Components of Resistance to *Magnaporthe grisea* in 'Coyote' and 'Coronado' Tall Fescue

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ABSTRACT

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The components of resistance in tall fescue to *Magnaporthe grisea*, the causal agent of gray leaf spot, were measured in growth chamber experiments. Cultivars ranging in susceptibility to *M. grisea* were selected: 'Kentucky 31' (susceptible), 'Rebel III' (moderately susceptible), 'Coronado' (resistant), and 'Coyote' (resistant). Plants were inoculated with nine *M. grisea* isolates representing five clonal lineages associated with tall fescue in Georgia. Compared to Kentucky 31, Coronado and Coyote exhibited longer incubation and latent periods, reduced rates of disease progress and lesion expansion, and lower final disease incidence, final foliar blight incidence, final mean lesion length, area under the lesion expansion curve, and area under the disease progress curve. No evidence of hypersensitive response was observed, all *M. grisea* isolates completed the disease cycle by producing secondary inoculum, and no differential response to isolates from different clonal lineages was detected in Coronado and Coyote. These results indicate that Coronado and Coyote have partial resistance to *M. grisea*. Measurement of resistance components using primary parameters and derived parameters yielded similar results. Foliar blight incidence data exhibited increased variation relative to other parameters and was less powerful for detection of *M. grisea* resistance. Measurements of incubation period, latent period, final disease incidence, and final mean lesion length were the most effective and efficient methods for detecting *M. grisea* resistance in tall fescue.

Additional keywords: *Pyricularia*, turfgrass

Gray leaf spot, caused by *Magnaporthe grisea* (T.T. Hebert) Yaegashi & Udagawa (anamorph *Pyricularia grisea* (Cooke) Sacc.), is a disease of increasing importance in the turfgrass industry. Before 1991, gray leaf spot was limited to St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze), a warm-season grass grown in subtropical and tropical climates (18). Since 1991, the occurrence of gray leaf spot has become a persistent problem in tall fescue (*Festuca arundinacea* Schreb.) in the southeastern United States (11) and in perennial ryegrass (*Lolium perenne* L.) in the northeastern, mid-Atlantic, and midwestern United States (8).

Variation in *M. grisea* resistance among tall fescue cultivars was identified in North Carolina in 1995 (10,11). Two cultivars, 'Coyote' and 'Coronado', exhibited a high level of resistance to *M. grisea*, whereas

'Kentucky 31' tall fescue was severely defoliated (10,11). However, Coyote and Coronado are highly susceptible to *Rhizoctonia solani* Kühn, the causal agent of brown patch, which is the most severe disease of tall fescue in the southeastern United States. In order to be commercially viable for the southeastern United States, a cultivar with resistance to *M. grisea* must also be resistant to *R. solani*. Therefore, the *M. grisea* resistance in Coronado and Coyote must be transferred into a genetic background with *R. solani* resistance.

In rice, resistance to *M. grisea* may be complete or partial (19). Complete resistance to *M. grisea* in rice is qualitative in nature, rendering the plant immune to pathogen attack through a hypersensitive response, is controlled by single genes, and is insensitive to environmental conditions. However, single genes for complete resistance are only effective against a subset of *M. grisea* races. As a result, complete resistance is unstable over time and often results in resistance breakdown after 2 to 3 years of use in the field (21).

Most sources of partial resistance to *M. grisea* in rice are effective against all races of the pathogen and therefore stable over time (19). The use of partial resistance has been limited in the breeding of rice for resistance to *M. grisea*. During intense selection of inbred rice lines for complete resistance, genes for partial resistance to

the pathogen may have been lost (24). The inheritance and genetic mechanisms of partial resistance to *M. grisea* in rice are poorly understood (29). Some sources of partial resistance are polygenic (17), whereas others are apparently controlled by major genes (13). This lack of understanding makes the transfer of partial resistance into commercial cultivars difficult (20,28,29). In addition, detection of partial resistance in breeding lines with genes for complete resistance is difficult due to epistasis (24).

Tall fescue is an outcrossing, self-sterile allohexaploid ($2n=6x=42$) species (1), as opposed to rice, which is a self-pollinating diploid (2). Cultivars of tall fescue are synthetic, produced by cross-pollination among selected parents to produce a heterogeneous population of genotypes. Many contemporary tall fescue cultivars originate from introductions from Europe made in the 1800s (1). Kentucky 31 tall fescue was selected from a pasture in Kentucky and released in 1942 (9), and it remains the most widely used cultivar for pastures and low maintenance turf areas (3). Turf-type varieties, such as Coronado, Coyote, and 'Rebel III', originate from selections made from old turf areas in the northeastern and southeastern United States (12,15,23).

