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Susceptibility of Chewings Fescue and Hard Fescue to Anthracnose Disease Caused by *Colletotrichum cereale*

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Abstract. **Anthracnose, caused by the fungal pathogen *Colletotrichum cereale* Manns sensu lato Crouch, Clarke & Hillman, can be a damaging disease on many cool-season turfgrasses; however, it has not been reported as an aggressive pathogen on fine fescue species (*Festuca* spp.). Symptoms and signs associated with anthracnose disease were observed in fine fescues on the Rutgers University Plant Science Research and Extension Farm in Adelphia, NJ, in Jun 2014. The objectives of this study were to identify the causal agent, determine if the isolate of *C. cereale* (FF1A) obtained from symptomatic Chewings fescue (*Festuca rubra* L. ssp. *commutata* Gaudin) plants was pathogenic to Chewings fescue and hard fescue (*F. brevipila* Tracey) turfs, and whether cultivars and accessions collected from Europe varied in disease susceptibility. Pathogenicity of this fine fescue isolate was evaluated using four Chewings fescue and four hard fescue cultivars or accessions in a growth chamber. Disease symptoms were first observed at 5 days post-inoculation, and evaluations continued to 17 days post-inoculation. Infection was confirmed by morphological evaluations, re-isolation from symptomatic tissues, and real-time polymerase chain reaction (PCR). Three noncommercial accessions (two Chewings fescues and one hard fescue) were very susceptible to the fine fescue *C. cereale* FF1A isolate, whereas 'Sword' and 'Beacon' hard fescues exhibited low susceptibility. In addition, an isolate of *C. cereale* (HF217CS) from annual bluegrass [*Poa annua* L. f. *reptans* (Hausskn) T. Koyama] was included, and our data demonstrated that this isolate was also able to infect Chewings fescue and hard fescue. This study confirmed that *C. cereale* can be a damaging pathogen of fine fescues, and that breeding for resistance to anthracnose should be considered when developing new cultivars.**

C. cereale is an ascomycete fungus that commonly inhabits grasses in the subfamily Pooideae (Crouch and Beirn 2009; Selby and Manns 1909). *C. cereale* has been reported on at least 14 grass hosts, where it is capable of surviving as a pathogen, saprophyte, or endophyte (Crouch and Beirn 2009; Crouch et al. 2006, 2009a; Tao et al. 2013). In addition to a wide host range, the fungus exists as two distinct lineages, known as clade A and clade B; however, the biological significance of these clades has yet to be determined (Beirn et al. 2014; Crouch et al. 2006, 2009a). As a pathogen, *C. cereale* can be particularly destructive on annual bluegrass (*Poa annua*) and creeping bentgrass (*Agrostis stolonifera* L.) putting green turfs, where it can cause a foliar blight and/or a basal rot disease called anthracnose (Sann 1998; Smiley et al. 2005). Anthracnose caused by *C. cereale* has also been reported on other cool-season turfgrasses including fine fescues (*Festuca* spp.), other bluegrasses (*Poa* spp.), ryegrasses (*Lolium* spp.), and tall fescue (*Festuca arundinacea* Schreb.); although, it is typically not considered a major disease on these hosts (Bonos et al. 2006). The disease is most severe when environmental conditions or management practices induce plant stress (Hempfling et al. 2017; Inguagiato et al. 2008, 2009; Roberts et al. 2011; Schmid et al. 2018; Wang et al. 2018).

Fine fescue is an economically important group of perennial, cool-season turfgrasses that are known for their very fine leaf texture and ability to survive under stressful conditions (e.g., low fertility, shade, and drought) (Braun et al. 2020; Turgeon 1991). This group includes five major turfgrass species: strong creeping red fescue (*F. rubra* L. ssp. *rubra* Gaudin), slender creeping red fescue [*F. rubra* L. ssp. *littoralis* (G. Mey.) Auquier], Chewings fescue (*F. rubra* L. ssp. *commutata*), hard fescue (*F. brevipila*), and sheep fescue (*F. ovina* L.) (Braun et al. 2020; Christians 2004). Chewings fescue and hard fescue originated from Europe and are very well adapted to drought, cold, shade, and low fertility conditions, making them excellent low-maintenance grasses for lawns in cool, humid climates (Beard 1973; Emmons 2008). As cultivated turfgrasses, fine fescues are susceptible to severe damage from several diseases, including summer patch [*Magnaporthiopsis poae* (Landsch. & N. Jacks.) J. Luo & N. Zhang and *M. meyerifestucae* sp. nov.], dollar spot (*Clarireedia jacksonii* C. Salgado, L.A. Beirn, B.B. Clarke, & J.A. Crouch sp. nov.), and red thread [*Laetisaria fuciformis* (Berk.) Burds.] (Bonos et al. 2005, 2006; Clarke et al. 2006; Fraser and Rose-Fricker 2001; Hodges et al. 1975; Luo and Zhang 2013; Luo et al. 2017; Ruemmele et al. 1995; Salgado-Salazar et al. 2018; Smiley et al. 2005). Turfgrass breeders have sought to incorporate improved resistance to many of these diseases when developing new cultivars, because disease susceptibility has typically been regarded as the principal disadvantage of fine fescue species (Bonos et al. 2006).

