Received: 29 April 2020 | Accepted: 10 September 2020 | Published online: 12 January 2021

DOI: 10.1002/csc2.20371

Crop Science

SPECIAL ISSUE: INTERNATIONAL TURFGRASS RESEARCH CONFERENCE

Turfgrass Genetics and Breeding

Identification of sources of resistance to gray leaf spot in *Stenotaphrum* germplasm

Esdras M. Carbajal[1] | **Bangya Ma**[2] | **M. Carolina Zuleta**[1] | **W. Casey Reynolds**[3] | **Consuelo Arellano**[4] | **Lane P. Tredway**[5] | **Susana R. Milla-Lewis**[1]

[1] Dep. of Crop and Soil Sciences, North Carolina State Univ., Box 7620, Raleigh, NC 27695, USA

[2] SePRO Research and Technology, 16013 Watson Seed Farm Rd., Whitakers, NC 27891, USA

[3] Turfgrass Producers International, 444 E. Roosevelt Rd. Suite 346, Lombard, IL 60148, USA

[4] Dep. of Statistics, North Carolina State Univ., Box 8203, Raleigh, NC 27695, USA

[5] Dep. of Plant Pathology, North Carolina State University, Box 7616, Raleigh, NC 27695, USA

Correspondence
Susana R. Milla-Lewis, Dep. of Crop and Soil Sciences, North Carolina State Univ., Box 7620, Raleigh, NC 27695, USA.
Email: susana_milla-lewis@ncsu.edu

Assigned to Associate Editor Bradley Bushman.

Funding information
North Carolina Crop Improvement Association, Grant/Award Number: NA; North Carolina State University Center for Turfgrass Environmental Research and Education, Grant/Award Number: NA

Abstract

St. Augustinegrass [*Stenotaphrum secundatum* (Walter) Kuntze] is a popular warm-season turfgrass in the southern United States. Gray leaf spot (GLS), caused by the fungal pathogen *Pyricularia oryzae* Cavara, is one of the major diseases in St. Augustinegrass. Although previous studies have reported polyploid lines with resistance to GLS, no comprehensive evaluations of sources of resistance have been performed in the genus. Such evaluations will enable breeders to identify resistant parents for cultivar development. In this study, 58 genotypes of St. Augustinegrass and two genotypes of pembagrass [*Stenotaphrum dimidiatum* (L.) Brongn.] were screened for resistance to three different *P. oryzae* sources of inoculum under controlled environmental conditions. The parameters evaluated were incubation period, number of leaves with lesions, mean lesion length, area under the disease progress curve (AUDPC), and area under the lesion expansion curve (AULEC). Significant differences among genotypes were identified. Polyploid genotypes PI 365031, PI 290888, PI 300129, PI 300130, and cultivar 'FX-10' and diploid genotype PI 410353 consistently showed high levels of resistance across trials, inoculum sources, and parameters. The identification of resistance genes in diploid genotypes is of great importance for future St. Augustinegrass breeding efforts, as this germplasm pool can be more readily exploited because of the lack of reproductive barriers with most commercial cultivars and plant introductions.

1 | INTRODUCTION

Gray leaf spot (GLS) is a turfgrass disease caused by the fungus *Pyricularia oryzae* Cavara. Gray leaf spot is one of the most common diseases of St. Augustinegrass [*Stenotaphrum secundatum* (Walter) Kuntze], a warm-season turfgrass widely distributed in tropical and subtropical regions of the world. St. Augustinegrass is well adapted to shady, humid and sandy environments which make this grass a good candidate for lawns and landscapes in coastal areas (Busey, 1995).

Gray leaf spot develops when warm temperatures coincide with long periods of leaf wetness (Moss & Trevathan,

Abbreviations: AUDPC, area under the disease progress curve; AULEC, area under the lesion expansion curve; GLS, gray leaf spot; INCID D7, incidence on Day 7 after inoculation; RH, relative humidity.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2020 The Authors. *Crop Science* published by Wiley Periodicals, Inc. on behalf of Crop Science Society of America

1987; Uddin, Serlemitsos, & Viji, 2003). The disease initially appears on leaf blades as small, water-soaked lesions that become necrotic spots. As the disease progresses, necrotic spots coalesce and cause partial or complete blighting of the leaf blades (Harmon, Datnoff, Nagata, Brecht, & Stiles, 2005; Smiley, Dernoden, & Clarke, 2005). Under favorable conditions, GLS develops rapidly, and entire grass swards can be killed within a few days. Cultural management practices can slow disease development (Datnoff, Brecht, Stiles, & Rutherford, 2005; Harmon et al., 2005) but do not provide adequate control when disease pressure is high. Certain fungicides provide effective GLS control but often are not economical for sod production, and limited products are registered for application to residential lawns. Furthermore, *P. oryzae* can quickly develop resistance to single-site fungicides (Vincelli & Dixon, 2002). Development of St. Augustinegrass cultivars with genetic resistance to GLS would be a potential alternative for managing the disease (Atilano & Busey, 1983; Harmon et al., 2005). Breeding for GLS resistance has been successful in other turfgrass species including perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Festuca arundinacea* Schreb.) (Tredway, Stevenson, & Burpee, 2003).

Although GLS was first reported in St. Augustinegrass in 1957 (Malca & Owen, 1957), the first evaluation of germplasm for GLS resistance was performed in 1964, and it was not until the 1980s when a more significant number of genotypes, including African and Caribbean plant introductions, gulf coast selections, hybrids, and mutants, were evaluated (Atilano & Busey, 1983; Freeman, 1964). Both studies identified pembagrass [*Stenotaphrum dimidiatum* (L.) Brongn.], a wild relative of St. Augustinegrass, accessions PI 289729 and PI 365031, as highly resistant (Atilano & Busey, 1983). Later in 2005, a GLS evaluation reported the polyploid cultivar FX-10 as the most resistant genotype (Harmon et al., 2005). Metz, Chandra, Genovesi, and Campos (2011) reported polyploids FX-10, TAES 5382, and TAES 5384, along with interploids DALSA 0605 and DALSA 0607 to be highly resistant and resistant, respectively.

