Pathogenicity of Some Ectotrophic Fungi with *Phialophora* Anamorphs that Infect the Roots of Turfgrasses

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ABSTRACT

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Magnaporthe poae, *Gaeumannomyces incrustans*, *G. cylindrosporus*, and *Phialophora graminicola* (anamorph of *G. cylindrosporus*) were identified on turfgrasses in Rhode Island and New York. *M. poae* was isolated from *Poa annua* and *Poa pratensis*, and *G. incrustans* was isolated from *P. pratensis* exhibiting symptoms of summer patch disease. *G. cylindrosporus* or *P. graminicola* were obtained from *P. pratensis* and *Lolium perenne* exhibiting only a decline in quality. At 28 C, *M. poae* was highly pathogenic to 8-wk-old turf of *P. pratensis* and *P. annua* and to 7-yr-old turf of *P. pratensis*. *G. incrustans* was mildly pathogenic to *P. pratensis* and *P. annua*, whereas *G. cylindrosporus* or *P. graminicola* were parasitic but not pathogenic to the same hosts. *M. poae* also was pathogenic to *Agrostis palustris*, *Festuca rubra* var. *commutata*, *Festuca arundinacea*, *L. perenne*, *Hordeum vulgare*, *Triticum aestivum*, *Avena sativa*, and *Secale cereale*. When inoculated into mature (22-mo-old) turf of *P. pratensis*, *M. poae* produced symptoms of summer patch. *M. poae* was reisolated from the roots and crowns of diseased plants, confirming a causal relationship between *M. poae* and summer patch disease.

Additional keywords: annual bluegrass, Kentucky bluegrass, root pathogens, turf disease.

Patch diseases caused by ectotrophic root- and crown-infecting fungi are among the most destructive turfgrass diseases in the United States. Couch and Bedford (2) coined the name Fusarium blight for a patch disease of Kentucky bluegrass (*Poa pratensis* L.) that occurred in the late 1950s and early 1960s. The disease has remained a major management problem of Kentucky bluegrass turf. After failing to confirm *Fusarium* spp. as primary pathogens of turf exhibiting typical Fusarium blight symptoms, Smiley (14) designated the disease in New York State as distinct and named it Fusarium blight syndrome, with an undetermined causal agent. In 1984, Smiley and Craven Fowler (16) reported that two fungi with ectotrophic growth habits similar to *Gaeumannomyces graminis* (Sacc.) Arx & Olivier were associated with the Fusarium blight syndrome of Kentucky bluegrass turf in New York. The two fungi, *Leptosphaeria korrae* Walker & Smith and *Phialophora graminicola* (Deacon) Walker, induced disease symptoms indistinguishable from Fusarium blight syndrome. Smiley (15) proposed the name summer patch for the disease caused by *P. graminicola* and endorsed necrotic ring spot for the disease caused by *L. korrae* (23). A crown rot, root rot, and leaf blight disease caused by various *Fusarium* spp. also occurs on Kentucky bluegrass; however, this disease is distinct from Fusarium blight syndrome (14).

Although Smiley and Craven Fowler (16) reported that *P. graminicola* was the primary incitant of summer patch, others had concluded that *P. graminicola* was a parasitic but nonpathogenic, root-inhabiting fungus of grasses and cereals, with potential as a biocontrol agent against take-all disease of wheat (*Triticum aestivum* L.) and take-all patch of bentgrasses (*Agrostis* spp.) (1,3,4,13,21,22). Smiley et al (17) suggested that this apparent contradiction could be explained by the different temperatures used by the various investigators. Whereas European and Australian biocontrol investigations with *P. graminicola* were conducted at 22 C or below, Smiley et al (17) concluded that expression of summer patch symptoms required temperatures >25 C and also suggested that isolates of *P. graminicola* in the United States might be more virulent than those from Europe or Australia.

More recently, Landschoot (9) found that several isolates designated by R. W. Smiley (*personal communication*) as *P. graminicola* (ATCC 64413, ATCC 56773, ATCC 60239, and 258 and 197) and implicated as summer patch incitants actually were *Magnaporthe poae* Landschoot & Jackson. This finding prompted reexamination of the etiology of summer patch disease. The objectives of our present study were to isolate and identify those anamorphs of *Phialophora* present on roots and crowns of *Poa* spp. exhibiting summer patch symptoms, determine the relative pathogenicity of the individual species, and confirm the identity of the causal agent.

MATERIALS AND METHODS

Isolation and identification of fungi. Turf of *Poa* spp. exhibiting symptoms of summer patch disease in Rhode Island was surveyed for the presence of fungi with ectotrophic runner hyphae and anamorphs of *Phialophora* on the roots and crowns of affected plants. In addition, plant pathologists from other locations in the United States were solicited for isolates of similar fungi. Some isolates were obtained from swards of *Poa* spp. and perennial ryegrass (*Lolium perenne* L.) exhibiting an overall decline in quality but not necessarily showing summer patch symptoms.

Fungi from turfgrass plants were isolated from colonized 3- to 5-mm segments of root or crown tissue. Segments were surface disinfested by immersion in 1.0% $AgNO_3$ for 30–60 sec, followed by a brief transfer to 5.0% NaCl to precipitate the $AgNO_3$. They then were rinsed in sterile distilled water, blotted on a clean paper towel, and placed on a medium selective for *Gaeumannomyces* (SM-GGT3) (8). Developing colonies were transferred to potato-dextrose agar (PDA).