In Jun 2014, fine fescue cultivars and accessions in a field trial seeded the previous September at the Rutgers University Plant Science Research and Extension Farm in Adelphia, NJ, began exhibiting symptoms (chlorotic leaf lesions and foliar necrosis) and signs (dark elongated black setae and acervuli containing hyaline, one-celled, lunate conidia) similar to anthracnose disease on other turfgrass hosts. Symptoms quickly spread throughout the field trial, resulting in large patches of blighted turf that were straw-colored in appearance (Fig. 1A and B). Affected turf often died several weeks after symptoms appeared. Isolations from symptomatic tissue revealed a fungus resembling *C. cereale* in morphology. Because *C. cereale* had not been reported as an aggressive pathogen of fine fescue turf in the field (Bonos et al. 2006; Crouch et al. 2009a; Ruemmele et al. 1995), the objectives of this study were 1) to identify the causal agent of symptomatic fine fescue plants in the Adelphia field trial, and 2) to assess its pathogenicity on commercially available Chewings fescue and hard fescue cultivars and some accessions collected from Europe, and determine whether an isolate of *C. cereale* known to be pathogenic to annual bluegrass is capable of infecting fine fescues

and causing disease symptoms in a growth chamber study.

Materials and Methods

Fungal isolates. Two fungal isolates were used in this study. Isolate FF1A, suspected of being *C. cereale*, was collected Jul 2014 from Chewings fescue plants exhibiting symptoms and signs of anthracnose in fine fescue research trials at the Rutgers University Plant Science Research and Extension Farm in Adelphia, NJ. *C. cereale* isolate HF217CS was collected in Oct 2010 from annual bluegrass maintained as putting green turf at the Rutgers University Horticultural Farm No. 2 in North Brunswick, NJ. Fungi were stored as desiccated conidia on silica gel in 1 mL cryogenic tubes (Fisher Scientific, Pittsburgh, PA, USA) at −80 °C. Fungal isolates were allowed to grow on potato dextrose agar (PDA) until they covered the plate. Three milliliters of sterile 7.5% Skim milk solution (Difco Skim Milk, BD Life Sciences, Franklin Lakes, NJ, USA) was pipetted onto the plate. A sterile glass rod was used to gently dislodge fungal tissue. Five hundred microliters of the fungal milk slurry was transferred to cryogenic tubes (Nalgene, Rochester, NY, USA) containing sterile silica gel and stored in −80 °C.

Turfgrass lines. Three commercially available cultivars and five experimental accessions of fine fescues, including four Chewings fescues and four hard fescues (Table 1) were seeded (0.13 g of seeds per pot) into 10-cm pots filled with Fafard Canadian Grow Mix 2 (Agawam, MA, USA). Seeds were covered with a thin layer of the growth mix to prevent desiccation and ensure seed to soil contact. Pots were moved to a greenhouse and watered daily to ensure uniform germination. Cultivars and accessions used in this study ranged from low to high susceptibility (Table 1) based on field observations of a natural outbreak of anthracnose disease in turf breeding evaluation trials at Rutgers University in Adelphia, NJ, as well as an initial pathogenicity screening in the growth chamber with isolate FF1A (S. Yin, unpublished data).

Inoculation procedure. Fungal isolates were grown on PDA (Fisher Scientific) for 7 d under continuous florescent light at room temperature. When fungal growth covered the plates (∼8–10 d), 25 mL sterile dH_2O was poured onto the plates, and mycelia and conidia were gently dislodged by scraping with a sterile glass rod, creating a mycelia–spore suspension. A sterile inoculation loop was dipped into the suspension and was streaked onto fresh PDA plates. Plates were covered but not parafilmed, and placed under continuous florescent light at room temperature to induce sporulation (∼3–4 d).

Conidia were harvested from 7-d-old, sporulating cultures of *C. cereale* and quantified using a hemacytometer (Hausser Scientific, Horsham, PA, USA) to create a 5×10^4 mL^{-1} conidial suspension in 10% potato dextrose broth (PDB; Fisher Scientific). Before inoculation, 2-week-old plants were transferred to a growth chamber maintained at 30 °C, 60% relative humidity (RH), 500 μmol·m^{-2}·s^{-1} daylight, and allowed to acclimate for 4 h. Fine fescue plants were inoculated with a 20-mL spore suspension of isolate FF1A or HF217CS using a hand spray bottle (Fisher Scientific). Additional plants sprayed with 10% PDB served as noninoculated controls. There were three replicates (pots) per treatment and control. After inoculation, pots were placed in sealed 60 cm × 90 cm translucent plastic autoclave bags (Fisher Scientific), and moved to a growth chamber maintained at a 30 °C day and 26 °C night temperature, 60% RH, and 16 h daylight (500 μmol·m^{-2}·s^{-1}) until symptoms developed (∼5–11 d). A wet paper towel was placed in the bottom of each bag to maintain high humidity for infection. Four 10-mL pipettes (Fisher Scientific) were inserted tip down into each pot to prevent the bag from touching inoculated plants. The bags were moved to different locations in the growth chamber each day to minimize potential growth chamber effects. Plants were watered with tap water every other day at the base of the pot to maintain adequate soil moisture and avoid wetting the foliage during the experiment. The experiment was repeated, and the two runs of the experiment were treated as blocks in a generalized randomized block design for data analysis.

Confirmation of pathogenicity. Identification and pathogenicity of both fungal isolates was confirmed using three methods: visual inspection on plants for signs of the fungus (appressoria and acervuli), re-isolation of the pathogen from symptomatic plants, and use of real-time PCR, clade-specific assays developed with the *Apn2* marker (Beirn et al. 2014) to verify the presence and clade of *C. cereale* in infected tissue.