Since first reported, most St. Augustinegrass breeding efforts have concentrated in the diploid gene pool, with only a few polyploid cultivars released. Interploid breeding in St. Augustinegrass is limited because of sexual hybridization barriers among different ploidy levels and also, to a lesser extent, because of restricted germplasm availability. Thus, examples of successful introgression of traits of interest from polyploid genotypes into commercial cultivars are few, with FX-10 being one example. Early attempts at using conventional breeding methods to cross genotypes of different ploidy levels resulted in failure (Busey, 2003), until more recently when in vitro embryo rescue techniques were used to successfully develop interploid populations (Genovesi, Russel, Engelke, & Burson, 2009).

Core Ideas

- Significant variation for GLS response was identified in diploid and polyploid germplasm.
- Polyploid genotypes PI 365031, PI 290888, PI 300129, PI 300130, and FX-10 were confirmed as highly resistant.
- Diploid PI 410353 had high levels of resistance comparable with those of polyploid materials.
- This is the first report of high levels of GLS resistance in diploid germplasm of St. Augustinegrass.

Given the difficulties in exploiting the genetic variation present among *Stenotaphrum* polyploids and the lack of a comprehensive evaluation of GLS resistance in the species, the objectives of the present study were (a) to evaluate a diverse collection of *Stenotaphrum* germplasm for resistance to isolates of *P. oryzae*, and (b) to determine if resistance to GLS is expressed in diploid genotypes and, if so, how it compares to that of polyploids.

2 | MATERIAL AND METHODS

2.1 | Plant material, establishment, and maintenance

Fifty eight St. Augustinegrass genotypes were included (Table 1): 21 commercial cultivars, 17 plant introductions (PI) from the National Plant Germplasm System (NPGS, Griffin, GA), and 20 accessions from the North Carolina State University turfgrass breeding program germplasm collection. Additionally, the only two available accessions of pembagrass [*Stenotaphrum dimidiatum* (L.) Brongn.] in the U.S. germplasm collection were included in this study as resistant controls. Genotypes were vegetatively propagated from greenhouse-grown plants by planting five two-node stolons into 473-ml (16-oz.) styrofoam cups (Dart Container Corporation) filled with Profile Greens Grade (Profile Products). Plants were maintained in a walk-in growth chamber at the Southeastern Plant Environment Laboratory (North Carolina State University Phytotron) under 12-h days at 30 ˚C and 75% relative humidity (RH), and 12-h nights (no light) at 24 ˚C and 100% RH for ∼3 mo until pots reached 100% coverage. During this period, plants were watered daily with a standard nutrient solution (Saravitz, Downs, & Thomas, 2009) and mowed weekly to a height of 7 cm.

Due to space limitations in the growth chamber, the 60 genotypes were divided into two trials (Table 1). Additionally, a selection of the best and worst performers from each trial was included in a third trial to compare responses. The diploid

TABLE 1 *Stenotaphrum* genotypes evaluated for resistance to three inoculum sources of *Pyricularia oryzae* under controlled environmental conditions over three separate trials

No.	Identity	Type[a]	Ploidy[b] species	Source[c]	Trial evaluated
1	106SVT3	Col	Diploid, *secundatum*	NCSU	II, III
2	200Elm4	Col	Diploid, *secundatum*	NCSU	II
3	904AT4	Col	Diploid, *secundatum*	NCSU	II, III
4	TR 6-10 (Amerishade®)	Cu	Diploid, *secundatum*	UF	I
5	Bitterblue	Cu	Triploid, *secundatum*	UF	I, III
6	C1	Col	Diploid, *secundatum*	NCSU	II, III
7	C2	Col	Diploid, *secundatum*	NCSU	II
8	C3	Col	Diploid, *secundatum*	NCSU	II
9	C4	Col	Diploid, *secundatum*	NCSU	II
10	C6	Col	Diploid, *secundatum*	NCSU	II
11	C7	Col	Diploid, *secundatum*	NCSU	II
12	NUF-76 (Captiva®)	Cu	Diploid, *secundatum*	UF	I
13	VNS (Classic™)	Cu	Diploid, *secundatum*	UF	I, III
14	Clem	Col	Diploid, *secundatum*	NCSU	II
15	Co2	Col	Diploid, *secundatum*	NCSU	II
16	Craig	Col	Diploid, *secundatum*	NCSU	II
17	6-72-99 (Delmar™)	Cu	Diploid, *secundatum*	UF	I
18	DeltaShade	Cu	Diploid, *secundatum*	UF	I
19	Eclipse (syn. MSA-31)	Cu	Diploid, *secundatum*	MSU	I
20	Floralawn	Cu	Aneuploid, *secundatum*	UF	I, III
21	Floratam	Cu	Aneuploid, *secundatum*	UF	I
22	Floratine	Cu	Triploid, *secundatum*	UF	I
23	Floraverde	Cu	Diploid, *secundatum*	UF	I
24	FX-10	Cu	Aneuploid, *secundatum*	UF	I, III
25	GF2	Col	Diploid, *secundatum*	NCSU	II, III
26	6-72-182 (Jade™)	Cu	Diploid, *secundatum*	UF	I
27	Jones	Col	Diploid, *secundatum*	NCSU	II
28	FA-40 (Mercedes™)	Cu	Diploid, *secundatum*	UF	I
29	SS-100 (Palmetto®)	Cu	Diploid, *secundatum*	UF	I
30	PI 289729	PI	Tetraploid, *dimidiatum*	NPGS	I, III
31	PI 290888	PI	Aneuploid, *secundatum*	NPGS	I, III
32	PI 291594	PI	Triploid, *secundatum*	NPGS	I, III
33	PI 300129	PI	Triploid, *secundatum*	NPGS	I, III
34	PI 300130	PI	Aneuploid, *secundatum*	NPGS	I, III
35	PI 365031	PI	Hexaploid, *dimidiatum*	NPGS	I, II, III
36	PI 410353	PI	Diploid, *secundatum*	NPGS	II, III
37	PI 410355	PI	Diploid, *secundatum*	NPGS	II
38	PI 410357	PI	Diploid, *secundatum*	NPGS	II, III
39	PI 410360	PI	Diploid, *secundatum*	NPGS	II, III
40	PI 410361	PI	Diploid, *secundatum*	NPGS	I, III
41	PI 410363	PI	Diploid, *secundatum*	NPGS	I, III
42	PI 410364	PI	Diploid, *secundatum*	NPGS	II, III
43	PI 414079	PI	Diploid, *secundatum*	NPGS	II
44	PI 509038	PI	Diploid, *secundatum*	NPGS	II, III
45	PI 509039	PI	Diploid, *secundatum*	NPGS	II, III