Single-ascospore cultures were derived by immersing mature perithecia in 10% sodium hypochlorite for 20–30 sec, followed by a rinse in sterile distilled water. Perithecia then were pipetted with 2–3 ml of sterile distilled water onto PDA fortified with 50 μg/ml of streptomycin sulfate. The perithecia were crushed and their contents dispersed with a sterile glass rod. Germinating ascospores were transferred to fresh PDA with a fine-tipped glass rod.

All cultures were maintained on 15 ml of PDA in 100-ml screw-capped bottles at 5–10 C, with transfers to fresh PDA every 3–4 mo. Cultures used for temperature and pathogenicity studies were cycled through wheat roots once each year (19).

Identifications of all fungi except *P. graminicola* were based on teleomorph characteristics. Teleomorphs of *M. poae* and *G. incrustans* Landschoot & Jackson were produced in culture by placing 6-mm-diameter plugs from colonies of two mating types on opposite sides of propylene oxide-sterilized wheat stems embedded in Sach's agar in petri dishes (12). All dishes were sealed with Parafilm and incubated at room temperature near a west-facing window. Perithecia of *G. cylindrosporus* Hornby, Slope, Gutteridge, & Sivanesan were found on partially decomposed root and stem tissue in the thatch of fairway turf. Attempts to produce perithecia from single-ascospore isolates under axenic conditions failed. The identity of *P. graminicola* was based on anamorphic characteristics (that is, growth rates, colony morphology, hyphopodia, phialospores).

Effect of temperature on radial growth. Isolates of *M. poae*, *G. incrustans*, *P. graminicola*, and *G. cylindrosporus* were transferred from medicine bottles to 90-mm-diameter plastic petri dishes containing half-strength PDA. Cultures were incubated in darkness for 8 days at 25 C. Agar plugs (5 mm diameter) were cut from the actively growing margins of each colony and transferred to the center of 90-mm-diameter plastic petri dishes containing approximately 20 ml of half-strength PDA. Four replicate plates were used for each isolate, and the plates were incubated at 5, 10, 15, 20, 25, 30, 35, and 40 C. Growth rates were determined by measuring radial growth every 24 hr for 8 days, or until the colony margin reached the edge of the plate. Two measurements of colony radius were taken at right angles to each other on each plate, and the values were averaged. Growth measurements used for temperature comparisons were from the 24-hr period showing maximum growth for each isolate. The experiment was a completely randomized design, and the test was repeated once. The data from each test were analyzed separately. Because the results were similar for both tests, only the results from the first test are presented.

Pathogenicity studies. Relative pathogenicity of *M. poae*, *G. incrustans*, *P. graminicola*, and *G. cylindrosporus* was evaluated on 8-wk-old Kentucky bluegrass cultivar Merion and annual bluegrass (*Poa annua* L.) and 7-yr-old Kentucky bluegrass cultivar Merion. Annual bluegrass seed was obtained from R. W. Smiley, superintendent of the Columbia Basin Agricultural Research Center, Pendleton, OR. The fungal species and isolate designations are listed in Table 1. The eight isolates of the three fungus species used in the pathogenicity tests were randomly selected from a larger group of approximately 40 isolates that showed stability of growth in culture and the ability to successfully colonize wheat roots in culture (19). Inoculum was produced by transferring mycelium from the margins of actively growing colonies to 125-ml Erlenmyer flasks containing 1.5 g of autoclaved perennial ryegrass grains and 5 ml of sterile distilled water. Flasks were incubated in the dark at 25 C for 4 wk to generate the inoculum.

Clay pots (10-cm diameter) were filled with washed Terra-Green calcined clay (Oil-Dry Corporation of America, Chicago, IL) (N, 7 mg/kg; P, 20 mg/kg; K, 842 mg/kg; pH 5.4), planted with 0.12 g of nondisinfested seed of Kentucky bluegrass and annual bluegrass, and placed in a mist house until seeds had germinated (approximately 2 wk). The pots of seedling grasses were transferred to a greenhouse for 8 wk. A 7.6-cm-diameter cup cutter was used to remove plugs of 7-yr-old Kentucky bluegrass from plots at the University of Rhode Island Turfgrass Research Farm in the spring of 1987. All plugs were cut to a depth of 3.0 cm, planted in 10-cm-diameter pots containing calcined clay, and placed in the greenhouse for 10 days before inoculation. Both the seedling grasses and the 7-yr-old Kentucky bluegrass were maintained at a height of 3.0 cm. All pots were watered daily with approximately 150 ml of water and fertilized biweekly with a 20-10-20 fertilizer at 0.5 g/150 ml of H_2O per pot. Pots of 8-wk-old grasses were inoculated with 1.0 g of air-dried inoculum at the surface in strips on two diameters at right angles. Seven-year-old Kentucky bluegrass plugs were inoculated by cutting two narrow 1.0-cm-deep slits on two diameters at right angles and placing 1.0 g of air-dried inoculum at the bottom of the slits. Four replicates were inoculated with each isolate. Controls were seeded with 1.0 g of uncolonized, autoclaved, air-dried perennial ryegrass grains.