To isolate the pathogen, three to five symptomatic leaves were collected and cut into 1-cm-long pieces. Harvested tissue was washed with 10% commercial bleach (6% NaClO) for 1 min, 70% EtOH for 30 s, rinsed with sterile dH_2O, and then blotted on sterile filter paper to remove excess water. Surface sterilized leaf tissues were placed on PDA, and the petri dishes were parafilmed and incubated under continuous florescent light at room temperature for 1 week. The presence of *C. cereale* was verified using microscopy (Olympus BX41 clinical microscope; New York/New Jersey Scientific, Middlebush, NJ, USA) by examining and measuring conidia, appressoria, and setae and comparing results to descriptions of *C. cereale* by Crouch et al. (2006).

For real-time PCR evaluation, genomic DNA from inoculated tissue displaying symptoms of anthracnose disease was extracted with the OmniPrep DNA Extraction Kit (G-Biosciences, St. Louis, MO, USA) using a modified protocol. Briefly, small sections of plant tissue (5 to 8 cm^2) were placed in a 2-mL microcentrifuge tube containing eight 2.5-mm glass beads (BioSpec Products, Bartlesville, OK, USA). A BioSpec bead-beater (BioSpec Products) was then used to shake samples for 1 min at the medium speed. Next, 600 μL of Genomic Lysis Buffer (G-Biosciences) containing 1% Proteinase K was added to each tube and incubated at 60 °C for 1 h while mixing by inversion every 15 min. Following incubation, samples were cooled to room temperature and 200 μL of chloroform was added to each tube. The manufacturer's protocol was followed for the remaining procedure. DNA quality and quantity were assessed using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and DNA was diluted to a final concentration of 15 ng·μL^{-1}. In addition, DNA samples from pure culture of *C. cereale* isolate FF1A or HF217CS were used as positive controls in the real-time PCR evaluation. Samples were screened against real-time PCR probes capable of accurately genotyping *C. cereale* isolates as belonging to either clade A or clade B in as little as 6 hours (Beirn et al. 2014). Briefly, real-time PCR reactions were performed as 20-μL reactions in 96-well plates using the StepOnePlus System (Applied Biosystems, Foster City, CA, USA). Roche's Light Cycler 480 Probes Master Mix (Roche, Indianapolis, IN, USA) was used for all reactions with primers and probe concentrations of 20 μM and 2 μM, respectively. The cycling program was as follows: 95 °C for 120 s, followed by 45 cycles of 95 °C for 5 s, 60 °C annealing for 30 s, and 72 °C extension for 1 s. Samples were considered to contain *C. cereale* (a positive reaction) when the fluorescent threshold (30) was crossed before cycle 40, and a negative reaction was verified when samples had a cycle threshold (C_T) equal to zero.

Disease ratings. Plants were visually inspected every 2 days from 1 to 17 d postinoculation for symptoms of anthracnose disease (i.e., chlorosis or necrosis). Disease incidence was estimated for each pot as the percentage of leaves exhibiting disease symptoms (leaf lesions, chlorosis, necrosis, and dieback from the leaf tips). An average disease severity was estimated for all symptomatic leaves in a pot using a 1 to 10 scale, where 1 represented no disease symptoms; 2 = 1%–4%; 3 = 5%–14%; 4 = 15%–29%; 5 = 30%–49%; 6 = 50%–69%; 7 = 70%–84%; 8 = 85%–94%; 9 = 95%–99%; 10 = 100% of the leaf surface area with chlorosis or necrosis. A disease severity × incidence product was calculated for each pot by multiplying disease incidence by disease severity. Turf quality was measured for each pot using a visual scale of 1 to 9, where 9 represented the best quality and 5 was the minimal acceptable

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Fig. 1. Symptoms and signs of anthracnose disease on fine fescue (*Festuca* spp.) turf. (**A** and **B**) Symptoms of anthracnose disease on a newly seeded fine fescue research trial at the Rutgers University Plant Science Research and Extension Farm in Adelphia, NJ, in 2014. (**C**) Plant inoculated with *Colletotrichum cereale* isolate FF1A obtained from Chewings fescue. (**D**) Noninoculated control. (**E**) Lesions on fine fescue leaf. (**F**) Acervuli of *C. cereale* on necrotic leaf tissue. (**G**) Conidia of *C. cereale* isolate FF1A (Bar = 10 μm). (**H**) Appressoria of *C. cereale* on an inoculated leaf (Bar = 10 μm). (**I**) *C. cereale* isolate FF1A in culture on potato dextrose agar.

quality, and included an assessment of disease severity, turf color, and uniformity.

Statistical analyses. For the purpose of evaluating the pathogenicity of the *C. cereale* isolates on two fine fescue species, noninoculated controls were excluded in the statistical analyses. To test for main effects and interactions of turfgrass line, fungal isolate, and rating date, the MIXED procedure of the SAS System (version 9.4; SAS Institute Cary, NC, USA) was used to fit a mixed effects repeated measures analysis of covariance. Blocks (runs) of the generalized randomized block design were included in the model as a random effect. The fixed effects in the model were the classification variables turfgrass line and fungal isolate; rating date was the continuous covariate. All two- and three-way interactions among the fixed effects were included in the model. The significant three-way interaction of turfgrass line with fungal isolate and rating date was further investigated by performing pairwise comparisons of the slopes across days after inoculation (DAI). For each isolate, data were compared between all pairs of turfgrass lines using single-degree-of-freedom contrasts based within the mixed model analysis. Model adequacy was assessed using graphical analysis of studentized residuals to evaluate error distributional assumptions, and graphical assessment of the raw data to determine additivity of the block effect.

