Soil and Atmospheric Moistures Associated with Fusarium Crown Rot and Leaf Blight of *Poa pratensis*

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ABSTRACT

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Kentucky bluegrass (*Poa pratensis*) sod produced in the field was used to study the sequential effects of turfgrass watering regimes and atmospheric humidities on the incidence and severity of *Fusarium* diseases in the greenhouse. Severity of leaf blight was significantly increased by drought stress, especially when followed by periods of flooding, high humidity, or both. Plants that had not been predisposed by drought were resistant to leaf blight even when subsequently flooded but sustained low levels of infection during prolonged periods (up to 8 days) of high relative humidity. *Fusarium* spp. were principal colonists of crowns but not of tiller bases, whereas the reverse was true for *Nigrospora* spp. Inoculating the turfgrass with a conidial suspension of mixed *Fusarium* spp. did not increase disease levels above those in uninoculated treatments of field-produced sod.

Fusarium spp. are ubiquitous inhabitants of organic litter (thatch) in Kentucky bluegrass (19). They become the dominant facultative parasites in thatch during the summer (2,14) and are therefore likely to become the principal colonists of grasses subjected to stresses at that time. Foliar blight and crown and root rot are caused by a large group of these fungi, including *F. crookwellense, F. avenaceum, F. equiseti,* and others (17). It is important to differentiate these diseases of diffuse to irregular patches from the group of distinctly shaped patch diseases named Fusarium blight (7) or Fusarium blight syndrome (17). The latter were recently divided and renamed summer patch and necrotic ringspot (18,20).

Fusarium crown rot has been associated with drought stress conditions in the field (10,11). Crown rot and foliar blight increased when inoculated plants were subjected to drought conditions in the greenhouse (9,14), but drought stress before inoculation of bluegrasses with *Fusarium* spp. did not increase blighting (7). In these earlier studies, only single isolates of *Fusarium* had been added to plants grown in sterile sand or pasteurized sandy soil mixture, and the relative humidity around the foliage was elevated by enclosing the pots in plastic bags or in dew cabinets as long as 6 days. Inocula for these earlier studies included applications of conidial suspensions (9) or an infested cornmeal:sand mixture (7). Although the pathogenicity of individual isolates was demonstrated under experimental conditions, the pathogenic capabilities of the fungi in drier atmospheres under natural droughts or in wet soils or the competition of *Fusarium* with other members of the turfgrass microflora were not defined.

We are unaware of greenhouse studies of *Fusarium*-caused diseases in turfgrass ecosystems that approximate field conditions. The development of Fusarium

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crown rot and leaf blight should be conducted on mature, field-grown bluegrass in carefully managed soil moisture regimes. These grasses typically have thatch layers 1–4 cm thick (3) where living roots and stems are surrounded by organic litter in various stages of decomposition. Thatch becomes the primary rooting medium for plants in mature turfs. Thatch layers are subject to wide fluctuations in temperature, moisture, and nutrition (3). Thatch typically dries much faster than the underlying soil (13), but when thatch is wet and warm for even short periods, it may become poorly oxidized and deleterious to roots (21). Therefore, very wet thatch could be as effective as dry thatch in predisposing plants to infections by facultative parasites.

The purpose of this investigation was to examine the relative importance of atmospheric humidity and soil flooding and drying in the greenhouse on the development of Fusarium crown rot and leaf blight of field-grown Kentucky bluegrass.

MATERIALS AND METHODS

Ten-cm-diameter cores of 6-yr-old Kentucky bluegrass cultivar Fylking with 13-mm thatch and pH 5.1 were collected from a field plot at the Cornell University Turfgrass Field Laboratory, Ithaca, NY. The soil on which the turfgrass was growing was a moderately well-drained Hudson silty clay loam of the fine, illitic mesic family of Glossaquic Hapludolfs. Cores were embedded in a peat-sand-soil mix (1:1:1, v/v) in 13-cm-diameter clay pots and placed in a greenhouse at 19 C. The turf was cut to 5 cm high once each week and watered and fertilized as necessary. After 5 wk, the pots were moved to a greenhouse at 32 C and inoculated.