Given the breeding system in tall fescue and the origins of Coronado and Coyote, it is likely that these cultivars possess partial resistance to *M. grisea* as opposed to complete resistance. Inheritance studies have shown that the resistance in Coronado and Coyote are controlled by several genes (14), providing additional support for this hypothesis. Partial resistance may be an effective tool for management of gray leaf spot. In turfgrasses, a single, isolated lesion does not cause significant reductions in stand quality. Visible damage to the stand occurs when lesions expand and coalesce to girdle the leaf blade, producing a foliar blight symptom. Therefore, if the number of lesions or the rate of lesion expansion could be reduced, the impact of gray leaf spot on turfgrass quality may be reduced to acceptable levels. In a perennial crop, such as turfgrasses, complete resistance may break down rapidly due to high selection pressure for alternative races of *M. grisea*.

In the rice and wheat pathosystems, *M. grisea* populations are composed of genetically isolated groups of individuals called clonal lineages (16). These clonal lineages

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correspond to distinct races of the pathogen or groups of races with similar virulence profiles (6,7,16). From tall fescue stands in Georgia, five clonal lineages of *M. grisea* have been identified using amplified fragment length polymorphism (AFLP) markers, and they are designated E, G1, G2, G4, and H (27). Lineage G4 was dominant in each of four populations from tall fescue in 1999 and 2000, comprising 65 to 100% of the populations. Lineages E and H were present in one location, whereas lineages G1 and G2 were isolated in three and four locations, respectively. It is unknown if these clonal lineages from tall fescue vary in virulence or correspond to distinct races that will exhibit differential responses to the resistance in Coronado and Coyote.

Accurate detection of disease resistance in breeding programs requires careful selection of the parameters to be measured and the methods of data analysis (29). Disease resistance may be expressed through several different phenotypes, or components of resistance: infection efficiency, incubation period, lesion expansion, latent period, and secondary inoculum production (22). Parameters for quantifying resistance components are either primary, using the raw data, or derived, calculated from raw data using formulas or regression analysis. For example, infection efficiency may be quantified using disease incidence (primary parameter) or disease progress rate and area under the disease progress curve (derived parameters). Similarly, lesion expansion may be quantified using mean lesion length (primary parameter) or lesion expansion rate and area under the lesion expansion curve (derived parameters). Other parameters, such as foliar blight incidence, combine several resistance components (incubation period, infection efficiency, lesion expansion) into a single measurement.

All of the resistance components have been detected in sources of partial resistance to *M. grisea* in rice (5,24,25,29,30). Genetic analysis of partial resistance to *M. grisea* in rice indicates that unique loci are responsible for conferring the individual resistance phenotypes (28). Parameters for measuring partial resistance to *M. grisea* in tall fescue must be selected not only based on efficiency of data collection and amount of variation, but also based on the resistance phenotypes that are expressed by the resistance sources.

In the following growth chamber experiments, we measured the components of resistance in Coronado, Coyote, Rebel III, and Kentucky 31 tall fescue inoculated with *M. grisea* isolates representing the five clonal lineages identified from tall fescue in Georgia. The specific objectives of this study were to: (i) identify the resistance components that are responsible for the *M. grisea* resistance observed in Coyote and Coronado tall fescue; (ii) determine if Coronado and Coyote tall fescue

possess complete or partial resistance to *M. grisea*; (iii) determine if the resistance in Coronado and Coyote exhibits differential responses to isolates belonging to different clonal lineages; and (iv) investigate the relationship between virulence and frequency of clonal lineages in *M. grisea* populations associated with tall fescue.