Results

Real-time PCR identification and confirmation. Real-time PCR genotyped isolate FF1A obtained from Chewings fescue as belonging to *C. cereale* clade A (Fig. 2A), whereas *C. cereale* isolate HF217CS previously obtained from annual bluegrass was identified as a member of *C. cereale* clade B (Beirn et al. 2015). At the

Table 1. Origin and susceptibility of fine fescue species, cultivars, and accessions to *Colletotrichum cereale*.

Scientific name	Common name	Cultivar/Accession	Origin[i]	Disease susceptibility[ii]
Festuca rubra ssp. *commutata*	Chewings fescue	Ambrose	Commercial cultivar	Moderate
	Chewings fescue	54219-13	Italy, 2013	High
	Chewings fescue	54259-13	Italy, 2012	Moderate
	Chewings fescue	54280-13	Italy, 2012	High
Festuca brevipila	Hard fescue	Sword	Commercial cultivar	Low
	Hard fescue	Beacon	Commercial cultivar	Low
	Hard fescue	52494-12	Italy, 2012	High
	Hard fescue	54415-13	Turkey, 2013	High

[i] Origin of cultivars and accessions and the year that accessions were collected.
[ii] Relative susceptibility of fine fescue cultivars and accessions to *C. cereale* based on results from previous field evaluations and pre-screening of fine fescue cultivars and accessions to this pathogen in the growth chamber.

conclusion of the inoculation experiment, *C. cereale* was detected from all inoculated plants (Fig. 2), with average C_T values of 30.31 for isolate FF1A and 33.81 for isolate HF217CS. No cross contamination was detected between plants inoculated with isolates FF1A or HF217CS. Noninoculated controls produced C_T values of 0.00.

Morphological confirmation. Disease symptoms were observed on all inoculated cultivars and accessions 5 days following inoculation, except for 'Sword' hard fescue, which began to exhibit symptoms 7 and 11 DAI with *C. cereale* FF1A and HF217CS, respectively. Plants inoculated with either isolate exhibited necrotic leaf tips and appeared chlorotic (Fig. 1C) compared with noninoculated controls (Fig. 1D). In addition, accessions 52494-12, 54259-13, and 54280-