Half of the pots were drenched with 50 ml of water and the other half with an equal volume of suspension supplying 1.98×10^6 conidia per pot, including conidia of *F. graminearum, F. equiseti, F. sambucinum, F. culmorum, F. heterosporum,* and *F. poae* in a 1:2:2:1:2:2 ratio. Conidia were produced from single-spore isolates grown on potato-dextrose agar medium. The isolates were from crowns and foliage of uninoculated field sod that developed a severe foliar blight when moved to the greenhouse and grown under a plastic cover. The *Fusarium* isolates were identified according to Booth's (4) taxonomic key.

One week after inoculation in the greenhouse (32 C), the first incubation treatment (phase 1) was initiated. Half of the inoculated and half of the uninoculated pots were watered daily with 250 ml of water. The other half were allowed to dry until wilting symptoms (folded leaves) began to appear in more than 90% of the pots. At that time (6 days after the start of the drying cycle), all of the pots were watered thoroughly. Two complete drying cycles were performed over a 16-day period. Four new treatments (phase 2) were then superimposed on the previous four treatments. The new treatments consisted of pots that were flooded (to 5 mm below the thatch surface) or not flooded and either enclosed by polyethylene bags or left open. These treatments continued for either 4 or 8 days. Six repetitions were performed for each of the 32 treatments.

Measurements of temperatures, redox potentials, and concentrations of oxygen, carbon dioxide, and ethylene in the turfgrass thatch were made as previously reported (21).

Foliar blighting was evaluated at the end of the 4- and 8-day sampling periods. The disease rating was based on a scale of 1–10, where 1 = no diseased tissue and 10 = totally necrotic tissue. Ten blighted plants from each pot were removed and isolations made from tiller bases and crowns that had been surface-disinfested in 0.5% sodium hypochlorite for 45–60 and 180 sec, respectively. Samples were then rinsed in distilled water and plated onto half-strength, acidified (pH 5.3) potato-dextrose agar medium. Fungi isolated from tissues were transferred to additional plates for identification.

RESULTS

Symptoms on blighted bluegrass leaves and stems were as described previously (7,12). The dark green, water-soaked areas enlarged rapidly under conditions of high humidity, but when the humidity was reduced, the lesions became light tan and often had darker brown to reddish brown margins. Lesions were irregular in shape and size. Disease ratings were less than 4 (Table 1) even though the inocula used were from heavily blighted sods incubated under similar conditions of wet soil and high humidity. Leaf blight was significantly affected by phase 1 and 2 watering characteristics, by duration of phase 2 incubation, and by atmospheric humidity (Table 2). Leaf blight in pots inoculated with *Fusarium* spp. did not differ significantly from that in uninoculated pots.

Watering schedules that induced wilt symptoms had the greatest effect on disease severity; drought-stressed turf had significantly higher levels of disease than frequently watered, nonwilted turf (Tables 1 and 3). Leaf blight was more severe in plants subjected to drought stress followed by long-term flooding than in plants subjected to drought stress followed by a moist but nonflooded state. Disease severity was higher when grass was incubated at very high humidity than

Table 1. Fusarium leaf blight ratings on mature Kentucky bluegrass cultivar Fylking growing in various environmental conditions in the greenhouse

Phase 1 watering[a]	Phase 2 watering[b]	Atmospheric humidity[c]	Disease rating[d] for phase 2 incubation	
			4 Days	8 Days
Cyclic	Flooded	Increased	2.8	3.9
Cyclic	Nonflooded	Increased	2.3	3.1
Cyclic	Flooded	Unmodified	1.8	3.2
Cyclic	Nonflooded	Unmodified	2.1	2.4
Daily	Flooded	Increased	1.5	2.6
Daily	Nonflooded	Increased	1.1	1.6
Daily	Flooded	Unmodified	1.0	1.3
Daily	Nonflooded	Unmodified	1.0	1.3

[a] To produce drying and rewetting cycles, watering was done either daily or when drought-stressed grasses wilted.
[b] Pots were maintained in a moist state or "flooded" by submerging the turf in water to a level about 5 mm below the thatch surface.
[c] Humidity was unmodified in the greenhouse or increased by enclosing pots in a plastic bag.
[d] Disease rating based on a scale of 1–10, where 1 = no diseased tissue and 10 = totally necrotic tissue. Data are means of plants inoculated or not inoculated with a suspension of *Fusarium* spp. conidia.