After inoculation, pots of Kentucky bluegrass were placed immediately in a growth chamber at 28 ± 2 C, with a 12-hr photoperiod (620 μE $m^{-2}s^{-1}$) and 60–100% relative humidity. Temperature and relative humidity were recorded with a hygrothermograph. Plants were watered daily, and the medium was kept moist by placing water-absorbent pads beneath the pots.

The experimental design was a completely randomized design. The experiment was terminated after 6 wk. The test was repeated once, and each test was analyzed separately. Although actual values differed, the trends were similar; therefore, only the results from the first experiment are presented.

Criteria for evaluating pathogenicity included weekly quality ratings, clipping yields (shoot dry weights), and estimates of root colonization and rot. Quality ratings were based on a scale of 1–9, with 1 = dead plants (straw-yellow color and no apparent growth) and 9 = dense, dark green, actively growing turf. Leaf growth above 3.0-cm height was harvested once each week, air dried at 60 C for 48 hr, and weighed. Root colonization and rot were assessed visually by removing five tillers from each pot, boiling the roots and crowns in 3.0% KOH for 30–60 sec, and examining under a compound microscope at ×10. Values were assigned on a scale of 1–5, with 1 = no colonization or rot, 2 = colonization and slight rot, 3 = colonization and moderate rot, 4 = colonization and moderately severe rot, 5 = colonization and severe rot. All data were subjected to analysis of variance, and means were compared with Duncan's multiple range test ($P = 0.05$). Of the three criteria used to evaluate pathogenicity, quality ratings were the least important because the disease was not directly evaluated. However, because quality is important in the assessment of turf performance, it becomes valuable when combined with other data used to determine the extent of disease injury. Also, weekly quality ratings provide a nondestructive

TABLE 1. Influence of root-infecting fungi with anamorphs of *Phialophora* on turf quality of 8-wk-old Kentucky bluegrass cultivar Merion incubated for 6 wk at 28 C

Fungus	Isolate	Turf quality at indicated week[y]					
		1	2	3	4	5	6
Magnaporthe poae	ATCC 64413	9.0 a[z]	6.5 b	3.5 b	1.8 c	1.0 d	1.0 d
M. poae	ATCC 64412	9.0 a	6.8 b	3.3 b	2.3 c	1.5 d	1.0 d
M. poae	ATCC 60259	9.0 a	6.3 b	4.0 b	1.3 c	1.0 d	1.0 d
M. poae	ATCC 64131	9.0 a	6.5 b	3.5 b	1.8 c	1.0 d	1.0 d
Gaeumannomyces incrustans	ATCC 64418	9.0 a	7.8 a	7.5 a	5.3 b	4.3 c	2.8 c
Phialophora graminicola	ATCC 64414	9.0 a	8.3 a	8.3 a	7.8 ab	6.8 b	4.5 b
P. graminicola	ATCC 64415	9.0 a	8.0 a	8.5 a	8.0 a	7.0 ab	4.5 b
G. cylindrosporus	ATCC 64420	9.0 a	8.0 a	8.5 a	8.0 a	7.0 ab	4.8 b
Control	...	9.0 a	8.0 a	8.3 a	7.8 ab	7.5 a	5.3 a

[y] Based on a scale of 1–9, with 1 = dead plants (straw-yellow color) and 9 = dense, dark green, actively growing turf.
[z] Means within a column and followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. Values represent means of quality of four replicate pots per treatment from one trial.

means of determining differences between inoculated and uninoculated turf over time. Yield is a measure of the effect of the disease on plant growth. This is an important criterion when combined with quality and root rot data. The most valuable criterion for assessing disease was root rot ratings. Rating for the degree of root rot provided a direct measure of disease severity.

One isolate of *M. poae* (ATCC 64413) was used to inoculate several species of turfgrasses and cereals. Isolate ATCC 64413 was cycled on Kentucky bluegrass roots 1 mo before initiation of this study, and inoculum was prepared as described previously. Paired comparisons (*t* test) of parameters used to assess pathogenicity were made between the inoculated and control plants for each species (18).

Clay pots (10 cm diameter) were filled with calcined clay and seeded with 0.07 g of creeping bentgrass (*Agrostis palustris* Huds. 'Penncross'), 0.20 g of Chewing's fescue (*Festuca rubra* var. *commutata* Gaud. 'Jamestown'), 0.20 g of tall fescue (*Festuca arundinacea* Schreb. 'Rebel'), and 0.20 g of perennial ryegrass (cultivar Yorktown II). Seeds were not disinfested before planting. Pots were placed on a mist bench until seeds had germinated and then transferred to a greenhouse. Turf was maintained at a height of 3.0 cm and watered and fertilized as described previously. After 8 wk, all pots of turfgrasses were inoculated and placed in growth chambers under the same conditions as described previously. Four replicates of inoculated and control plants were included for each species in a completely randomized design. The criteria for evaluating pathogenicity and statistical analyses were described previously. The experiment was repeated once, and the results from each experiment were analyzed separately. The pathogenicity of *M. poae* on each species was similiar in each experiment; hence, only the results of the first experiment are reported.