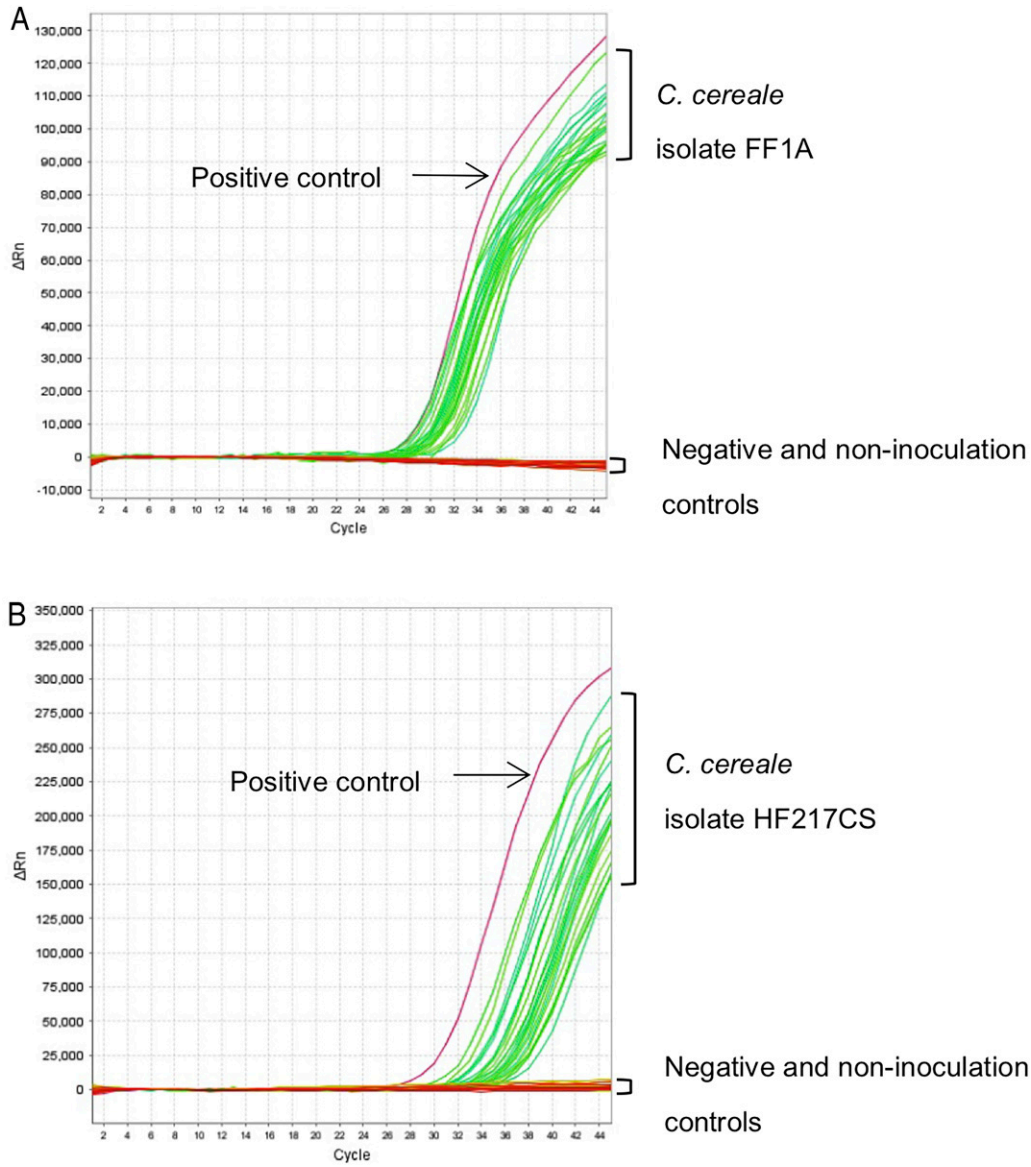

Fig. 2. Real-time polymerase chain reaction amplification plots from using *Colletotrichum cereale* probes to screen a 96-well plate of *C. cereale* infected samples, positive controls (cultured *C. cereale* isolate FF1A or HF217CS sample) and negative controls as labeled. (**A**) Clade A probe for detection of *C. cereale* FF1A. (**B**) Clade B probe for detection of *C. cereale* HF217CS.

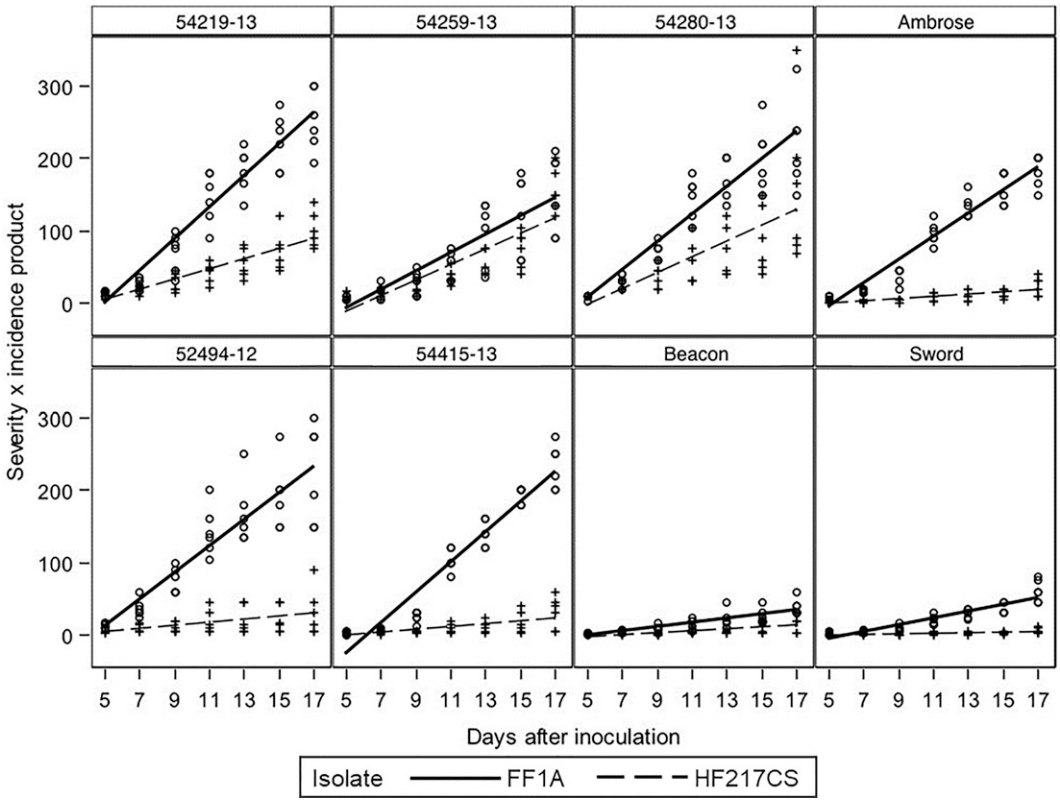

Fig. 3. Plots of the disease severity × incidence product for Chewings fescues (54219-13, 54259-13, 54280-13, and Ambrose) and hard fescues (52494-12, 54415-13, Beacon, and Sword) from 5 to 17 d after inoculation for fungal isolates *Colletotrichum cereale* FF1A and HF217CS.

13 exhibited small tan-colored lesions at the midvein of inoculated foliage (Fig. 1E). Acervuli with black setae were present on all cultivars and accessions inoculated with either isolate of *C. cereale* (Fig. 1F). Microscopic observation of infected leaves showed the presence of masses of conidia and appressoria matching the description of *C. cereale* (Fig. 1G and H).

Conidia were single-celled, hyaline, falcate, one to multiguttulate, and measured from 13.8 to 35.3 µm long × 2.4 to 4.9 µm wide. Appressoria were randomly distributed on the leaf surfaces, dark brown to black, irregular (rounded or lobed) in shape, and ranged from 8.6 to 12.5 µm long × 6.1 to 7.5 µm wide. Black setae tapered at the tip and measured 65.6 to 128.7 µm. These morphological characteristics conformed to those of *C. cereale* as reported by Crouch et al. (2006). *C. cereale* was successfully isolated on PDA from all inoculated plants (Fig. 1I). No chlorosis, necrosis, leaf lesions, or acervuli were present on noninoculated control plants, although there was some limited dieback at the leaf tips (Fig. 1D) at the end of each run due to prolonged exposure to heat stress encountered in the growth chamber. *C. cereale* was never recovered on PDA or identified with real-time PCR from noninoculated control plants.

