal of the plastic cover and exposure to lower humidities and full sunlight, lesions and affected areas often bleached to a light tan.

Fusarium roseum and *F. tricinctum* isolates were more virulent at 33 C and 26 C than at 12 C. Merion and Fylking Kentucky bluegrass were more severely diseased than Delta Kentucky bluegrass. The *F. roseum* 'Culmorum' isolates, except in one case, were the most virulent, whereas the *F. tricinctum* isolates, except for three, were the least virulent. The remaining isolates ranged from avirulent to highly virulent, with no consistent pattern developing between isolate identification and virulence.

Consistent differences in kinds of symptom expression; i.e., crown infection, leaf spot, or foliar blighting, could not be detected among isolates. In general, the more virulent isolates induced larger lesions and more severe foliar blighting than the less virulent ones. In other words, an increase in disease incidence was accompanied by increased severity.

Significant differences occurred in the isolate × temp interaction indicating that each isolate had a specific temp range in which it was most virulent on seedling turf (ANOV, $P = 0.01$). Most isolates preferred a high temp, but a few had higher virulence ratings at 12 C than at 33 C.

—*Mature 1-yr-old plants grown in pots.*—Statistical analysis of the pathogenicity tests on mature turfgrass plants revealed significant differences (ANOV, $P = 0.010$ among the mean virulence ratings for the isolates, temp, cultivars, isolate × temp, temp × cultivar, and isolate × temp × cultivar interactions, as well as significant differences (ANOV, $P = 0.05$) among the isolate × cultivar interactions.

All isolates induced significantly more severe symptoms than appeared on the uninoculated check plants maintained under the same conditions. The most virulent isolate was R1302 of *F. roseum* 'Culmorum.' The two least virulent isolates were also *F. roseum* 'Culmorum.' The five other isolates consisting of one of *F. roseum,* one of *F. tricinctum,* an Avenaceum, and two Graminearum cultivars of *F. roseum* were moderately virulent. Major disease symptoms were expressed as a general gray-green foliage collapse due to crown rot with minimal visible leaf lesions or tip diebacks; however, foliar lesions present were similar to those reported with seedling turf. Isolations from surface sterilized diseased crown areas were made and *F. roseum* or *F. tricinctum* was recovered, in accordance with the inoculum originally introduced.

Higher pathogenicity ratings were obtained on the Merion than on either the Fylking or the Delta. Differences among the isolate × temp × cultivar interaction is exemplified by R1302, which was highly virulent at 26 C on Merion, but only mildly virulent on Fylking, and avirulent on Delta at 12 C (Table 1).

Laboratory experiment.—Effect of temperature on the growth rate of eight Fusarium isolates.—The growth rates of all isolates increased gradually as the temp increased until maximum growth rates were reached in the 24 to 27 C range, and then dropped off rapidly. All isolates grew slowly at 12 C and only R1302 and T120 grew at 36 C. Isolates R1146, R1495, and R1684 achieved their fastest mean radial growth rates at 24 C, while the remaining five isolates grew fastest at 27 C.

Inoculation of field-grown Merion Kentucky bluegrass sod under greenhouse conditions.—In two experiments, mature Merion sod sections inoculated with infested clippings exhibited the most severe symptoms. Symptoms in the plots inoculated with a conidial suspension were less severe and noninoculated plots had minimal infections. Mean severity ratings were 3.4 and 2.8, respectively. Inoculation with infected clippings resulted in significantly more severe symptoms than the conidial

TABLE 1. Severity evaluation of eight *Fusarium* isolates on three 1-year-old Kentucky bluegrasses at three temp

	Temperature									Mean
	12 C			26 C			33 C			
Isolate	M[a]	D[b]	F[c]	M	D	F	M	D	F	All temp for all cultivars
R1137[d]	1.3[e]	2.0	2.0	4.0	2.7	2.3	2.0	2.0	2.0	2.26 BC[f]
R1146	1.3	2.0	1.3	2.7	2.3	2.3	3.0	3.7	2.3	2.30 AB
R1302	1.0	1.3	1.3	4.7	3.0	2.0	3.3	3.0	3.3	2.56 A
R1495	1.0	1.0	1.7	2.7	2.3	2.3	2.0	2.0	3.0	2.00 CD
R1497	1.0	1.0	1.0	2.3	2.7	2.3	3.3	2.0	1.7	1.93 D
R1655	1.3	2.0	2.0	3.0	1.7	3.0	3.3	2.3	2.0	2.30 AB
R1684	1.0	1.0	1.0	3.3	2.3	2.7	3.3	2.0	1.7	2.15 BCD
T120	1.3	1.7	1.7	4.0	1.7	2.7	3.0	2.3	1.3	2.18 BCD
Check	1.0	1.0	1.0	2.0	1.0	1.0	1.7	1.3	1.0	1.22 E

LSD = 0.5 for isolates ($P = 0.01$, ANOV)

[a] M = 'Merion' Kentucky bluegrass.