MATERIALS AND METHODS

Turfgrass establishment and maintenance. Tall fescue cultivars ranging in susceptibility (10,11) to *M. grisea* were selected: Coyote (resistant), Coronado (resistant), Rebel III (moderately susceptible), and Kentucky 31 (susceptible). Each cultivar was seeded at a rate of 4.4 mg cm⁻² into 946.4-cm³ Styrofoam cups containing calcined clay (Turface Allsport, Profile Products LLC, Buffalo Grove, IL). The cups were transferred to a greenhouse, covered with paper, and misted four times per day to encourage rapid seed germination. Using this method, seed germination is typically observed within 4 to 5 days after seeding. The grass was maintained in the greenhouse for 10 weeks after seeding by cutting to a height of 9 cm weekly and fertilizing weekly with 1.3 g N m⁻² (Peters 20-10-20, Scotts-Sierra Horticultural Products Co., Marysville, OH). Prior to inoculation, the number of leaves in 10 cups of each cultivar was counted to provide an estimate of the number of leaves in each cup for calculation of disease incidence.

Inoculum production. Nine *M. grisea* isolates representing the five clonal lineages associated with tall fescue in Georgia were selected randomly from a collection of 491 isolates collected in 1999 and 2000. The isolates were stored at -80°C on infested rye grains (*Secale cereale* L.). Isolates were revived by placing three to five infested grains on the surface of a 90-mm petri dish containing potato dextrose agar (PDA), amended with 50 mg each of tetracycline, streptomycin, and chloramphenicol per liter. After 1 week of incubation at 20°C with no light, each isolate was transferred to five 90-mm petri dishes containing 1.5% water agar. The agar surface was overlaid with 15 to 20 sterilized alfalfa stem sections (6 cm long), with leaves and petioles removed. The cultures were placed at room temperature (23 to 25°C) with continuous fluorescent illumination. After 3 weeks, conidia were harvested by transferring the alfalfa stem sections to 50-ml conical centrifuge tubes (Corning Inc., Corning, NY) containing 20 ml of H₂O, vortexing for 10 s, then filtering the suspension through a single layer of cheesecloth. The conidial suspension was adjusted to a concentration of 2×10^5 conidia ml⁻¹ using a hemacytometer.

Inoculation and incubation conditions. A factorial treatment arrangement and a split-plot, randomized complete block experimental design were used in this experiment, with the tall fescue culti-

vars representing subplots and individual *M. grisea* isolates applied to main plots. Ten weeks after seeding, one cup of each tall fescue cultivar was randomly arranged in plastic containers (41.2 × 28.7 × 25.3 cm) that had a single 6.35-mm-diameter hole at a height of 2.54 cm to permit water drainage. The grass in each container was inoculated with 20 ml of conidial suspension from a single isolate applied with an air-brush (Model 350, Badger Air-Brush Co., Franklin Park, IL) powered with compressed CO₂ at 2.2 kg m⁻². Immediately after inoculation, the lids were placed on the plastic containers, which were transferred to a single growth chamber and arranged in a randomized complete block with three replications. Incubation conditions for the first 24 h were 24°C, 100% relative humidity (RH), and no light. Subsequently, the growth chamber was programmed for 12-h days at 30°C and 75% RH and 12-h nights (no light) at 24°C and 100% RH. The lids were removed from the plastic containers for each day cycle. At the beginning of each night cycle, the cups were misted with water and the lids were placed on the containers to maintain continuous and uniform humidity and leaf wetness. An auxiliary humidifier (Herrmidifier 500, Trion Inc., Sanford, NC) was used to maintain 100% RH in the growth chamber during the initial incubation and during each night cycle.

Disease development. Components of disease development data were collected as described by Seebold et al. (25) with modifications. Incubation period was determined by examining the foliage in each cup for gray leaf spot symptoms daily at 1000 h. The incubation period was considered lapsed when one or more gray leaf spot lesions were observed in a cup. The number of days between inoculation and symptom expression was recorded as the incubation period for each cup.

Two distinct symptoms of gray leaf spot were observed in inoculated tall fescue plants in this study. Initial symptoms of the disease were distinct leaf spots that were tan in color with dark brown borders. In some cases, the lesions expanded and coalesced to girdle the leaf blade, producing a foliar blight symptom, where the distal portions of the leaf blade became twisted, shriveled, and necrotic. Both symptoms were considered when assessing disease incidence (leaf spot incidence + foliar blight incidence), but the incidence of foliar blight was also assessed separately (foliar blight incidence).

Disease incidence and foliar blight incidence were recorded daily at 1000 h for 7 days beginning 24 h after inoculation. Disease incidence was determined by counting the number of leaves exhibiting gray leaf spot symptoms in each cup and dividing by the average number of leaves per cup for that cultivar. Foliar blight incidence was determined by counting the number of leaf

blades that were shriveled, twisted, and necrotic in each cup and dividing by the average number of leaves per cup for that cultivar.