Inoculation with C. cereale FF1A. The disease severity × incidence product increased among all cultivars and accessions over the course of the experiment (Fig. 3). The rate at which the disease severity × incidence product increased over time ranged from 3.01 ('Beacon'

Table 2. Disease severity × incidence product means at 5 and 17 d after inoculation (DAI) and slopes across DAI for eight lines of fine fescues inoculated with two isolates of *Colletotrichum cereale*, FF1A and HF217CS.

		Disease severity × incidence product[i]				Slope of disease severity × incidence product[ii]	
		5 DAI		17 DAI			
Turfgrass species	Turfgrass line	FF1A	HF217CS	FF1A	HF217CS	FF1A	HF217CS
Chewings fescue	Ambrose	8.00	0.67	182.50	23.33	14.98 bc	1.84 c
	54219-13	12.67	12.67	253.33	100.83	20.60 a	7.27 ab
	54259-13	7.00	7.67	152.50	155.83	12.34 c	11.92 a
	54280-13	8.67	10.00	221.67	159.17	18.19 a	12.00 a
Hard fescue	Beacon	1.67	0.67	38.33	17.33	3.01 d	1.37 c
	Sword	1.67	0.00	60.83	6.00	4.87 d	0.49 c
	52494-12	13.33	5.33	224.17	32.00	17.84 ab	2.20 bc
	54415-13	2.67	3.00	232.50	30.50	19.68 a	2.24 bc

[i] Disease severity × incidence product was calculated by multiplying disease incidence by disease severity. Disease incidence was estimated for each pot as the percentage of leaves exhibiting disease symptoms. An average disease severity was estimated for all of the symptomatic leaves in a pot using a 1 to 10 scale, where 1 represented no disease symptoms; 2 = 1%–4%; 3 = 5%–14%; 4 = 15%–29%; 5 = 30%–49%; 6 = 50%–69%; 7 = 70%–84%; 8 = 85%–94%; 9 = 95%–99%; 10 = 100% of the leaf surface area with chlorosis or necrosis.

[ii] For each isolate, the slope of disease severity × incidence product compared between all pairs of turfgrass lines using single-degree-of-freedom contrasts based within the mixed model analysis. Different letters indicate a difference in slope within each isolate at $\alpha = 0.05$ for disease severity × incidence product.

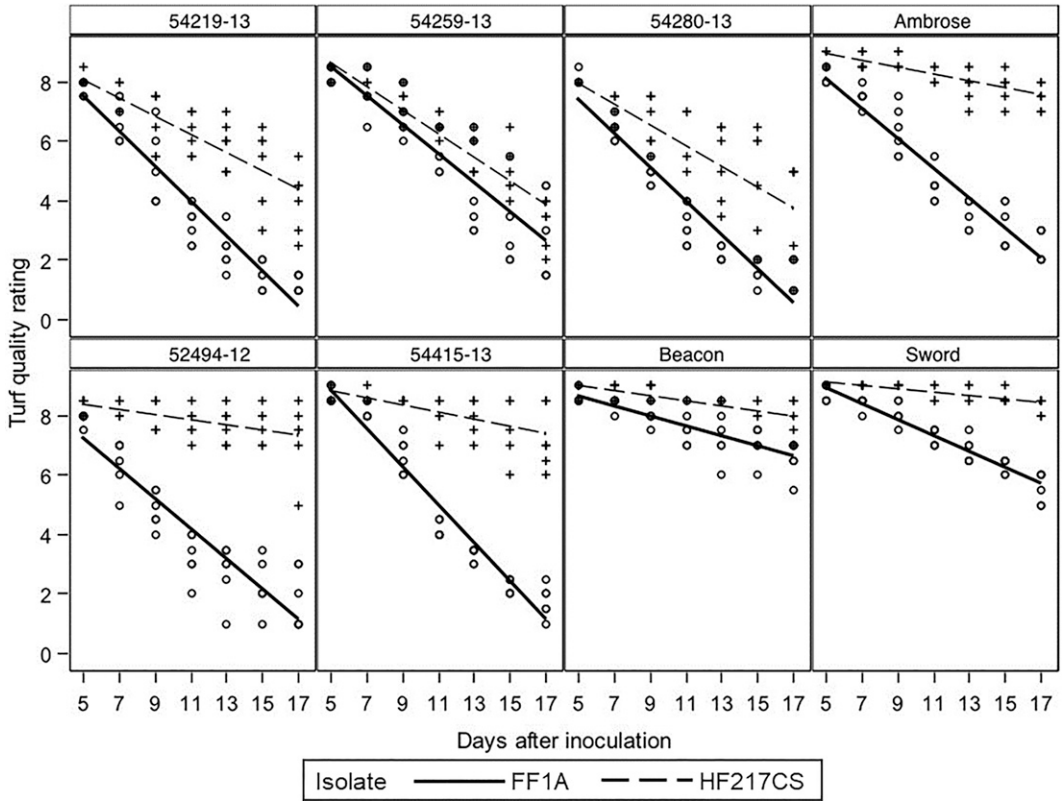

Fig. 4. Plots of the turf quality ratings for Chewings fescues (54219-13, 54259-13, 54280-13, and Ambrose) and hard fescues (52494-12, 54415-13, Beacon, and Sword) from 5 to 17 d after inoculation for fungal isolates *Colletotrichum cereale* FF1A and HF217CS. Turf quality was based on a visual scale of 1 to 9, where 9 represented the best quality and 1 represented completely dead turf, which included an assessment of disease severity, turf color, and uniformity.

hard fescue) to 20.60 (54219-13 Chewings fescue) (Table 2). Hard fescue 'Beacon' and 'Sword' exhibited low susceptibility to isolate FF1A (disease severity × incidence product at 17 DAI = 38.33 and 60.83; slope = 3.01 and 4.87, respectively), whereas Chewings fescues 54219-13 and 54280-13, and hard fescue 54415-13 were the most susceptible (disease severity × incidence product at 17 DAI = 253.33, 221.67, and 232.50; slope = 20.60, 18.19, and 19.68, respectively) (Table 2). The wide range of slopes illustrated large differences in the susceptibility of the fine fescues tested (Table 2, Fig. 3).