(Continues)

TABLE 1 (Continued)

No.	Identity	Type[a]	Ploidy[b] species	Source[c]	Trial evaluated
46	PI 600734	PI	Diploid, *secundatum*	Seed	II, III
47	PI 647924	PI	Diploid, *secundatum*	NPGS	II, III
48	PI 647925	PI	Diploid, *secundatum*	NPGS	I
49	Polaris	Cu	Diploid, *secundatum*	MSU	I
50	Raleigh	Cu	Diploid, *secundatum*	UF	I
51	Ray	Col	Diploid, *secundatum*	NCSU	II
52	Rebok	Col	Diploid, *secundatum*	NCSU	II
53	B12 (Sapphire®)	Cu	Diploid, *secundatum*	UF	I
54	615866 (Seville™)	Cu	Diploid, *secundatum*	UF	I, III
55	6-72-130 (Sunclipse™)	Cu	Diploid, *secundatum*	UF	I, II, III
56	SV20	Col	Diploid, *secundatum*	NCSU	II, III
57	T638	Col	Diploid, *secundatum*	NCSU	II
58	T644	Col	Diploid, *secundatum*	NCSU	II
59	T672	Col	Diploid, *secundatum*	NCSU	II
60	Texas Common	Cu	Diploid, *secundatum*	UF	I, III

[a]Germplasm type; Col, collection; Cu, cultivar.
[b]Ploidy levels according to Milla-Lewis, Zuleta, Van Esbroeck, Quesenberry, and Kenworthy (2013).
[c]NCSU, North Carolina State University, Raleigh, NC; UF, University of Florida, Gainesville, FL; MSU, Mississippi State University, Starkville, MS; NPGS, National Plant Germplasm System, Griffin, GA.

cultivar '6-72-130' (Sunclipse™) and hexaploid PI 365031 were included as the susceptible and resistant controls, respectively, in each trial. Each trial was evaluated twice (two runs) and chlorothalonil (Daconil Ultrex, Syngenta Crop Protection) was applied at a rate of 94.64 ml in 37.85 L per 92.9 m^2 (3.2 fluid oz. in 10 gal per 1,000 ft^2) two times 10 d apart in between runs to allow plants to recover completely from GLS infection. Plants were mowed after fungicide application to discard any remaining infected tissue and were allowed to recover for 10 wk before reinoculation.

2.2 | Fungal isolates and inocula production

Three inoculum sources of *P. oryzae*, obtained from the North Carolina State University turfgrass pathology program's collection, were used in this study. Each inoculum source consisted of two individual *P. oryzae* isolates: LWS consisted of isolates LWS1 and LWC3 collected from North Carolina State University's Lake Wheeler Turf Field Laboratory (Raleigh, NC), SRS consisted of isolates SRS1 and SRS3 collected from the Sandhills Research Station (Jackson Springs, NC), and GA consisted of isolates 1173 and 1345 collected from Georgia (Tredway, Stevenson, & Burpee, 2005). Conidia were produced based on the method developed by Ma and Uddin (2009) with modifications. Briefly, pieces of dry filter paper stored at −80 ˚C containing *P. oryzae* fungal mycelia were transferred to potato dextrose agar (PDA) (Difco, Becton, Dickinson and Company) and incubated at room temperature for five days. PDA blocks (5 mm^3) containing actively growing mycelium were removed and placed on oatmeal agar (20 g Difco agar and 20 g of Gerber oatmeal) in 1 L of diluted tall fescue extract. Tall fescue extract was prepared by boiling 20 g of fresh tall fescue leaf blades in 1 L water for 10 min and then diluted twofold by adding an equal volume of distilled water. Plates were sealed with Parafilm M all-purpose laboratory film (Bemis) and incubated at 26 ˚C with 12-h day/night cycles of fluorescent light (76 μmol m^{-2} s^{-1}) for 5–6 d until the growth of *P. oryzae* was ∼50 mm in diameter. The Parafilm was then removed and the culture plates were placed upside down and incubated for an additional 10 d to induce sporulation. Conidia were harvested by adding 5 ml of distilled water to each culture plate, scraping the surface of the fungal colony with a soft brush, and filtering the suspension through four layers of cheesecloth. The final concentration of conidial suspension of each strain was adjusted to 4×10^4 conidia ml^{-1} using a hemacytometer.

2.3 | Inoculation and incubation conditions

A split-plot experimental design with three replications was established, where main plots were the three inoculum sources and the subplots were the St. Augustinegrass genotypes. Each main plot contained a total of 96 pots sprayed with a particular inoculum source, distributed in a set of 12 containers holding eight pots each, for a total of 32 genotypes distributed in three replications. Before inoculation, the total

number of leaves in each cup was counted and genotypes were randomly arranged in four plastic containers per replication. The inoculation methods and conditions were as described by Tredway et al. (2005). Briefly, a conidial suspension was applied uniformly with an Badger Air-Brush Model 350 (Badger Air-Brush Company) powered with compressed CO_2 at 2.2 kg m^{-2}. After inoculation, the plastic containers were covered, and incubation conditions for the first 24 h were set at 24 ˚C, 100% RH, and no light. Subsequently, the growth chamber was programmed for 12 h of light (450–500 µmol m^{-2} s^{-1}) at 30 ˚C and 75% RH and 12 h of darkness at 24 ˚C and with RH between 70 and 90%. Plastic containers were uncovered at the beginning of each day cycle. At the end of the day, plants were sprayed with a mist of water and containers were covered again to maintain leaf wetness and humidity overnight. Additional mist was constantly applied in the growth chamber during the first 48 h after inoculation and every night until the end of the experiment using three Herrmidifier 707-U series atomizing humidifiers (Trion).