Seeds of barley (*Hordeum vulgare* L. 'Arivat'), wheat (*T. aestivum* 'Redcoat'), oats (*Avena sativa* L., cultivar unknown), and rye (*Secale cereale* L., cultivar unknown) were surface disinfested with 10% sodium hypochlorite for 1–2 min, placed on moistened paper towels in glass dishes, and left to germinate (24–48 hr). Five pregerminated seeds were placed over 1.0 g of inoculum in 10-cm-diameter pots filled with calcined clay and covered with an additional 2 cm of this medium. Controls were planted over 1.0 g of sterilized, air-dried perennial ryegrass grains. After inoculation, all pots of cereals were placed in growth chambers under the same conditions as described previously. Four replicate pots of inoculated and control plants were included for each species in a completely randomized design. All plants were harvested after 6 wk and evaluated for the degree of pathogenicity. Criteria for evaluating pathogenicity included measuring the longest leaf on each plant, shoot dry weights, root dry weights, and degree of root colonization and rot as described previously. This experiment was repeated once, and similar results were obtained. Only the results from the first test are presented.

Mature (22-mo-old), field-grown Kentucky bluegrass sod (50% cultivar Baron, 20% cultivar Ram I, 15% cultivar Majestic, and 15% cultivar Touchdown) was transplanted to a ground bed (9.1 m long × 0.4 m wide × 0.18 m deep) filled with a compost-sand (50-50) mix in a greenhouse. The mix was steam sterilized and left fallow for 1 yr before the sod was established. Sod was established on 23 May 1985 and inoculated on 10 June 1985 with three isolates of *M. poae* (ATCC 64413, ATCC 56773, and MI-1); three isolates of *G. incrustans* (ATCC 64418, CREST, and ATBM); one isolate of *G. graminis* var. *avenae* (LLGga); one isolate of *G. cylindrosporus* (SAK); and one isolate of *P. graminicola* (REY). The control consisted of uncolonized, autoclaved ryegrass grains. Inoculum was prepared as described previously and consisted of 1.0 g placed at the soil-thatch interface (1.0–1.5 cm below the sod surface). Inocula for individual treatments were placed in the center of 43-cm^2 plots. The experimental design was a randomized complete block with three replicates. Sod was maintained at 4.5-cm height, watered approximately three times per week (more frequently in summer months), and fertilized biweekly with a 20-10-20 fertilizer at 0.66 g/L of H_2O. Where symptoms occurred, efforts were made to reisolate the causal fungus. Isolation of fungi from the roots of grass plants in the control plots also was done to determine the species present. Attempts to reproduce this experiment were unsuccessful, presumably due to the inability to obtain high temperatures for extended periods of time, conditions required for summer patch development.

RESULTS AND DISCUSSION

Three species of root-infecting fungi with ectotrophic runner hyphae and anamorphs of *Phialophora* were isolated from the roots and crowns of turfgrasses in New York and Rhode Island. Two isolates of *M. Poae* (ATCC 64413 and ATCC 60259) were isolated by R. W. Smiley from Kentucky bluegrass in New York. *M. poae* (ATCC 64412) was a single-ascospore isolate from a perithecium produced from a cross between ATCC 64413 and an undesignated isolate of *M. poae*. *M. poae* (ATCC 64131) was obtained from annual bluegrass in Rhode Island. *G. incrustans* (ATCC 64418), *P. graminicola* (ATCC 64414), and *G. cylindrosporus* (ATCC 64420) were isolated from Kentucky bluegrass in Rhode Island. *P. graminicola* (ATCC 64415) was isolated from perennial ryegrass in Rhode Island. All isolates of *M. poae* and *G. incrustans* were collected from *Poa* spp. affected with summer patch. *G. cylindrosporus* or *P. graminicola* were isolated from *Poa* spp. or perennial ryegrass, sometimes exhibiting a decline in quality but no summer patch symptoms.

All isolates of *M. poae* and *G. incrustans* produced mature perithecia when crossed with isolates of opposing mating types. Teleomorph characteristics conformed to those described by Landschoot and Jackson (10,11). Isolates of *P. graminicola* conformed to Deacon's (5) description of *P. graminicola* var. *graminicola* and Walker's (20) description of *P. graminicola*. Identity of two isolates of *P. graminicola* (ATCC 64414 and ATCC 64415) was confirmed by James Deacon, University of Edinburgh, U.K. Teleomorph characteristics of *G. cylindrosporus* conformed to those described by Hornby et al (7). Identity of *G. cylindrosporus* was confirmed by John Walker, Biological and Chemical Research Institute, Rydalmere, Australia.

Effect of temperature on radial growth. Growth rates varied among the fungus species included in this test. Radial growth rates for *M. poae* (ATCC 64413), *G. incrustans* (ATCC 64418), *P. graminicola* (ATCC 64415), and *G. cylindrosporous* (ATCC 64420) are compared in Figure 1. *M. poae* and *G. incrustans* grew much faster than *P. graminicola* and *G. cylindrosporus* at 25 and 30 C. Optimal growth for *M. poae* occurred at 30 C, whereas no growth occurred at 10 or 40 C. Optimal growth of *G. incrustans* occurred at 25 C, but no growth occurred at 10 or 40 C. Optimal growth of *P. graminicola* and *G. cylindrosporus*