Turf quality decreased as disease severity × incidence product increased over time when plants were inoculated with *C. cereale* isolate FF1A (Figs. 3 and 4). Average turf quality declined dramatically and varied among the cultivars and accessions (Table 3). Slopes ranged from −0.17 for 'Beacon' hard fescue (turf quality at 17 DAI = 6.6) to −0.60 for 54415-13 hard fescue (turf quality at 17 DAI = 1.8) (Table 3). Plots of the individual responses are presented in Fig. 4. The turf quality means ranged from 9.0 to 7.9 at 5 DAI when anthracnose symptoms first started to appear, which were above the general acceptable minimum of >5.0 for all turfgrass lines including noninoculated controls and plants inoculated with *C. cereale* isolate FF1A. At 17 DAI, when the experiment was concluded, the turf quality means ranged from 1.3 to 6.6 for turf inoculated with FF1A (clade A) compared with 5.8 to 8.4 for noninoculated controls (Table 3).

Inoculation with C. cereale HF217CS. Compared with inoculation with *C. cereale* FF1A,

Table 3. Turf quality means at 5 and 17 d after inoculation (DAI) and slopes across DAI for eight lines of fine fescues noninoculated controls and plants inoculated with two isolates of *Colletotrichum cereale*, FF1A and HF217CS.

		Turf quality[i]						Slope of turf quality[ii]	
		5 DAI			17 DAI				
Turfgrass species	Turfgrass line	Control	FF1A	HF217CS	Control	FF1A	HF217CS	FF1A	HF217CS
Chewings fescue	Ambrose	8.92	8.17	8.83	8.42	2.50	7.50	−0.48 cd	−0.11 a
	54219-13	8.67	7.92	8.00	6.25	1.25	4.00	−0.57 c	−0.33 b
	54259-13	8.58	8.25	8.33	6.25	2.75	3.25	−0.47 c	−0.42 c
	54280-13	8.42	8.08	8.00	5.83	1.50	3.42	−0.56 d	−0.37 bc
Hard fescue	Beacon	9.00	8.58	8.83	8.33	6.58	7.75	−0.17 a	−0.09 a
	Sword	9.00	8.75	9.00	8.33	5.58	8.33	−0.26 b	−0.06 a
	52494-12	8.75	7.92	8.33	7.33	1.83	7.25	−0.51 cd	−0.09 a
	54415-13	8.83	8.67	8.67	6.83	1.75	7.25	−0.60 c	−0.12 a

[i] Turf quality rated on a visual scale of 1 to 9, where 9 represented the best quality and 1 represented completely dead turf, which included an assessment of disease severity, turf color, and uniformity.

[ii] For each isolate, the slope of turf quality compared between all pairs of turfgrass lines using single-degree-of-freedom contrasts based within the mixed model analysis. Different letters indicate a difference in slope within each isolate at $\alpha = 0.05$ for turf quality.

the disease severity × incidence product was lower when fine fescue cultivars and accessions were inoculated with isolate HF217CS (Fig. 3). The Chewings fescue 'Ambrose' showed minimal susceptibility (disease severity × incidence product = 23.33 at 17 DAI) (Table 2). However, three accessions, 54219-13, 54259-13, and 54280-13, all of which are Chewings fescues, displayed moderate susceptibility to isolate HF217CS (disease severity × incidence product = 100.83, 155.83, and 159.17 at 17 DAI; slope = 7.27, 11.92, and 12.00, respectively) (Table 2). The remaining cultivars and accessions displayed low susceptibility to isolate HF217CS, with minimal symptoms developing throughout the duration of the experiment. The hard fescues tested in this study showed minimal susceptibility to isolate HF217CS with the highest disease severity × incidence product (32.00) observed at 17 DAI for accession 52494-12 (Table 2).

The average turf quality of plants inoculated with *C. cereale* HF217CS declined over time (Fig. 4), and ranged from 8.3 to 3.3 for the eight cultivars and accessions at the conclusion of the experiment (17 DAI) (Table 3). Three accessions (54219-13, 54259-13, and 54280-13) exhibited unacceptable turf quality (turf quality rating <5) of 4.0, 3.3, and 3.4, respectively. The remaining five cultivars and accessions exhibited turf quality ratings ≥7.3 at the end of the experiment (Table 3).

Discussion

The objectives of this study were to determine if *C. cereale* can be a destructive pathogen on Chewings fescue and hard fescue, and if different cultivars and accessions of these two fine fescue species exhibit varying susceptibility to the pathogen. *C. cereale* was previously found in association with fine fescue hosts in early research (Ruemmele et al. 1995), but has not been reported as a major pathogen on fescue species in the past 20 years. The results from this study confirm that *C. cereale* isolated from symptomatic fine fescue plants from the Rutgers University Plant Science Research and Extension Farm is the causal agent responsible for the symptoms observed in the field, and that *C. cereale* can be a damaging pathogen on Chewings fescue and hard fescue, a finding that has important implications for turfgrass breeding programs.