2.4 | Data collection and analysis

Beginning 2 d after inoculation, symptoms were evaluated for 5 d. The number of leaves with lesions was recorded daily. Disease incidence was then estimated as number of leaves with lesions (LWL) over the total number of leaves per subplot (cup) counted within 24 h prior to inoculation. Mean lesion length (LL) was determined by measuring the length of at least five lesions randomly selected per subplot using a digital fractional caliper (General Tools and Instruments; Tredway et al., 2003). Incubation period was recorded as the number of days from inoculation to first lesion appearance. Based on LWL and LL, derived parameters of disease progress rate, and lesion expansion rate, area under the disease progress curve (AUDPC), and area under the lesion expansion curve (AULEC) – were calculated. Disease progress rate and lesion expansion rate were determined for each plant from the five daily observations of disease incidence and mean lesion length, respectively. The AUDPC and AULEC were calculated from the disease incidence and mean lesion length data, respectively, using the formula $\sum_i^n (y_i + y_{i/2}/2)(t_{i+1} - t_i)$ developed by Shaner and Finney (1977), where i = rating day, y_i is disease incidence or mean lesion length on the ith rating date, and t_i is the day of the ith rating.

The PROC MIXED procedure (SAS version 9.2, SAS Institute) was used to fit the linear mixed model, estimate the covariance parameter for random effects, and run the Type 3 test of fixed effects to test the significance of main effects (genotype and isolate) and their interaction for the primary and derived parameters evaluated. Genotype, inoculum sources, and their interaction were considered fixed effects. Trial, run nested within trial, replication nested within trial and run, and their interactions with fixed effects were considered random effects. The Kenward–Roger's approximation was used to improve estimation of standard errors and degrees of freedom in the final model when covariance parameters were estimated as zero. Main effect and interaction effect least squares means were calculated and mean separation tests were conducted through multiple pairwise means comparisons using the Tukey–Kramer test at a significance level of .05 (Kramer, 1956). In order to detect correlations among the different traits evaluated, Pearson's coefficients were calculated using the CORR procedure in SAS 9.2 (SAS version 9.2, SAS Institute).

3 | RESULTS

Given the number of genotypes evaluated and size of the environmental chambers used for the study, the evaluation was split into three trials: Trial I, which included half of the genotypes; Trial II, which included the other half; and Trial III, which included the accessions with best and worst disease responses from each trial in order to make side-by-side comparisons of performance (Table 1). Although seven parameters were evaluated and analyzed, only incubation period, incidence on Day 7 after inoculation (INCID D7), AUDPC, and AULEC are presented here.

3.1 | Trial I

In this trial, significant differences among genotypes were identified for all parameters evaluated except incubation period (Table 2). Meanwhile, significant differences among inoculum sources were detected only for INCID D7. The interaction effect between genotype and inoculum source was significant only for AULEC. Plant Introductions 289729, 291594, 300129, and 300130 and cultivar FX-10 were not significantly different from resistant check PI 365031 for any of the traits (Supplemental Table S1). Cultivars VNS (Classic™), 'FA-40' (Mercedes™), and '6-72-182' (Jade™) were consistently the most susceptible genotypes across all parameters. Inoculum LWS was the most virulent across runs, achieving the highest scores across traits. Inocula SRS and GA obtained rank two and three, respectively (data not shown). Covariance parameter estimates are presented in Supplemental Table S2.

3.2 | Trial II

In general, disease pressure was higher for Trial II as compared with Trial I. In Trial II, disease developed faster as reflected by lower numbers for incubation period, and reached

TABLE 2 ANOVA for evaluation trials of *Stenotaphrum* germplasm evaluated for resistance to three sources of inoculum of *Pyricularia oryzae* under controlled environmental conditions over three trials

		Incubation period		Incidence on Day 7		Sq Rt AUDPC[a]		AULEC[a]	
Source	Num DF[a]	Den DF[b]	*F* value	Den DF	*F* value	Den DF	*F* value	Den DF	*F* value
Trial I									
Inoculum (Inoc)	2	2	7.98NS[†]	10.5	12.09**	2.01	14.5NS	2	1.28NS
Genotype (Gen)	30	30	1.26NS	29.7	6.67***	29.5	5.47***	29.9	14.58***
Gen × Inoc	60	60	0.98NS	60.8	1.24NS	60.2	1.49NS	58.1	2.18**
Trial II									
Inoculum	2	2	0.19NS	2	0.39NS	2	0.07NS	2	0.54NS
Genotype	31	31	2.54**	31	4.06***	31	3.29***	93	6.54***
Gen × Inoc	62	446	0.99NS	62	1.57*	62	1.39NS	93	1.91**
Trial III									
Inoculum	2	2	0.1NS	2	0NS	2	0.01NS	2.97	0.17NS
Genotype	27	26.6	4.85***	27.1	4.46***	27	5.96***	27.2	10.36***
Gen × Inoc	54	374	1.98***	53.9	0.9NS	54	1.38NS	54.4	1.36NS

[a]Sq Rt AUDPC, square-root-transformed area under the disease progress curve; AULEC, area under the lesion expansion curve.
[b]Num DF, numerator degrees of freedom; Den DF, denominator degrees of freedom.
*Significant at the .05 probability level. **Significant at the .01 probability level. ***Significant at the .001 probability level. †NS, nonsignificant.

higher levels, which was evidenced by higher values for incidence, AULEC, and AUDPC. Genotypes were found to be significantly different for all parameters (Table 2). There was a significant interaction effect between genotype and inoculum for INCID D7 and AULEC. As in Trial I, PI 365031 was among the top performers across parameters. Genotypes PI 410353 and PI 410364 had similar or better performance than PI 365031 for all variables measured (Supplemental Table S3).

3.3 | Trial III

The ANOVA results showed significant differences among genotypes for all traits (Table 3). The genotype × inoculum source interaction was significant for incubation period only. Although PI 365031 was once again in the best performance group for all parameters, PIs 300129, 300130, and 410353 and cultivar FX-10 exhibited comparable performance and were not significantly different from this PI for any of the parameters, with the exception of incubation period for PI 410353 with inoculum SRS (Table 3). Although not the worst, cultivar 6-72-130 (Sunclipse™) remained a poor performer for all parameters.