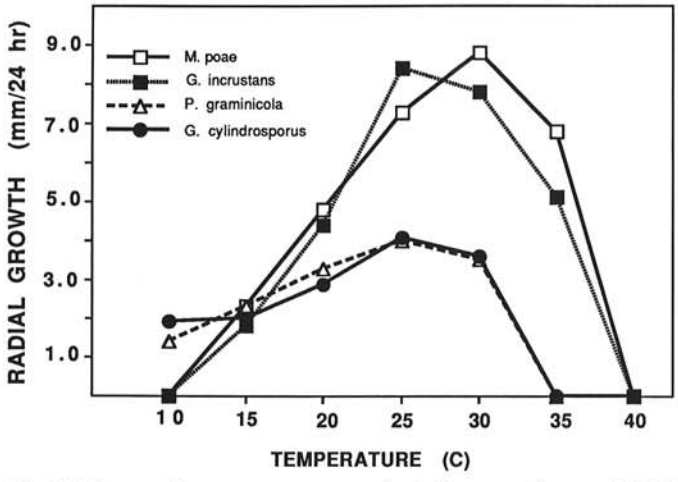

Fig. 1. Influence of temperature on growth of *Magnaporthe poae* (ATCC 64413), *Gaeumannomyces incrustans* (ATCC 64418), *Philaphora graminicola* (ATCC 64415), and *G. cylindrosporus* (ATCC 64420) on half-strength potato-dextrose agar.

occurred at 25 C. In contrast to *M. poae* and *G. incrustans*, *P. graminicola* and *G. cylindrosporus* grew at 10 C but not at 35 C.

The growth rate of *P. graminicola* (ATCC 64415) was only slightly faster than those reported for isolates of *P. graminicola* from the United Kingdom and Australia (21). Enhanced growth rates at higher temperatures reported by Smiley et al (17) may be discounted because it now is apparent that the American isolates used by these authors were misidentified and actually were *M. poae* (9).

Pathogenicity studies. Quality ratings of 8-wk-old Kentucky bluegrass, of annual bluegrass, and of 7-yr-old Kentucky bluegrass turfs inoculated with *M. poae*, *G. incrustans*, *G. cylindrosporus*, or *P. graminicola* are shown in Tables 1–3. Clipping yields and estimates of root colonization and rot are provided in Table 4.

M. poae was the most pathogenic species in 8-wk-old Kentucky bluegrass and annual bluegrass and 7-yr-old Kentucky bluegrass. A significant decline in quality of 8-wk-old Kentucky bluegrass and annual bluegrass was apparent after 2–3 wk at 28 C with all isolates of *M. poae* when compared with controls. A similar trend was found on 7-yr-old Kentucky bluegrass; however, significantly lower quality ratings did not occur until the third or fourth week of the test. Clipping yields (shoot dry weights) of the test grasses inoculated with *M. poae* were significantly lower

TABLE 2. Influence of root-infecting fungi with anamorphs of *Phialophora* on turf quality of 7-yr-old Kentucky bluegrass cultivar Merion incubated for 6 wk at 28 C

		Turf quality at indicated week[y]					
Fungus	Isolate	1	2	3	4	5	6
Magnaporthe poae	ATCC 64413	9.0 a[z]	7.8 a	6.3 cd	4.0 cd	1.8 d	1.0 c
M. poae	ATCC 64412	9.0 a	8.0 a	6.5 cd	5.0 c	3.3 bc	1.8 c
M. poae	ATCC 60259	9.0 a	7.8 a	5.0 f	3.8 d	1.5 d	1.0 c
M. poae	ATCC 64131	9.0 a	7.5 a	5.5 ef	4.0 cd	2.0 cd	1.0 c
Gaeumannomyces incrustans	ATCC 64418	9.0 a	8.0 a	7.5 ab	5.5 ab	3.3 bc	2.0 c
Phialophora graminicola	ATCC 64414	9.0 a	8.3 a	7.8 a	6.5 a	6.3 a	5.5 a
P. graminicola	ATCC 64415	9.0 a	7.5 a	6.5 cd	5.5 ab	4.5 b	3.8 b
G. cylindrosporus	ATCC 64420	9.0 a	7.8 a	7.0 bc	5.5 ab	6.0 a	4.3 b
Control	···	9.0 a	8.0 a	8.0 a	6.5 a	4.3 b	3.5 b

[y]Based on a scale of 1–9, with 1 = dead plants (straw-yellow color) and 9 = dense, dark green, actively growing turf.
[z]Means within a column and followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. Values represent means of quality of four replicate pots per treatment from one trial.

TABLE 3. Influence of root-infecting fungi with anamorphs of *Phialophora* on turf quality of 8-wk-old annual bluegrass incubated for 6 wk at 28 C

		Turf quality at indicated week[y]					
Fungus	Isolate	1	2	3	4	5	6
Magnaporthe poae	ATCC 64413	9.0 a[z]	7.8 ab	5.8 b	1.5 b	1.0 c	1.0 c
M. poae	ATCC 64412	9.0 a	6.5 c	4.3 c	1.0 b	1.0 c	1.0 c
M. poae	ATCC 60259	9.0 a	8.0 a	4.8 c	1.5 b	1.0 c	1.0 c
M. poae	ATCC 64131	9.0 a	7.3 b	4.5 c	1.5 b	1.0 c	1.0 c
Gaeumannomyces incrustans	ATCC 64418	9.0 a	7.8 ab	7.3 a	4.8 a	2.0 b	1.3 bc
Phialophora graminicola	ATCC 64414	9.0 a	8.0 a	6.8 a	5.5 a	3.0 a	2.0 b
P. graminicola	ATCC 64415	9.0 a	8.0 a	7.8 a	5.8 a	3.8 a	3.0 a
G. cylindrosporus	ATCC 64420	9.0 a	8.0 a	7.3 a	5.5 a	3.5 a	1.8 bc
Control	···	9.0 a	8.0 a	7.8 a	5.5 a	3.3 a	1.8 bc

[y]Based on a scale of 1–9, with 1 = dead plants (straw-yellow color) and 9 = dense, dark green, actively growing turf.
[z]Means within a column and followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. Values represent means of quality of four replicate pots per treatment from one trial.