Mean lesion length was assessed daily at 1000 h for 7 days beginning 24 h after inoculation. Mean lesion length was determined by measuring the length of 10 lesions per cup using a handheld digital caliper (Series 500, Mitutoyo Corp., Aurora, IL). The lesions to be measured were selected arbitrarily on each day, and thus the same set of lesions was not measured every day. When fewer than 10 lesions were present in a cup, the lengths of all lesions in that cup were measured.

Latent period was determined by examining all lesions in each cup daily at 1000 h for a visible change in color from tan to gray, indicating sporulation of *M. grisea* within the lesion. When a color change was detected in a lesion, the presence of conidia was confirmed microscopically by pressing clear cellophane tape to the lesion, removing the tape, affixing the tape to a glass microscope slide, and examining the slide under a compound microscope for the presence of conidia. The latent period was considered lapsed for a cup when sporulation within a single lesion was confirmed. The number of days between inoculation and sporulation was recorded as the latent period for each cup.

Secondary inoculum production was assessed 3 days after the latent period had elapsed for each individual cup. Ten symptomatic leaves from each cup were placed in screw cap vials (17 × 60 mm) containing 2 ml of a 1% CuSO₄, 1% Triton X-100 (Sigma-Aldrich Co., St. Louis, MO) solution, then vortexed for 1 min to disperse the conidia. If fewer than 10 symptomatic leaves were present in a cup, then all symptomatic leaves were collected. The concentration of conidia in each sample was estimated with a hemacytometer, and the total number of conidia per sample was calculated. The symptomatic leaves were removed from the conidial suspension, blotted dry with paper towels, arranged on

a piece of black construction paper, and affixed to the construction paper with clear packing tape. A digital image of each sample was obtained using a flatbed scanner (Astra 2200, UMAX Technologies, Inc., Fremont, CA). The total lesion surface area in each sample was measured using UTHSCSA ImageTool v. 2.03 (Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio). The number of conidia produced per unit lesion surface area was calculated for each sample.

Viability of conidia. The viability of conidia produced by each isolate was assessed in vitro. Conidial suspensions were produced as described above, adjusted to 2×10^5 conidia per ml of H₂O using a hemacytometer, and spread in 100-μl aliquots on four petri dishes containing 1.5% water agar. The cultures were incubated for 24 h at 20°C with no light. Between 100 and 150 conidia were examined under a compound microscope and rated as either germinated or not germinated based on the presence or absence of a germ tube. The frequency of viable conidia was determined by dividing the number of germinated conidia by the total number examined.

Data analysis. All statistical analyses were conducted using SAS v. 8.2 (SAS Institute, Cary, NC). The primary parameters analyzed were incubation period, latent period, final disease incidence (7 days after inoculation [DAI]), final foliar blight incidence (7 DAI), final mean lesion length (7 DAI), secondary inoculum density, and conidial viability. Several derived parameters were also assessed: disease progress rate, lesion expansion rate, area under the disease progress curve (AUDPC), and area under the lesion expansion curve (AULEC). Disease progress rate and lesion expansion rate were determined for each cup from the seven observations of disease incidence and mean lesion length, respectively, using simple linear regression (4). AUDPC and AULEC were calculated from the disease incidence and mean lesion

length data, respectively, using the formula $\sum_{i=1}^{n-1} [(y_i + y_{i+1})/2](t_{i+1} - t_i)$, where $i = 1, 2, 3, 4, 5, 6, 7$, y_i is disease incidence or mean lesion length on the i th rating date, and t_i is the day of the i th rating (4). For all primary and derived parameters, analysis of variance (ANOVA) was used to test the significance of main effects (cultivar and isolate) and the first-order interaction (cultivar × isolate). Means separations were conducted using the Waller-Duncan k -ratio t test ($k = 100$). The experiments were repeated twice, and the results of each experiment were analyzed separately.

RESULTS

Turfgrass establishment. In experiment 1, leaf counts in 10 cups of each cultivar yielded the following mean (and standard deviation) for each cultivar: Coronado, 204.6 (24.0); Coyote, 220.9 (24.8); Kentucky 31, 107.7 (18.0); and Rebel III, 157.0 (23.6). In experiment 2, leaf counts yielded the following mean (and standard deviation): Coronado, 279.6 (12.2); Coyote, 290.7 (9.4); Kentucky 31, 172.4 (11.2); and Rebel III, 249.1 (16.2).