3.4 | Consistency in performance

Genotypes with consistent performance across trials and parameters could be identified (Table 4). The resistant check, PI 365031, ranked in the top five for 10 out of 12 trial by parameter combinations. Plant Introductions 300129, 300130, and 410353 were also consistently among the top performers. Plots of AULEC vs. INCID D7, AUDPC vs. INCID D7, and AUDPC vs. AULEC by incubation period (Figures 1a, 1b, and 1c, respectively) revealed a wide range in response to infection to *P. oryzae*. A group of genotypes (PI 365031, PI 300129, PI 300130, PI 291594, PI 289729, and PI 410353) was identified as having consistent performance for AUDPC, AULEC, and INCID D7. Performance for incubation period was more variable. Furthermore, the plots of AUDPC vs. AULEC, INCID D7 vs. AULEC, and AUDPC vs. AULEV by ploidy level (Figure 2a, 2b, and 2c, respectively) identified PI 410353 as the best-performing diploid genotype.

3.5 | Trait correlation

Pearson's correlation coefficients were calculated between each pair of traits. Results indicated that AUDPC, AULEC, and INCID D7 are highly correlated ($p < .0001$) (Table 5). On the other hand, incubation period had a significant association ($p = .0002$) with AUDPC and INCID D7 only.

4 | DISCUSSION

Significant differences in incubation period, INCID D7, AUDPC, and AULEC were observed among *Stenotaphrum* genotypes evaluated in this study. These results confirmed, as has been previously reported in other studies (Atilano &

TABLE 3 Genotype by inoculum least squares means for incubation period, disease incidence on Day 7, area under the disease progress curve (AUDPC), and area under the lesion expansion curve (AULEC) of *Stenotaphrum* genotypes evaluated for resistance to *Pyricularia oryzae* in Trial III

	Incubation Period			Incidence on Day 7			Sq Rt AUDPC[a]			AULEC		
Genotype	**GA**	**LW**	**SRS**	**GA**	**LW**	**SRS**	**GA**	**LW**	**SRS**	**GA**	**LW**	**SRS**
106SVT3	4.17B	4.17A	4.33BCD	37.09A	33.28A	23.62A	7.29ABC	7.04A	5.73A–E	4.10A–F	4.39A–D	4.05A–D
904AT4	4.5B	4.67A	4.33BCD	38.62A	54.77A	44.26A	7.92ABC	8.91A	8.10A–E	5.24A–E	6.11AB	5.11A–D
Bitterblue	4.67AB	5.00A	4.33BCD	13.07A	13.24A	30.09A	5.25ABC	4.66A	7.44A–E	2.83A–F	2.41A–D	5.01A–D
C1	4.67AB	4.50A	5.00A–D	31.91A	32.50A	29.81A	6.99ABC	6.35A	6.59A–E	4.70A–F	3.99A–D	3.63A–D
Classic	4.33B	4.83A	4.17CD	49.64A	50.12A	41.49A	9.01AB	8.29A	8.10A–E	5.97A–D	4.77A–D	5.43A–D
Floralawn	4.50	4.67A	4.17CD	13.81A	28.38A	45.97A	5.38ABC	6.60A	8.45A–E	4.24A–F	3.95A–D	6.20AB
FX-10	5.17AB	4.67A	6.67AB	14.91A	9.82A	3.90A	5.09ABC	4.27A	1.65DE	2.30C–F	1.70BCD	1.13CD
GF2	4.17B	4.67A	4.50BCD	59.65A	47.91A	47.35A	10.04A	7.93A	8.70A–D	4.95A–F	4.97A–D	4.49A–D
PI 289729	4.50B	4.25A	4.33BCD	24.83A	19.10A	23.44A	6.65ABC	5.77A	6.20A–E	4.64A–F	4.09A–D	4.04A–D
PI 290888	2.92B	3.50A	3.33D	5.21A	17.05A	20.20A	4.62ABC	5.87A	7.74A–E	0.56EF	0.69D	1.06CD
PI 291594	3.05B	5.17A	3.67D	24.47A	22.13A	36.03A	7.48ABC	5.76A	8.68A–D	4.68A–F	2.34A–D	5.63ABC
PI 300129	5.00AB	5.67A	5.67A–D	7.70A	6.51A	6.04A	3.78ABC	2.92A	2.92B–E	2.38B–F	2.07A–D	2.21BCD
PI 300130	5.33AB	5.67A	6.33ABC	3.12A	7.88A	3.68A	2.60BC	3.11A	1.85CDE	1.65DEF	1.45BCD	1.31CD
PI 365031[b]	**5.00AB**	**5.67A**	**7.00A**	**6.24A**	**5.75A**	**3.38A**	**3.74ABC**	**3.04A**	**1.40E**	**3.08A–F**	**1.61BCD**	**0.74D**
PI 410353	7.00A	4.00A	4.17CD	4.76A	11.28A	9.26A	1.54C	5.39A	4.54A–E	0.33F	1.03CD	1.89BCD
PI 410357	4.67AB	4.83A	4.17CD	29.79A	25.99A	14.77A	7.57ABC	6.07A	5.47A–E	3.01A–F	3.00A–D	3.13A–D
PI 410360	3.50B	4.17A	4.00CD	24.71A	34.00A	16.81A	7.81ABC	8.22A	5.44A–E	5.65A–E	3.97A–D	3.11A–D
PI 410361	4.00B	4.83A	3.67D	36.25A	35.74A	49.47A	8.25ABC	8.24A	9.88AB	5.11A–F	3.04A–D	5.27A–D
PI 410363	3.67B	4.83A	4.33BCD	49.80A	47.00A	15.09A	9.35AB	7.39A	5.40A–E	5.75A–E	4.75A–D	2.22BCD
PI 410364	4.00B	5.00A	4.00CD	17.29A	21.49A	32.72A	6.17ABC	5.82A	7.58A–E	3.91A–F	3.15A–D	5.46A–D
PI 509038	4.83AB	4.86A	4.50BCD	55.06A	51.87A	64.43A	9.28AB	8.70A	10.63A	6.65ABC	6.58A	7.08A
PI 509039	4.17B	4.67A	4.67A–D	58.18A	42.74A	58.69A	10.35A	7.83A	10.12A	7.29A	5.68ABC	6.40AB
PI 600734	4.00B	4.50A	4.00CD	50.09A	46.74A	38.57A	9.25AB	8.57A	8.98ABC	7.13AB	5.70ABC	5.72ABC
PI 647924	4.83AB	5.33A	4.17CD	17.43A	19.32A	23.21A	5.06ABC	4.91A	6.21A–E	4.10A–F	3.31A–D	3.01A–D
Seville	4.50B	4.50A	4.67A–D	45.30A	45.07A	39.46A	8.72AB	7.94A	7.87A–E	5.50A–E	4.78A–D	4.52A–D
Sunclipse	5.00AB	5.17A	4.33BCD	36.33A	34.89A	20.25A	7.22ABC	7.12A	6.21A–E	5.24A–E	5.39A–D	3.89A–D
SV20	4.67AB	4.50A	4.83A–D	29.45A	33.24A	33.74A	6.30ABC	6.91A	6.55A–E	3.30A–F	3.55A–D	4.13A–D
TX Common	5.17AB	5.17A	4.00CD	44.59A	49.00A	39.22A	8.04ABC	7.96A	8.03A–E	5.84A–D	4.90A–D	4.93A–D