[b] D = 'Delta' Kentucky bluegrass.

[c] F = 'Fylking' Kentucky bluegrass.

[d] R1137 = *F. roseum* 'Avenaceum'; R1302, R1495, R1497 = *F. roseum* 'Culmorum'; R1146, R1655, R1684 = *F. roseum* unidentified cultivars; T120 = *F. tricinctum*.

[e] All ratings are the mean of three replications. The rating system which was used is as follows: 1 = no disease symptoms; 2 = mild foliar symptoms occurring on less than 10% of the plants; 3 = moderately severe foliar symptoms occurring on 10 to 40% of the plants; 4 = severe foliar blighting and crown decay symptoms occurring on 40 to 70% of the plants; 5 = severe blighting, crown decay, and death occurring on more than 70% of the plants.

[f] Means not followed by the same letter are significantly different as determined by Duncan's Modified Least Significant Difference Test ($P = 0.05$).

inoculation (ANOV, DMLST, $P = 0.05$).

Use of infested clippings as inoculum resulted in severe crown infection and death of entire tillers. Symptoms appeared as dark-green or gray-green areas on the leaves and rapidly involved the entire tiller. Death of the tiller often appeared to be due solely to crown infection. Similar symptoms appeared on plants inoculated with conidial suspensions, but affected tillers were scattered throughout and fewer tillers were involved. Scattered affected tillers were also present in the field-grown sod controls which may have been naturally infected. In addition, all treatments were beneath a common cover which would allow movement of inoculum, especially airborne conidia, from area to area. After removal of the plastic cover, affected foliage began to shrivel and dry, and the color shifted from gray-green to tan. Several of the clippings-inoculated plots had irregularly shaped areas 10- to 15-cm across within which all the tillers had been killed. These were very similar to field symptoms occurring from natural sources of infection.

DISCUSSION.—The results of the first experiments involving inoculation of seedling bluegrass plants are in general agreement with those of previous researchers (1, 2, 3, 4, 5, 7). Isolates of both *F. roseum* and *F. tricinctum* were pathogenic on Merion, Delta, and Fylking Kentucky bluegrass. Symptoms appeared at both low and high temp with the isolates generally being more virulent at higher air temp. A wide range of virulence was also present among isolates, some being more virulent at low temp. The findings reported in the present investigation are in general accord with field observations (1, 4) and greenhouse studies by previous researchers (2, 4, 5) concerning the role of temp in disease development. The in vitro growth-temp responses for the isolates tested also support previous data.

Our field observations in Pennsylvania generally indicate that high humidity and warm temp (27 C or above) are necessary in the 7 to 14 days prior to the development of Fusarium blight symptoms; outbreaks of Fusarium blight are almost always preceded by a period of warm, wet weather followed by dry weather. Turf areas in the warm humid southern and southeastern portions of the state are more severely affected by Fusarium blight than those in the northern areas of Pennsylvania. Where Fusarium blight occurs the first symptom is a wilt and darkening of scattered areas of turf in the heat of the day. These may be from 15- to 30-cm across in circular-to-serpentine patterns. Within 48 h the darkened areas may take on a permanently wilted gray-green appearance and then collapse and bleach to a straw color. Distinct lesions are usually not present on plants in the major part of the affected area but can be found; in a few cases they may be detected on plants at the margins of a dead area. Occasionally there is a small patch of unaffected grass in the center of the diseased circles. The size of the affected area does not increase from the time the darkened patch is first observed to the time when the plants collapse and turn straw color. In areas where many severe infections occur, the patches overlap and result in large areas of dead grass. Blight is more common on older (>5-yr-old) turfgrass than on newly established areas. Fusarium blight is seldom observed on turfgrass areas less than 2 yr old.

Past reports on inoculation of turfgrass have dealt with seedling inoculation and symptom expression; field symptoms on mature turf have not been demonstrated by artificial inoculation. In the present experiments, a crown rot and blighting of 1-yr-old plants in pots was induced by inoculation with massive numbers of conidia. On sections of sod transferred to a greenhouse bench, inoculation with infested clippings produced diseased areas that closely resembled field symptoms which developed under natural conditions with natural sources of inoculum. In these areas, entire tillers including roots and crowns were killed. To our knowledge this is the first report of field-symptom induced on mature turf. These results are a natural extension of Bean's conclusion from prior experiments (2) that a food base greatly accentuated disease severity. Although conidial inoculation did result in scattered dead tillers and foliar blighting, the symptoms were not expressed as totally dead areas of turf.

It is of interest that killing of complete tillers occurred in mature plants grown in steam-treated soil. The sod squares inoculated with clippings were not evaluated for nematode populations at the time of inoculation; however, previous nematode evaluations in the site from which the sod was taken had not revealed significant populations of stylet-bearing species of nematodes. However, this is not to imply that nematode feeding may not be a factor in development of Fusarium blight under field conditions, since this relationship has been demonstrated for other *Fusarium*-induced root diseases.

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