Disease development. In experiment 1, significant differences among cultivars were detected for all parameters measured except secondary inoculum density, disease progress rate, and lesion expansion rate (Table 1). Compared with other cultivars, Kentucky 31 exhibited significantly shorter incubation and latent periods and higher final disease incidence, final foliar blight incidence, final mean lesion length, AUDPC, and AULEC (Table 2). Coronado, Coyote, and Rebel III were similar in their responses to *M. grisea* inoculation. Incubation period was significantly longer for Coronado than for Coyote and Rebel III, whereas final disease incidence and AUDPC were significantly lower for Coyote than for Rebel III and Coronado.

In experiment 1, significant differences among isolates were detected in final disease incidence, final foliar blight incidence, final mean lesion length, secondary inoculum density, disease progress rate,

Table 1. *P* values for cultivar, isolate, and cultivar × isolate interactions according to analysis of variance conducted on components of resistance of tall fescue to *Magnaporthe grisea*

Resistance component	Experiment 1					Experiment 2				
	Cultivar	Isolate	Cultivar × isolate	CV ^a	R ²	Cultivar	Isolate	Cultivar × isolate	CV ^a	R ²
Incubation period	<0.0001	0.7455	0.8632	26.84	0.45	<0.0001	0.1618	0.1871	23.08	0.55
Latent period	<0.0001	0.4842	0.2357	11.18	0.48	<0.0001	0.007	0.1447	9.92	0.59
Final disease incidence	<0.0001	0.0011	0.4475	42.78	0.61	<0.0001	0.1954	0.7602	39.90	0.86
Final foliar blight incidence	0.0007	0.0271	0.1256	153.47	0.50	<0.0001	0.0017	0.0025	107.54	0.80
Final mean lesion length	<0.0001	0.0121	0.0456	22.71	0.62	<0.0001	0.0182	0.2761	19.06	0.72
Secondary inoculum density	0.2152	<0.0001	0.1153	77.55	0.53	0.6796	0.1243	0.7563	87.46	0.32
Disease progress rate	0.1485	0.0425	0.4825	58.44	0.39	<0.0001	0.2283	0.683	39.69	0.87
Lesion expansion rate	0.5575	0.0932	0.8665	32.62	0.31	<0.0001	0.0016	0.2558	20.12	0.64
AUDPC ^y	<0.0001	<0.0001	0.4435	45.95	0.69	<0.0001	0.0081	0.4013	39.89	0.88
AULEC ^z	<0.0001	0.4472	0.8852	23.58	0.59	<0.0001	0.007	0.5115	16.33	0.84

^a Coefficient of variation according to analysis of variance.

^y Area under the disease progress curve.

^z Area under the lesion expansion curve.

and AUDPC (Table 1). Isolates within a lineage were similar in virulence, except for lineage E, for which significant differences among isolates were detected for AUDPC, and lineage G4, for which significant differences among isolates were detected for final mean lesion length and secondary inoculum density (Table 3). Inoculation with isolates 1210-17 and 1213-26 (lineage G2) and 533-78 (lineage G1) resulted in lower AUDPC and final disease incidence compared with other isolates. Isolate 1207-59 (lineage G4) produced significantly greater mean final lesion length and secondary inoculum production than all other isolates.

In experiment 1, a significant cultivar $\times$ isolate interaction was detected for final mean lesion length (Table 1). On Kentucky 31, isolate 1213-59 induced lesions that were significantly longer than those induced by all isolates except 1213-90, 533-78, 1210-17, and 1210-57 (Fig. 1). However, on Coronado, Coyote, and Rebel III, the lesions induced by isolates 1213-59 and 1213-37 were either significantly shorter or not significantly different when compared with all other isolates.

The coefficient of variation ranged from 11.18 to 153.47, and R^2 values ranged from 0.31 to 0.69 for the parameters analyzed in experiment 1 (Table 1). Coefficients of

variation >35 were detected for incubation period, latent period, final mean lesion length, lesion expansion rate, and AULEC. R^2 values >0.50 were detected for final disease incidence, final mean lesion length, secondary inoculum density, AUDPC, and AULEC.