Note. Within a column, means followed by a common letter are not significantly different by Tukey–Kramer test at the .05 probability level.

[a]Sq Rt AUDPC, square-root-transformed AUDPC.

[b]Resistant check highlighted in bold

TABLE 4 Means across inocula for genotypes with high and low gray leaf spot area under the lesion expansion curve (AULEC), area under the disease progress curve (AUDPC), incubation period (INCPER) and incidence on Day 7 across runs and trials

	INCPER			Incidence on Day 7			Sq Rt AUDPC[a]			AULEC		
Genotype	**Trial I**	**Trial II**	**Trial III**	**Trial I**	**Trial II**	**Trial III**	**Trial I**	**Trial II**	**Trial III**	**Trial I**	**Trial II**	**Trial III**
106SVT3		3.56A	4.22AB		79.15AB	31.33A–F		12.12AB	6.69A–F		7.13D–G	4.18A–E
904AT4		3.67A	4.50AB		81.37AB	45.89ABC		12.53AB	8.31AB		8.23D–G	5.49ABC
Bitterblue	3.11AB		4.67AB	19.50A		18.80B–F	6.53A–E		5.78A–F	4.93A–D		3.41A–E
Captiva	2.84B			32.34A			7.85A–D			5.38A–D		
Classic	3.28AB		4.44AB	38.87A		47.08ABC	8.70AB		8.47A	6.26AB		5.39ABC
Floralawn	3.61AB		4.44AB	17.84A		29.39A–F	5.79A–E		6.81A–F	4.95A–D		4.80A–E
FX-10	**3.61AB**		**5.50A**	**13.06A**		**9.54DEF**	**4.88A–E**		**3.67C–F**	**3.31A–E**		**1.71CDE**
Jade	2.67B			38.17A			8.97A			7.03A		
PI 289729	**4.06AB**		**4.36AB**	**9.55A**		**22.58A–F**	**4.27B–E**		**6.21A–F**	**0.62E**		**4.25A–E**
PI 290888	3.22AB		3.28B	25.40A		14.66C–F	7.63A–D		6.11A–F	3.48A–E		0.79E
PI 291594	3.67AB		4.11AB	11.23A		27.55A–F	4.54A–E		7.31A–E	2.19B–E		4.22A–E
PI 300129	**3.72AB**		**5.44AB**	**6.90A**		**6.75EF**	**3.40DE**		**3.21DEF**	**1.98CDE**		**2.22B–E**
PI 300130	**3.89AB**		**5.78A**	**10.23A**		**4.90F**	**4.07CDE**		**2.52F**	**1.78DE**		**1.47CDE**
PI 365031[b]	**5.22A**	**3.44A**	**5.89A**	**6.93A**	**53.66B**	**5.13F**	**2.55E**	**10.29B**	**2.73EF**	**0.30E**	**7.16D–G**	**1.81CDE**
PI 410353		**3.17A**	**5.06AB**		**62.06AB**	**8.43EF**		**12.43AB**	**3.82B–F**		**5.45G**	**1.08DE**
PI 410357		**3.06A**	**4.56AB**		**72.27AB**	**23.52A–F**		**13.92AB**	**6.37A–F**		**5.59G**	**3.05A–E**
PI 410360		**3.28A**	**3.89AB**		**58.09AB**	**25.17A–F**		**12.03AB**	**7.15A–E**		**5.56G**	**4.19A–E**
PI 410361	2.39B		4.17AB	32.38A		40.49A–E	8.96A		8.79A	5.90A–D		4.47A–E
PI 410363	2.86B		4.28AB	33.66A		37.30A–F	7.97A–D		7.38A–D	5.85A–D		4.24A–E
PI 410364		**3.28A**	**4.33AB**		**61.39AB**	**23.84A–F**		**12.71AB**	**6.52A–F**		**6.26FG**	**4.17A–E**
PI 509038		3.61A	4.70AB		80.13AB	57.12A		12.61AB	9.54A		9.89A–F	6.77A
PI 509039		3.61A	4.50AB		87.64AB	53.20AB		12.92AB	9.43A		10.96A–E	6.45A
PI 600734		3.11A	4.17AB		74.01AB	45.14ABC		13.55AB	8.93A		8.28C–G	6.18AB
PI 647924		3.33A	4.78AB		62.36AB	19.99B–F		11.48B	5.39A–F		5.33G	3.47A–E
Seville	3.17AB		4.56AB	34.36A		43.27A–D	8.42ABC		8.18ABC	5.67A–D		4.93A–E
Sunclipse	3.06B	3.61A	4.83AB	30.40A	64.24AB	30.49A–F	7.42A–D	11.11B	6.85A–F	6.50A	6.72FG	4.84A–E
SV20		3.28A	4.67AB		67.71AB	32.14A–F		11.76B	6.59A–F		6.32FG	3.66A–E
TX Common				31.98A		44.27ABC	7.90A–D		8.01ABC	6.18AB		5.22A–D

Note. Genotypes highlighted in bold performed consistently among the top and were identified as highly resistant (HR). Within a column, means followed by a common letter are not significantly different by Tukey–Kramer test at the .05 probabilty level.