Table 2. Statistical analysis of Fusarium leaf blight ratings and main treatment variables

Treatment variable	df	Type I SS[a]	*F* value	$P > F$
Phase 1 watering schedule[b]	1	78.8	102.6	0.0001
Relative humidity[c]	1	18.1	23.6	0.0001
Duration of phase 2 incubation[d]	1	24.8	32.3	0.0001
Phase 2 watering characteristic[e]	1	7.1	9.3	0.0027
Inoculation with *Fusarium* spp.	1	1.5	2.0	0.1634

[a] Sums of squares have been rounded off.
[b] To produce drying and rewetting cycles, watering was done either daily or when drough-stressed grasses wilted.
[c] Humidity was unmodified in the greenhouse or increased by enclosing pots in a plastic bag.
[d] Duration of phase 2 incubation was either 4 or 8 days.
[e] Pots were maintained in a moist state or "flooded" by submerging the turf in water to a level about 5 mm below the thatch surface.

when incubated in an unmodified greenhouse environment. Plants that had not been stressed by drought did not become blighted unless enclosed in high-humidity chambers. Even then, disease severity increased significantly in only one combination of treatments: flooded plants followed by high humidity for 8 days.

We reported elsewhere (21) that the gaseous compositions of soil air at a depth of 13 mm and the redox potentials of the solution phase in thatch of flooded and nonflooded treatments in this study differed significantly. Flooding for 4 days caused the soil atmosphere to have a reduced potential and led to accumulations of carbon dioxide (mean of 11%, maximum of 14%) and ethylene (mean of 8 ppm, maximum of 20 ppm) at the thatch/mineral soil interface. In nonflooded soil, the potential was considered poorly oxidized, and the mean concentrations of carbon dioxide and ethylene were 0.3% and 0.2 ppm, respectively. This "flooding" treatment was accomplished by raising the water table to the thatch zone without entirely submerging it. Similar conditions occur on low, poorly drained turfs.

The predominant genera of fungi isolated from tiller bases and crowns were species of *Fusarium, Nigrospora,* and *Trichoderma* (Table 4). The dominant *Fusarium* spp. included *F. equiseti, F. graminearum,* and *F. heterosporum.* Other fungi isolated were species of *Rhizoctonia, Myrothecium, Aspergillus, Penicillium,* and *Curvularia* plus nonsporulating fungi producing white or dark mycelium. The frequency of isolations of *Fusarium* from leaves and crowns was influenced by phase 1 watering schedules (Table 3) but not by phase 2 watering characteristics or by differences in atmospheric humidity. Isolations of *Fusarium* spp. were significantly greater from grass that had been subject to wilting than from grass that had not been stressed to the wilting condition. Isolation frequencies of *Nigrospora* sp. from crowns were significantly influenced by all three watering variables, whereas only the phase 2 watering affected isolations of *Nigrospora* sp. from leaves. The longer incubation (8 days) of phase 2 watering was most important for isolation of this fungus from leaves and crowns. *Nigrospora* sp. was isolated more frequently from grass in nonflooded than in flooded soil. Statistical analyses for *Trichoderma* sp. are not reported in Table 3 because the relationships were not significant for phase 1 ($P = 0.36$–0.85) or phase 2 ($P = 0.83$–1.0) waterings or for the atmospheric humidity ($P = 0.18$–0.85)

Table 3. Linear comparison differences and levels of significance for Fusarium leaf blight or fungal isolation frequencies among main effect water treatments