Significant differences among cultivars were detected in experiment 2 for all pa-

rameters measured except secondary inoculum density (Table 1). Kentucky 31 was more susceptible to *M. grisea* than the other cultivars according to all resistance components except incubation period and secondary inoculum density (Table 4). Compared with Rebel III and Kentucky 31, Coronado and Coyote exhibited longer incubation and latent periods, and reduced

Fig. 1. Effects of tall fescue cultivar and *Magnaporthe grisea* isolate on final mean lesion length in experiment 1. Minimum significant difference (MSD) according to the Waller-Duncan k -ratio t test ($k = 100$) is 0.79.

Table 2. Components of resistance in tall fescue cultivars varying in susceptibility to *Magnaporthe grisea*, experiment 1

Cultivar	Incubation period (days)	Latent period (days)	Final dis. incidence (%)	Final foliar blight incidence (%)	Final mean lesion length (mm)	Secondary inoc. density (conidia/mm ²)	Disease progress rate (%/day)	Lesion expansion rate (mm/day)	AUDPC (% ²) ^x	AULEC (mm ²) ^y
Coronado	3.9 a ^z	6.2 a	8.9 bc	0.56 b	3.5 b	277 a	2.0 a	0.73 a	0.24 bc	8.5 b
Coyote	3.5 b	6.0 a	7.7 c	0.59 b	3.5 b	270 a	1.9 a	0.73 a	0.23 c	9.4 b
Kentucky 31	2.5 c	5.3 b	16.6 a	1.89 a	4.8 a	370 a	2.6 a	0.81 a	0.59 a	13.9 a
Rebel III	3.3 b	6.0 a	10.9 b	0.54 b	3.2 b	384 a	2.0 a	0.74 a	0.31 b	9.6 b

^x Area under the disease progress curve.

^y Area under the lesion expansion curve.

^z Within a column, means followed by the same letter are not significantly different according to Waller-Duncan k -ratio t test ($k = 100$).

Table 3. Virulence of *Magnaporthe grisea* isolates belonging to clonal lineages associated with tall fescue in Georgia, averaged over four tall fescue cultivars, experiment 1

Lineage	Isolate	Final disease incidence (%)	Final foliar blight incidence (%)	Final mean lesion length (mm)	Secondary inoculum density (conidia/mm ²)	AUDPC (% ²) ^y	Conidial viability (%)
E	1213-2	11.6 ab ^z	0.5 b	3.5 b	142 d	0.40 b	98.1 a
	1213-59	15.4 a	0.6 b	3.7 b	199 cd	0.53 a	96.9 ab
G1	533-78	8.4 bc	0.8 ab	3.8 b	316 bcd	0.25 cd	91.7 d
	1213-90	12.3 ab	1.5 ab	3.7 b	441 b	0.34 bc	95.5 bc
G2	1210-17	8.4 bc	0.5 b	3.7 b	314 bcd	0.22 cd	87.9 e
	1213-26	7.0 c	0.3 b	3.6 b	293 bcd	0.19 d	95.3 bc
G4	1207-59	12.2 ab	2.1 a	4.7 a	697 a	0.38 b	96.3 abc
	1210-57	12.7 a	1.3 ab	3.8 b	356 bc	0.37 b	94.9 c
H	1213-37	11.5 ab	0.5 b	3.3 b	170 cd	0.39 b	95.0 c

^y Area under the disease progress curve.

^z Within a column, means followed by the same letter are not significantly different according to Waller-Duncan k -ratio t test ($k = 100$).

final disease incidence, final mean lesion length, disease progress rate, AUDPC, and AULEC. Lesion expansion rates were similar in Coronado and Rebel III, but significantly greater than in Coyote. In addition, Coyote had significantly lower final disease incidence, final mean lesion length, disease progress rate, AUDPC, and AULEC compared with Coronado.

In experiment 2, significant differences were detected among isolates in latent period, final foliar blight incidence, final mean lesion length, lesion expansion rate, AUDPC, and AULEC (Table 1). Isolates within a lineage were similar in virulence, with the exception of isolates in lineage G1, which differed significantly in AUDPC, foliar blight incidence, and AULEC (Table 5). Isolates in lineage G4 were not consistently more virulent than isolates belonging to other lineages.