TABLE 4. Influence of root-infecting fungi with anamorphs of *Phialophora* on dry weight of leaf clippings and root colonization and rot of *Poa* spp. after 6 wk of incubation at 28 C

		8-wk-old Kentucky bluegrass cultivar Merion		7-yr-old Kentucky bluegrass cultivar Merion		8-wk-old Annual bluegrass	
Species	Isolate	Dry weight[x] of leaf clippings	Root rot[y]	Dry weight of leaf clippings	Root rot	Dry weight of leaf clippings	Root rot
Magnaporthe poae	ATCC 64413	0.80 c[z]	4.8 a	1.5 cd	4.8 a	1.09 bc	4.8 a
M. poae	ATCC 64412	0.86 c	4.4 b	1.6 bcd	4.6 ab	0.90 bc	4.6 b
M. poae	ATCC 60239	0.81 c	4.5 ab	1.3 d	4.7 ab	1.04 bc	4.9 a
M. poae	ATCC 64131	0.94 c	4.7 ab	1.3 cd	4.6 ab	0.79 c	4.7 ab
Gaeumannomyces incrustans	ATCC 64418	1.22 b	3.1 c	1.7 abc	3.2 c	1.18 b	3.4 c
Phialophora graminicola	ATCC 64414	1.99 a	2.1 d	2.0 ab	3.1 c	1.50 a	2.2 d
P. graminicola	ATCC 64415	1.93 a	2.3 d	1.9 ab	2.9 cd	1.78 a	2.1 d
G. cylindrosporus	ATCC 64420	2.08 a	2.1 d	2.0 ab	2.5 d	1.60 a	2.1 d
Control	···	1.93 a	1.0 e	2.1 a	2.8 cd	1.57 a	1.0 e

[x]Mean dry weight of leaf clippings in grams per 10-cm-diameter pot.
[y]Based on a visual rating scale of 1 to 5, with 1 = no colonization, 2 = colonization and slight root rot, 3 = colonization and moderate root rot, 4 = colonization and moderately severe root rot, 5 = colonization and severe root rot.
[z]Means within a column and followed by the same letter are not significantly different ($P = 0.5$) according to Duncan's multiple range test. Values are means of leaf clipping dry weight and root rot estimates from four replicate pots per treatment form one trial.

TABLE 5. Influence of *Magnaporthe poae* (ATCC 64413) on turf quality of four turfgrasses incubated for 6 wk at 28 C

Species	Treatment comparison	Turf quality at indicated week[y]					
		1	2	3	4	5	6
Creeping bentgrass (cultivar Penncross)	Control	9.0	9.0	7.5	6.0	4.8	4.8
	ATCC 64413	9.0 ns[z]	8.8 ns	7.3 ns	4.5*	2.3**	1.5**
Chewings' fescue (cultivar Jamestown)	Control	9.0	9.0	7.5	6.5	5.3	5.0
	ATCC 64413	9.0 ns	8.8 ns	6.0*	4.0***	2.8***	1.3***
Tall fescue (cultivar Rebel)	Control	9.0	9.0	8.0	7.8	6.8	6.0
	ATCC 64413	9.0 ns	9.0 ns	7.5 ns	6.8 ns	5.0*	4.3***
Perennial ryegrass (cultivar Yorktown II)	Control	9.0	8.8	8.5	7.3	6.0	5.0
	ATCC 64413	9.0 ns	9.0 ns	8.5 ns	7.3 ns	5.8 ns	4.0 ns

[y] Based on a scale of 1–9, with 1 = dead plants (straw-yellow color) and 9 = dense, dark green, actively growing turf.
[z] Significance levels are $P < 0.001$ (***), $P < 0.01$ (**), $P < 0.05$ (*), or $P > 0.05$ (ns) for treatment comparison within a turf species. Values are means of quality of four replicate pots per treatment from one trial.

than yields from uninoculated controls. Root rot was extensive on 8-wk-old bluegrass plants inoculated with *M. poae*, whereas control plants showed little evidence of root rot. Significant, but smaller, differences in root rot between plants inoculated with *M. poae* and controls were evident in the 7-yr-old turf. However, control plants of the mature turf showed more root rot than control plants of the 8-wk-old turf. This may have been due to a small amount of root infection by various root-inhabiting organisms that were present on the field-grown turf used in this study.

G. incrustans (ATCC 64418) was mildly pathogenic on both 8- and 7-yr-old turf. Significant differences in quality occurred between inoculated and uninoculated Kentucky bluegrass and annual bluegrass turfs after 4–5 wk of incubation at 28 C. Quality ratings of the plants usually were higher than those inoculated with *M. poae* although significant differences were not always apparent. Shoot dry matter yields of plants inoculated with *G. incrustans* were intermediate between plants inoculated with *M. poae* and uninoculated controls, and root rot from *G. incrustans* was much less marked.