Fine fescues are highly valued as cultivated, low-input turfgrasses, and turfgrass breeders are actively attempting to incorporate enhanced resistance to diseases such as red thread and dollar spot when developing new cultivars of fine fescues (Bonos et al. 2006). Based on our results, it is apparent that resistance to *C. cereale* should be considered when new germplasm is evaluated for disease susceptibility in the field. Red thread and anthracnose are more severe under low nitrogen fertility (Cahill et al. 1983; Inguagiato et al. 2008; Vargas 2005), and these diseases can limit the use of fine fescues as low-input turfs. Thus, improving resistance to these low fertility related diseases should be important objectives when breeding fine fescues. Incorporating resistance to *C. cereale* in fine fescues is promising because both commercially available hard fescue cultivars ('Sword' and 'Beacon') evaluated in this study exhibited good tolerance to *C. cereale* infection, whereas experimental accessions collected from Europe displayed high susceptibility to this pathogen. However, the commercially available Chewings fescue 'Ambrose' showed moderate susceptibility to the pathogenic fine fescue isolate. Among the Chewings fescue accessions, 54259-13 outperformed others with less disease when inoculated with the isolate from fine fescue. Such differences in susceptibility among these fine fescue accessions suggest that there may be natural genetic variation for resistance to anthracnose disease, and that breeding and selection techniques could be used to develop improved cultivars.

We evaluated infection at 30 °C and 60% RH to mimic what has been reported for successful infection of other grass inhabiting *Colletotrichum* species in switchgrass (*Panicum virgatum* L.) (Crouch et al. 2009b) and centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.] (Crouch and Tomaso-Peterson 2012; Fuke et al. 2006). Beirn et al. (2015) also used 30 °C for inoculating annual bluegrass with *C. cereale* isolates, including the HF217CS isolate. Although some dieback was observed at the leaf tips among control plants at the end of our experiment, repeated attempts to culture from these tissues did not identify any microorganisms, and real-time PCR results indicated a negative reaction of *C. cereale* for control plants (Fig. 2), thus these symptoms were likely the result of some heat stress from the high temperature (30 °C) in the growth chamber. As low-maintenance grasses, fine fescues are very tolerant to shade, drought, acid soils, and reduced fertility (Ruemmele et al. 1995), but they generally have poor tolerance to heat stress (Grimshaw et al. 2014). In our study, all control plants exhibited a decrease in turf quality over time, although this effect was more pronounced among the experimental accessions. The three commercial cultivars (Sword, Beacon, and Ambrose) were developed in the United States, whereas the five noncommercial accessions were collected from Europe, indicating that collections of fine fescues originating from Europe may be less tolerant to heat stress, although additional data are required to test this hypothesis.

In addition to determining that *C. cereale* FF1A is a pathogen of fine fescues, we were also able to infect the same fine fescue cultivars and accessions with *C. cereale* HF217CS obtained from annual bluegrass. We observed lesions on the leaves of Chewings fescue and hard fescue with both isolates, whereas in a previous study, isolate HF217CS did not cause lesions on annual bluegrass (Beirn et al. 2015). However, infection in our study was less severe with HF217CS than with the FF1A isolate of *C. cereale* and more variable, as evidenced by differences observed in the disease severity × incidence product ratings both within and between fine fescue species. Similar findings have been observed with isolates of *Colletotrichum* spp. from beans (*Phaseolus vulgaris* L. and *P. coccineus* L.) and the herbaceous perennial *Stylosanthes guianensis* (Abl.) SW. (Lenné and Burdon 1990; Sicard et al. 1997a, 1997b, 2007), where fungal isolates exhibit considerable variation in virulence and pathogenicity. Molecular analyses of *C. cereale* isolates obtained from putting green turf have suggested that pathogenic turfgrass isolates may exhibit a host preference, particularly in northern regions of the United States (Beirn et al. 2014). The reduced ability and the variability of *C. cereale* HF217CS to infect fine fescues in our study also suggested a possible host preference; however, additional cross-inoculation tests with a large collection of anthracnose isolates and host grass species are needed to test this hypothesis. Moreover, it is not known whether cross-infection occurs frequently in nature, or if it may only occur when plants are under environmental stresses.

The *C. cereale* isolate FF1A responsible for the disease outbreak on fine fescue at the Adelphia farm in New Jersey was identified as belonging to the clade A subgroup (Fig. 2A). Eleven specialized populations of *C. cereale* have been documented (Crouch et al. 2009a), with clade A composed of 10 distinct subgroups that correspond to ecosystem or host plant, whereas clade B encompasses one large subgroup of isolates that are from a diverse range of hosts and ecosystems (Crouch et al. 2009a). The molecular typing of isolate FF1A as clade A is consistent with other isolates collected from *Festuca* spp. from prairie or native grass settings (Beirn et al. 2014; Crouch et al. 2009a). Pathogenic isolates of *C. cereale* from putting green turf comprise both clades (Crouch et al. 2009a) and have often been found within the same putting green (Beirn et al. 2014). It remains unknown whether both clades of *C. cereale* can be found in nature on fine fescues grown as cultivated turfgrasses. Additional widespread sampling of fine fescue turfgrasses exhibiting symptoms of anthracnose will be required to better understand the distribution of fungal clades on this host. Future research is also needed to evaluate the susceptibility of fine fescue cultivars and accessions to anthracnose disease under field conditions. Anthracnose disease reported here as well as gray leaf spot disease caused by *Pyricularia oryzae* Cavara (Vines et al. 2022) were not significant problems on fine fescue species until recently, suggesting new objectives for breeding fine fescues for disease resistance.

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