In experiment 2, a significant cultivar $\times$ isolate interaction was detected for final foliar blight incidence (Table 1). Isolate 1210-57, which induced a moderate incidence of foliar blight on Kentucky 31 but the highest final foliar blight incidence on both Coronado and Rebel III, may have contributed to this interaction (Fig. 2). In addition, foliar blight symptoms were not observed in Coyote in experiment 2, but they were observed in Coronado, Coyote, and Rebel III.

The coefficient of variation ranged from 9.92 to 107.54, and R^2 values ranged from

0.32 to 0.88 for the parameters analyzed in experiment 2 (Table 1). Coefficients of variation <25 were detected for incubation period, latent period, final mean lesion length, lesion expansion rate, and AULEC. R^2 values >0.70 were detected for final disease incidence, final foliar blight incidence, final mean lesion length,

disease progress rate, AUDPC, and AULEC.

Viability of conidia. Significant differences in conidial viability were detected among the isolates utilized in this study. In experiment 1, conidial viability of isolates 533-78 and 1210-17 was significantly lower than that of all other isolates (Table

Fig. 2. Effects of tall fescue cultivar and *Magnaporthe grisea* isolate on final foliar blight incidence in experiment 2. Minimum significant difference (MSD) according to the Waller-Duncan k -ratio t test ($k = 100$) is 1.2.

Table 4. Components of resistance in tall fescue cultivars varying in susceptibility to *Magnaporthe grisea*, experiment 2

Cultivar	Incubation period (days)	Latent period (days)	Final disease incidence (%)	Final foliar blight incidence (%)	Final mean lesion length (mm)	Secondary inoc. density (conidia/mm ²)	Disease progress rate (%/day)	Lesion expansion rate (mm/day)	AUDPC (% ²) ^x	AULEC (mm ²) ^y
Coronado	3.4 a ^z	5.9 b	5.2 c	0.09 b	2.6 c	315 a	1.0 c	0.55 b	0.15 c	7.8 c
Coyote	3.7 a	6.3 a	2.7 d	0.00 b	2.0 d	385 a	0.4 d	0.43 c	0.08 d	5.5 d
Kentucky 31	2.7 b	5.3 d	22.3 a	4.49 a	3.7 a	314 a	4.6 a	0.68 a	0.79 a	12.5 a
Rebel III	2.7 b	5.6 c	8.6 b	0.47 b	3.0 b	296 a	1.7 b	0.57 b	0.28 b	9.9 b

^x Area under the disease progress curve.

^y Area under the lesion expansion curve.

^z Within a column, means followed by the same letter are not significantly different according to Waller-Duncan k -ratio t test ($k = 100$).

Table 5. Virulence of *Magnaporthe grisea* isolates belonging to clonal lineages associated with tall fescue in Georgia, average over four tall fescue cultivars, experiment 2

Lineage	Isolate	Latent period (days)	Final foliar blight incidence (%)	Final mean lesion length (mm)	Lesion expansion rate (mm/day)	AUDPC (% ²) ^x	AULEC (mm ²) ^y	Conidial viability (%)
E	1213-2	5.4 bc ^z	0.8 bc	2.6 bc	0.49 bc	0.40 a	9.1 a-d	96.6 a
	1213-59	5.2 c	0.5 c	2.5 c	0.50 bc	0.38 a	9.1 a-d	94.3 bc
G1	533-78	5.9 ab	0.9 bc	2.7 abc	0.54 abc	0.26 bc	8.2 cd	81.1 e
	1213-90	5.9 ab	2.4 a	2.9 abc	0.56 abc	0.40 a	9.9 a	92.5 cd
G2	1210-17	6.1 a	1.2 bc	3.1 ab	0.62 a	0.22 c	8.3 cd	91.3 d
	1213-26	5.8 ab	1.0 bc	2.8 abc	0.58 ab	0.28 abc	8.5 bcd	95.5 ab
G4	1207-59	5.8 ab	2.4 a	3.2 a	0.63 a	0.34 ab	9.7 ab	93.1 cd
	1210-57	5.8 ab	1.8 ab	3.0 abc	0.63 a	0.34 ab	9.5 abc	91.5 d
H	1213-37	5.8 ab	0.6 c	2.6 c	0.48 c	0.33 abc	7.9 d	96.7 a

^x Area under the disease progress curve.

^y Area under the lesion expansion curve.

^z Within a column, means followed by the same letter are not significantly different according to Waller-Duncan k -ratio t test ($k = 100$).