Quality ratings of 8-wk-old Kentucky bluegrass and annual bluegrass turf inoculated with *P. graminicola* or *G. cylindrosporus* were significantly lower than those of controls on only one occasion. Clipping weights of 8-wk-old Kentucky bluegrass and annual bluegrass and 7-yr-old Kentucky bluegrass turf inoculated with *P. graminicola* or *G. cylindrosporus* were not significantly different from controls but were consistently higher than turf inoculated with *M. poae*. Only slight root cortical rot occurred.

Quality ratings of four turfgrass species inoculated with *M. poae* (ATCC 64413) are presented in Table 5. Clipping yields and estimates of root colonization and rot are shown in Table 6. Shoot lengths, shoot dry weights, root dry weights, and estimates of root colonization and root rot of four cereal species inoculated with *M. poae* (ATCC 64413) and controls are presented in Table 7.

M. poae was more pathogenic on some turfgrass species than others. By the third week of this test, plants of Chewing's fescue inoculated with *M. poae* were significantly lower in quality than controls of the same species. Significantly lower quality ratings were apparent on inoculated creeping bentgrass by the fourth week of the test. Tall fescue inoculated with *M. poae* showed a significant decline in quality during the fifth and sixth weeks, whereas no differences occurred between inoculated plants and controls of perennial ryegrass. Except for creeping bentgrass, turfgrasses that showed a significant decline in quality also showed significantly lower clipping yields.

The species most adversely influenced by *M. poae* had the most root colonization and rot. Chewing's fescue roots showed moderate to moderately severe root rot, whereas roots of creeping bentgrass, tall fescue, and perennial ryegrass showed slight to moderate root rot.

M. poae was pathogenic to four cereal species but to varied degrees. Under the conditions imposed, barley was most susceptible, based on the greater degree of root discoloration, followed by wheat, oats, and rye. Although these results indicate a high degree of susceptibility of some cereal species, *M. poae* has not been isolated from cereal roots in the field. These pathogenicity studies were conducted on seedling plants under extended periods of high temperatures. Whether *M. poae* is able to cause root dysfunction on mature cereal plants under less severe environmental conditions is unknown.

TABLE 6. Influence of *Magnaporthe poae* (ATCC 64413) on dry weight of leaf clippings and root colonization and rot of four turfgrasses after 6 wk of incubation at 28 C

Species	Treatment comparison	Dry weight[x] of leaf clippings	Root[y] rot
Creeping bentgrass	Control	1.5	1.0
(cultivar Penncross)	ATCC 64413	1.3 ns[z]	2.8***
Chewings' fescue	Control	1.2	1.0
(cultivar Jamestown)	ATCC 64413	0.8**	3.6***
Tall fescue	Control	2.0	1.0
(cultivar Rebel)	ATCC 64413	1.5*	2.7***
Perennial ryegrass	Control	2.1	1.0
(cultivar Yorktown II)	ATCC 64413	1.8 ns	2.1***

[x] Mean dry weight of leaf clippings, grams per 10-cm-diameter pot.
[y] Based on a visual rating scale of 1 to 5, with 1 = no colonization, 2 = colonization and slight root rot, 3 = colonization and moderate root rot, 4 = colonization and moderately severe root rot, and 5 = colonization and severe root rot.
[z] Dry weight of leaf clippings analyzed by *t* tests. Significance levels are $P < 0.001$ (***), $P < 0.01$ (**), $P < 0.05$ (*), or $P > 0.05$ (ns) for treatment comparison within a turf species. Values are means of leaf clipping dry weights and root rot estimates from four replicate pots per treatment from one trial.

Patch symptoms first were observed on 24 July 1986 in plots inoculated with *M. poae* on 10 June 1985. Patches occurred in two of three replicate plots inoculated with isolates ATCC 56773 and ATCC 64413. One other isolate of *M. poae* (MI-1) included in the test did not induce patch symptoms. Inoculation with one isolate of *G. cylindrosporus* (SAK), one isolate of *P. graminicola* (REY), and three isolates of *G. incrustans* (ATCC 64418, CREST, and ATBM) failed to induce patch symptoms.

The first evidence of symptom development was the appearance of dark green patches of turf that wilted despite adequate soil moisture. The turf within these patches was stunted, turning a dark reddish brown over the course of 2 days. As symptoms progressed, the turf turned tan at the margins of the patches. Individual patches were roughly circular, ranging in size from 27 × 18 cm to 45 × 43 cm. (The size of these patches increased only 2–5 cm from the time symptoms first became visible.) Many live and apparently healthy tillers remained in the center of the patch. No distinct leaf lesions were apparent although some dieback was evident on many tillers. Roots and crowns of affected plants were rotted, most showing a distinct vascular discoloration. On some stem bases, large aggregates of swollen, spherical cells borne on pigmented hyphae were apparent.