[a]Square-root-transformed AUDPC

[b]Resistant check.

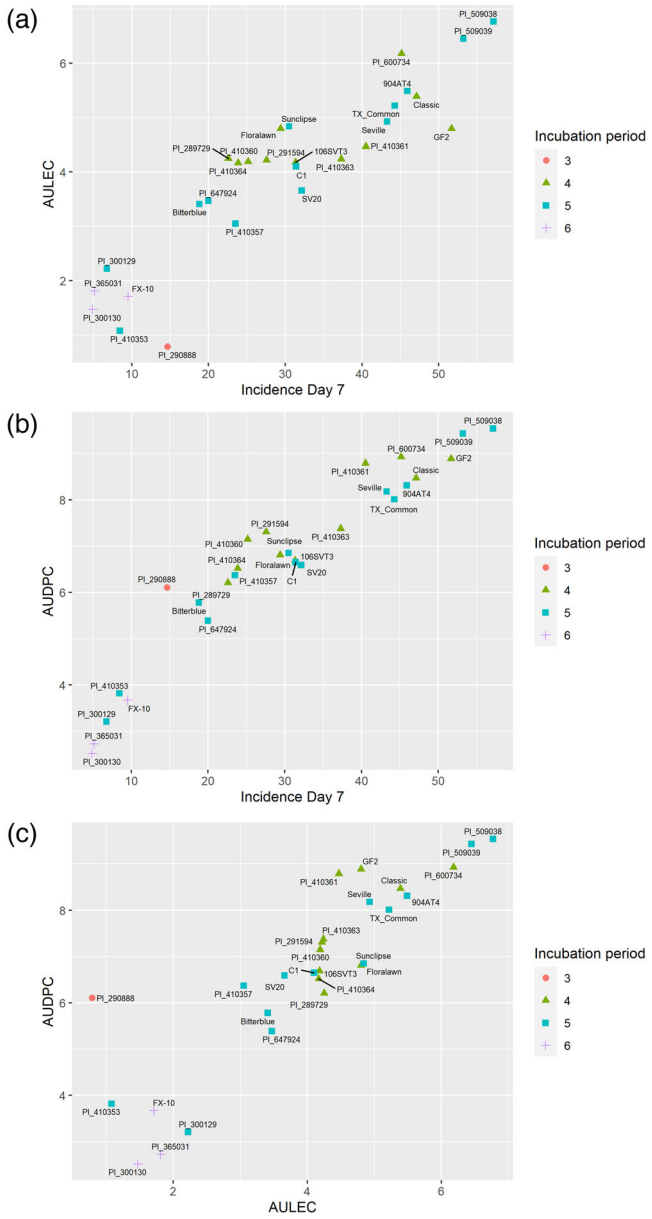


FIGURE 1 Least squares means for St. Augustinegrass germplasm evaluated for resistance to gray leaf spot under controlled environmental conditions: (a) area under the lesion expansion curve (AULEC) vs. disease incidence on Day 7 (Incidence Day 7, defined as the number of leaves with lesions seven days post inoculation over the total number of leaves per plant counted within 24 h prior to inoculation) by incubation period (defined as number of days from inoculation to when two or more lesions were present in a plant) and, (b) area under the disease progress curve (AUDPC) vs. Incidence day 7 by incubation period, and (c) AUDPC vs. AULEC by incubation period.

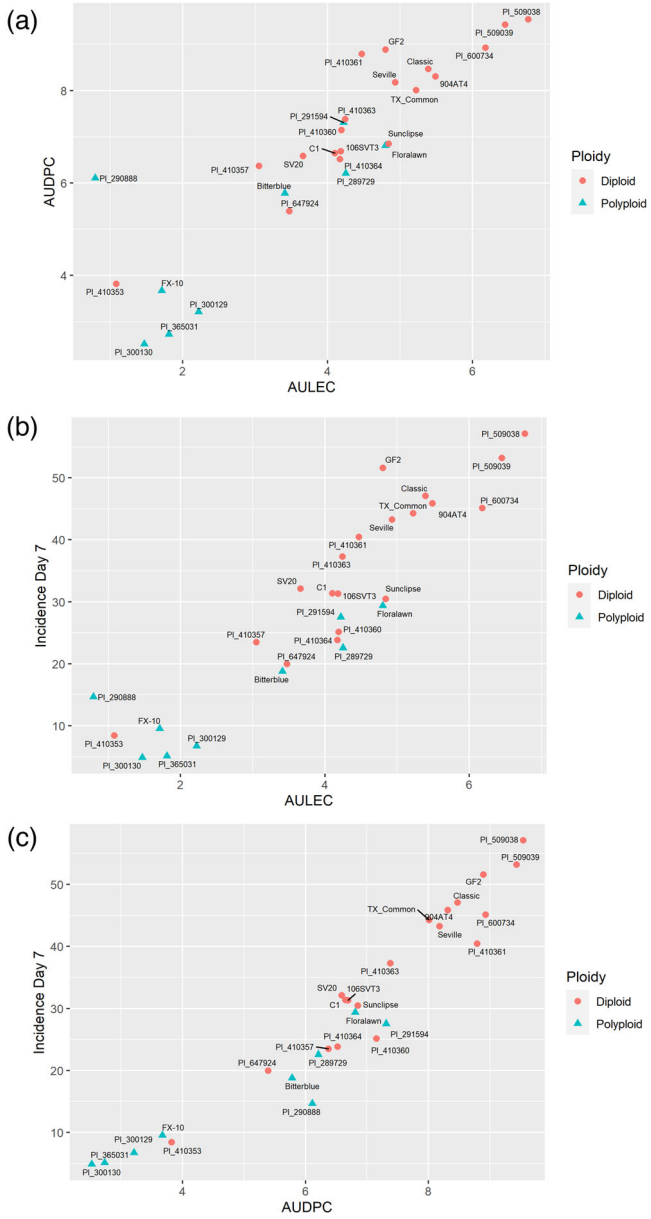


FIGURE 2 Least squares means for St. Augustinegrass germplasm evaluated for resistance to gray leaf spot under controlled environmental conditions: (a) area under the disease progress curve (AUDPC) vs. area under the lesion expansion curve (AULEC) by ploidy level, (b) disease incidence on Day 7 (Incidence day 7) vs. AULEC by ploidy level, and (c) AUDPC vs. Incidence day 7 by ploidy level