3). Viability of conidia produced by other isolates ranged from 94.9 to 98.1%. In experiment 2, conidial viability of isolate 533-78 was significantly lower than that of all other isolates, which ranged in viability from 91.3 to 96.7% (Table 5).

DISCUSSION

The results of this study provide additional support for the hypothesis that Coronado and Coyote tall fescue have partial resistance to *M. grisea*. No evidence of a hypersensitive response was observed on Coronado or Coyote following inoculation with *M. grisea*. Every *M. grisea* isolate used in this study completed the disease cycle on both resistant cultivars by producing secondary inoculum, which is a fundamental characteristic of partial resistance (22). No differential response in Coronado and Coyote to isolates from different clonal lineages of *M. grisea* was detected. Two significant cultivar $\times$ isolate interactions were detected in this study, but the interactions were not the result of a differential response by Coronado or Coyote and were not consistent among two independent experiments. Finally, several resistance components contributed to the observed resistance in Coronado and Coyote.

Compared to Kentucky 31, Coyote and Coronado consistently exhibited longer incubation and latent periods, and lower final disease incidence, final foliar blight incidence, final mean lesion length, AUDPC, and AULEC. Significant differences in disease progress rate and lesion expansion rate were observed in experiment 2 but not in experiment 1, and no significant difference in secondary inoculum density was observed in either experiment. The differences in resistance components observed in this study were similar to those reported among rice cultivars with and without partial resistance (5,25,29,30). Over repeated cycles of epidemic development, small numerical differences among cultivars are amplified. For example, six disease cycles may occur in a 30-day period on a cultivar with a latent period of 5 days, whereas only five cycles may occur on a cultivar with a latent period of 6 days in the same period of time.

Rebel III tall fescue varied in its response to *M. grisea* inoculation between the two experiments. In experiment 1, Rebel III was not significantly different from the resistant cultivars Coronado and Coyote for any of the measured parameters. However, in experiment 2, Rebel III was intermediate between the resistant cultivars and Kentucky 31. The results of experiment 2 more closely correspond to field results, where Rebel III appears to be moderately susceptible (10,11). Varying conditions in the greenhouse during the 10 weeks between seeding and inoculation may be responsible for differences between the two experiments. The cultivar Rebel III may possess partial resistance to *M. grisea*,

but the resistance may be influenced by environmental conditions to a greater extent than in Coyote and Coronado, resulting in the appearance of being moderately susceptible.

Similar methods that are used to quantify disease resistance may also be used to quantify the virulence of pathogen strains and detect differential responses of resistance sources to pathogen strains (26). In this study, *M. grisea* isolates within a clonal lineage were similar in their virulence to tall fescue cultivars. However, there were also many similarities among isolates from different lineages, and some significant differences within lineages were observed. One isolate from lineage G1 (533-78) and both isolates from lineage G2 (1210-17 and 1213-26) exhibited reduced virulence for most of the parameters measured when compared with isolates from other lineages. Reduced conidium viability may be partially involved in the reduced virulence of isolate 533-78, but isolates 1210-17 and 1213-26 did not consistently exhibit reduced conidium viability. Isolates from lineage G4, which was dominant in populations from tall fescue in Georgia (27), were among the most virulent isolates for each parameter measured, but were not significantly different from most other isolates. Based on the limited sample size in this study, the dominance of lineage G4 in *M. grisea* populations from tall fescue in Georgia cannot be explained by an increased virulence of the isolates in this lineage. Other factors, such as founder effects or enhanced overwintering ability, may be involved and must be investigated further.

All of the resistance components, except secondary inoculum density, were found to be present in Coronado and Coyote tall fescue in this study. The primary parameters and derived parameters that were measured provided very similar results. From a data collection standpoint, primary parameters are favored because they require a single measurement, as opposed to derived parameters, which require multiple measurements on regular intervals. Foliar blight incidence may be the most efficient parameter to measure, as it combines incubation period, infection efficiency, and lesion expansion components in a single measurement. However, measurements of foliar blight incidence had high variation relative to other parameters, and thus were less powerful for detection of *M. grisea* resistance. In experiment 2, no differences in foliar blight incidence were detected among Coronado, Coyote, and Rebel III, even though Rebel III was significantly more susceptible than Coronado and Coyote according to most other parameters. Measurements of incubation period, latent period, final disease incidence, and final mean lesion length appear to be the most effective and efficient methods for detecting resistance to *M. grisea* in tall fescue.

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