TABLE 7. Influence of *Magnaporthe poae* (ATCC 64413) on shoot length, shoot dry weight, root dry weight, and root rot of four cereal species after incubation for 6 wk at 28 C

Species	Treatment comparison	Shoot length (cm)	Shoot dry weight (g)	Root dry weight (g)	Root[y] rot
Barley	Control	56.1	3.2	1.1	1.0
(cultivar Arivat)	ATCC 64413	12.1***[z]	0.1***	0.1***	5.0***
Wheat	Control	47.0	1.9	0.6	1.0
(cultivar Redcoat)	ATCC 64413	30.1***	0.4***	0.2***	3.5***
Oats	Control	55.1	1.9	0.6	1.0
	ATCC 64413	46.4**	1.1***	0.4***	2.3***
Rye	Control	39.3	1.9	1.0	1.0
	ATCC 64413	37.4 ns	1.0*	0.4**	2.3***

[y] Based on visual ratings of 1 to 5, with 1 = no colonization, 2 = colonization and slight root rot, 3 = colonization and moderate root rot, 4 = colonization and moderately severe root rot, and 5 = colonization and severe root rot.

[z] Growth parameters analyzed with *t* tests. Significance levels are $P < 0.001$ (***), $P < 0.01$ (**), $P < 0.05$ (*), $P > 0.05$ (ns) for treatment comparison within a cereal species. Values are means of shoot length, shoot dry weight, root dry weight, and root rot estimates from four replicate pots per treatment from one trial.

Diseased roots and crowns yielded several common root-inhabiting fungi as well as *M. poae*, *G. incrustans*, and *P. graminicola*, but *M. poae* was the most consistent and predominant species isolated from infected plants. Isolates of *M. poae* that were recovered produced mature perithecia when mated with compatible mating types of the same species.

Because a synchronous wilt of plants in large patches was apparent at the onset of symptom development, it is likely that *M. poae* grew up to 22 cm from the point of inoculation over a 1 1/2-yr period and infected the roots without killing plants. When the turf became heat stressed after several days of temperatures >32 C, the fungus apparently gained an advantage over the suscept and began to invade the vascular system of the roots, causing dysfunction and eventually death.

It is not clear why symptoms did not appear in the third replicate for isolates ATCC 64413 or ATCC 56773, or why MI-1 did not induce patch symptoms in any plot. It is possible that soil microflora or microfauna inhibited the dissemination and colonization of the fungus, or that physical conditions were limiting at some inoculation sites in the sod or underlying soil.

The high degree of pathogenicity of *M. poae* to annual bluegrass confirms numerous observations by the authors of summer patch on annual bluegrass fairways and putting greens. Summer patch is also a serious disease of Kentucky bluegrass lawns (16) and, in some cases, fine-leaf fescues (P. H. Dernoeden, *personal communication*). Although creeping bentgrass was susceptible to *M. poae* in pathogenicity studies, summer patch is not typically a serious disease of bentgrasses in the field. Bentgrasses and perennial ryegrasses have been observed by the authors to colonize patches of turf damaged by summer patch.

The role of *G. incrustans* in patch disease development is more difficult to interpret. Although pot studies have shown that this fungus is capable of causing root rot in *Poa* spp., inoculation of mature turf did not result in patch symptoms. Under different environmental conditions, *G. incrustans* may be capable of causing turf disease. Studies currently are in progress to explore this possibility.

This study showed that *P. graminicola* and *G. cylindrosporus* are not pathogenic on *Poa* spp. Their presence on and in root tissue may well be beneficial and certainly does not warrant fungicidal control measures.

At high temperatures (>28 C), and with turf stressed by environmental and cultural factors, *M. poae* is able to exploit the roots and crowns of turfgrasses and cause a severe vascular dysfunction. Smiley et al (17) reported that the pathogenicity of their isolates (now known to be *M. poae*) were strongly influenced by temperature, the highest degree of pathogenicity occurring at 29 C. Cultural factors implicated in increasing the severity of summer patch included high levels of soil nitogren, low mowing heights, arsenical herbicides, and irrigated turf suddenly subjected to drought stress (17).

M. poae is able to colonize roots and crowns of turfgrasses as ectotrophic mycelium without causing disease symptoms. Inoculation of mature, field-grown sod has shown that this fungus can grow up to 22 cm over 1 1/2 yr in a greenhouse without causing visible disease symptoms. Garrett (6) noted that the growth of ectotrophic mycelium of some root-infecting fungi is accelerated after a decline in plant vigor. In particular, ectotrophic growth of *G. graminis* (presumably var. *tritici*) increased after the close grazing of young winter wheat (6). Close mowing of susceptible turfgrasses similarly may enhance the ectotrophic growth of *M. poae* over the root system.

The ectotrophic growth habit provides a competitive advantage for *M. poae* (as well as other ectotrophic, root-infecting fungi) in that the fungi are present on the root surface (infection court) when resistance breaks down. Another advantage afforded by the ectotrophic habit is that it provides a mechanism whereby the fungus can incite multiple infections in rapid succession along the length of the root, thus overwhelming the remaining host defenses (6).

Summer patch is one of several patch diseases with similar symptoms that occur under the same cultural and environmental conditions. In some instances, early investigators of this complex of diseases undoubtedly failed to identify *M. poae* or *L. korrae* from turfgrass roots, probably by using isolation techniques that favored the faster growing *Fusarium* spp. For many of these studies, the identity of the primary pathogen(s) remains unclear, and the conclusions drawn should be reexamined in light of current findings.

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