	Phase 1 watering[a]			Phase 2 watering[b]			Atmospheric humidity[c]		
Observations	**4 Days[d]**	**8 Days**	**Net**	**4 Days**	**8 Days**	**Net**	**4 Days**	**8 Days**	**Net**
Leaf blight rating[e]	1.08	1.48	1.28	−0.12	−0.65	−0.49	0.46	0.77	0.61
	(<0.01)[f]	(<0.01)	(<0.01)	(0.51)	(0.02)	(0.03)	(0.01)	(<0.01)	(<0.01)
Fusarium spp.[g]									
Crown	1.04	0.89	0.97	−0.04	0.19	0.07	−0.17	−0.35	−0.26
	(<0.01)	(0.01)	(<0.01)	(0.91)	(0.60)	(0.77)	(0.65)	(0.32)	(0.30)
Leaf	0.37	0.52	0.45	0.13	−0.15	−0.01	−0.08	0.06	−0.01
	(<0.01)	(0.02)	(<0.01)	(0.35)	(0.51)	(0.94)	(0.54)	(0.78)	(0.94)
Nigrospora spp.[g]									
Crown	−0.94	−0.72	−0.83	0.48	2.32	1.49	−1.06	−1.10	−1.09
	(0.09)	(0.23)	(0.04)	(0.39)	(<0.01)	(<0.01)	(0.05)	(0.07)	(0.01)
Leaf	0.54	−0.19	−0.36	0.12	0.94	0.54	−0.42	−0.06	−0.24
	(0.10)	(0.61)	(0.13)	(0.70)	(0.01)	(0.03)	(0.20)	(0.86)	(0.33)

[a] To produce drying and rewetting cycles, watering was done either daily or when drought-stressed grasses wilted.
[b] Pots were maintained in a moist state or "flooded" by submerging the turf in water to a level about 5 mm below the thatch surface.
[c] Humidity was unmodified in the greenhouse or increased by enclosing pots in a plastic bag.
[d] Duration of phase 2 incubation (4 and 8 days) or the overall (net) treatment effect.
[e] Disease rating based on a scale of 1–10, where 1 = no diseased tissue and 10 = totally necrotic tissue.
[f] Numbers in parentheses indicate levels of significance.
[g] Fungal isolation frequencies.

Table 4. Genera of fungi isolated from blighted, uninoculated Kentucky bluegrass cultivar Fylking growing in various environmental conditions in the greenhouse

Phase 1 watering[a]	Phase 2 watering[b]	Atmospheric humidity[c]	Phase 2 incubation (days)	Average number of fungal isolations[d]					
				C-Fus	**L-Fus**	**C-Tri**	**L-Tri**	**C-Nig**	**L-Nig**
Cyclic	Nonflooded	Increased	8	2.9	1.6	2.0	0.3	7.1	2.5
Cyclic	Nonflooded	Increased	4	2.7	0.5	3.6	0.3	5.1	2.6
Cyclic	Nonflooded	Unmodified	8	2.3	0.5	2.6	0.0	7.3	2.7
Cyclic	Nonflooded	Unmodified	4	2.8	0.8	2.4	0.3	6.5	2.6
Cyclic	Flooded	Unmodified	8	2.7	1.0	1.1	0.3	6.2	2.3
Cyclic	Flooded	Unmodified	4	2.4	0.5	2.8	0.0	5.6	2.8
Cyclic	Flooded	Increased	8	1.3	0.7	1.7	0.1	4.0	2.3
Cyclic	Flooded	Increased	4	2.4	0.3	4.1	0.3	4.0	1.8
Daily	Nonflooded	Increased	8	1.6	0.2	1.4	0.3	8.5	3.3
Daily	Nonflooded	Increased	4	1.3	0.1	3.6	0.1	6.3	3.4
Daily	Nonflooded	Unmodified	8	0.9	0.2	2.3	0.1	7.8	3.5
Daily	Nonflooded	Unmodified	4	1.4	0.2	3.7	0.0	6.1	2.5
Daily	Flooded	Unmodified	8	2.2	0.9	2.6	0.3	7.0	1.8
Daily	Flooded	Unmodified	4	1.9	0.0	2.9	0.2	7.0	3.8
Daily	Flooded	Increased	8	0.9	0.4	2.6	0.1	4.2	1.8
Daily	Flooded	Increased	4	1.6	0.3	3.6	0.3	5.5	2.3

[a] To produce drying and rewetting cycles, watering was done either daily or when drought-stressed grasses wilted.
[b] Pots were maintained in a moist state or "flooded" by submerging the turf in water to a level about 5 mm below the thatch surface.
[c] Humidity was unmodified in the greenhouse or increased by enclosing pots in a plastic bag.
[d] Average of 10 attempted isolations from crowns (C) and tiller bases (L); Fus = *Fusarium* spp., Tri = *Trichoderma* spp., and Nig = *Nigrospora* spp.

variable.

Regression analyses were performed to determine possible relationships between disease severity and the major groups of fungi isolated from turfgrass. *Fusarium* spp. isolated from crowns after 4 days of phase 2 incubation were positively related ($P = 0.0128$) to foliar blighting, whereas no significant relationships were found for *Nigrospora* spp. ($P = 0.6066$) and *Trichoderma* spp. ($P = 0.7860$). After 8 days, the relationships among these fungi in the crowns became more significant, with positive correlations at $P = 0.0115$ for *Fusarium* spp. and negative correlations at $P = 0.0001$ for *Nigrospora* spp. and 0.0068 for *Trichoderma* spp. Relationships among the disease and fungi isolated from tiller bases after 4 days differed somewhat in that there was a negative correlation with *Nigrospora* spp. ($P = 0.0116$) and no significant relationships with *Fusarium* spp. ($P = 0.5800$) and *Trichoderma* spp. ($P = 0.1322$). At 8 days, the relationships on tiller bases were insignificant ($P = 0.5604$–0.9070) for each of these fungi. Thus *Fusarium* spp. were principal colonists of bluegrass crowns and *Nigrospora* spp. were the principal colonists of tiller bases.

DISCUSSION

Drought stress was the most significant factor causing foliar blight of Kentucky bluegrass in this study. Disease severity increased when drought-stressed turf was subsequently flooded or incubated at high atmospheric humidity, and disease severity was highest in plants that were flooded as well as incubated under high humidity after being drought-stressed.

Combined flooding and high-humidity treatments also increased foliar blighting of plants that had not been wilted, but their effect was much less than that from drought stress. We previously indicated that the flooding treatment used in this study caused severe imbalances in the chemical environments of roots (21). Soil anaerobiosis and associated stresses in this greenhouse experiment were considerably higher than those measured in the field during similar studies. Therefore, we conclude that there is little likelihood that typical watering practices and rainfalls under field conditions would be directly responsible for predisposition of Kentucky bluegrasses to infection by *Fusarium* spp. The low frequency of foliar blights caused by *Fusarium* spp. in irrigated turfs, even in the humid temperate climatic zone, appears to support this conclusion.

Drought impairs various physiological processes in plants (8), including disease resistance (1). As stress levels increase, plants become increasingly susceptible to colonization by facultative parasites (15). The primary rooting medium in muture turfs (i.e., thatch) frequently becomes quite dry between irrigations or rainfalls, especially in semiarid zones. Therefore, the stresses caused by alternate drying and wetting should favor infection of root and coronal tissues by facultative parasites. *Fusarium* spp. are often the dominant facultatively parasitic fungi in turfgrass during the warm summer months (2,7,14) and are also well adapted for growth at highly negative water potentials (6). It is therefore likely that *Fusarium* spp. will be encountered in crowns of turfgrasses subjected to drought stress during the summer. They are also principal colonists of crowns and foliage of plants with vascular system dysfunction caused by soilborne pathogenic species of *Gaeumannomyces, Leptosphaeria,* and *Phialophora* (18,20). The failure to increase the level of disease by adding *Fusarium* spp. to sod in this study agrees with results of previous field studies (5,16). This is not surprising since large native populations of *Fusarium* spp. occur in the litter layer of turfgrasses (19).

Our finding that Fusarium crown rot and foliar blight were highest under drought stress or prolonged flooding and incubation at high humidity agree with similar studies conducted in the greenhouse (9) and mist chamber (7). The capacity of *Fusarium* spp. to incite crown rot and foliar blight was less in a natural soil-thatch system than in turfs where the native microflora were excluded (7,9,12). This may have been from the presence of fungi such as species of *Trichoderma* and *Nigrospora*, which may have restricted the extent of tissue colonization by *Fusarium* spp. The latter observation conflicts with that of Keohane (14), who found *F. culmorum* to be a more effective pathogen when mixed into a sterile soil system with *Trichoderma* spp.

Routine watering of bluegrasses during summer is therefore likely to minimize the severity of Fusarium crown rots. It also would appear that after a period of drought that heavy watering, especially at night or during periods of high atmospheric humidity, could increase the severity of this disease.

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