Busey, 1983; Metz et al., 2011), that diverse germplasm collections of St. Augustinegrass express differences in response to biotic stresses such as GLS. Previous studies have reported high levels of GLS resistance in *S. dimidiatum* plant introductions PI 365031 and PI 289729 (Atilano & Busey, 1983) and cultivar FX-10 (Metz et al., 2011). The present study confirmed these reports, but it also identified additional sources of resistance. Diploid PI 410353 exhibited levels of resistance comparable to that of polyploid resistant check PI 365031. Additionally, although PI 647924, PI 410357, PI 410360, and PI 410364 had higher levels of disease than PI 410353 in some runs, they still maintained consistent performance across trials for most parameters and were classified as moderately resistant. The GLS resistance in PI 410353 was corroborated in later studies (Mulkey, 2013; Yu et al., 2020).

TABLE 5 Pearson correlation coefficients among traits in the evaluation of *Stenotaphrum* genotypes for resistance to *Pyricularia oryzae*

Trait	AUDPC	AULEC	INCD7	INCPER
AUDPC	1	.8807	.9543	−.6560
		<.0001	<.0001	.0002
AULEC	.8807	1	.9123	−.3474
	<.0001		<.0001	.0701
INCD7	.9543	.9123	1	−.4338
	<.0001	<.0001		.0211
INCPER	−.6560	−.3474	−.4338	1
	.0002	.0701	.0211	

Note. AUDPC, area under the disease progress curve; AULEC, area under the lesion expansion curve; INCD7, disease incidence on Day 7; INCPER, incubation period.

The use of growth chambers for disease evaluations is beneficial in reducing space requirements and weather limitations associated with field trials (Metz et al., 2011). Moreover, by providing the specific environmental conditions required for fungal growth, development of high levels of disease epidemics can be achieved, which ultimately decreases the probability of escapes. A screening of different inoculation methods for the evaluation of GLS under field and controlled conditions revealed that results are equally valid on either situation (Metz et al., 2011). Our work supports previous reports (Metz et al., 2011; Mulkey, 2013; Yu et al., 2020) that the use of growth chambers is an effective way to evaluate large numbers of St. Augustinegrass genotypes for response to *P. oryzae*.

Primary (incubation period, INCID D7, and final lesion length) and derived (AUDPC and AULEC) parameters were consistent at identifying the most resistant genotypes. Although Tredway et al. (2003) found incidence to be the most efficient parameter because it combines incubation period, infection efficiency, and lesion expansion into one measurement, in our study, AULEC and AUDPC provided the best separation among genotypes and were therefore the most powerful for detection of high levels of GLS resistance. Ultimately, all three parameters were found to be highly correlated ($p < .001$), indicating that evaluation of one should be effective at also assessing response of the other two. As in different studies (Dallagnol, Rodrigues, Mielli, Ma, & Datnoff, 2009; Mulkey, 2013; Tredway et al., 2003; Yu et al., 2020), AULEC and AUDPC were useful criteria for uniform selection of resistant genotypes. In Trial III, polyploid genotypes such as PI 365031, PI 290888, PI 300130, FX-10, and PI 300129 were found to be more resistant to GLS than diploid plants as a group. However, AULEC and AUDPC for diploid genotypes PI 410353 and PI 410357 were consistently low, indicating that these genotypes are also resistant. Incubation period provided poor separation among genotypes. However, epidemic development is affected by incubation period and even small differences among cultivars can be amplified over multiple cycles of disease development (Tredway et al., 2003). Therefore, the average 1-d difference detected between highly resistant PI 365031 and susceptible cultivar 'Seville' might translate into the latter experiencing more disease cycles than the former over the same time period.

Even though the virulence of inoculum sources was assumed to be low (GA), medium (LWS), and high (SRS), results indicated similar levels of virulence for all inoculum sources on the genotypes evaluated. Comparable results were presented by Metz et al. (2011) when different *P. oryzae* isolates used separately and in combination were found to exhibit similar virulence and had no contribution to the overall variation among genotypes. The authors attributed these results to cross contamination of inoculum. In our study, genotypes were enclosed in plastic containers with the same inoculum, reducing the probability of spore dispersion to other containers. Tredway et al. (2003) identified similar virulence among isolates of *P. oryzae* to tall fescue cultivars even for isolates from different lineages. The significant interaction of genotype and inoculum identified for most traits in this study was mainly given by differences in rank among genotypes with intermediate performance. Highly resistant genotypes identified here demonstrated consistency in disease response across runs, trials, and parameters. Although polyploids PI 365031 and PI289729 had been previously identified as resistant this is the first report of high levels of resistance to GLS being observed among diploid materials. The identification and utilization of resistance genes in diploid genotypes is of great importance for future St. Augustinegrass breeding efforts as this germplasm pool can be more readily exploited due to the lack of reproductive barriers with most commercial cultivars and PIs.

ACKNOWLEDGMENTS

The authors wish to thank personnel at the North Carolina State University Environmental Facility for assistance with maintenance of plants and setup of the experimental chambers. This research was supported in part by funding provided by the North Carolina Crop Improvement Association and the North Carolina State University Center for Turfgrass Environmental Research and Education.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Lane P. Tredway https://orcid.org/0000-0001-8064-5607
Susana R. Milla-Lewis https://orcid.org/0000-0001-8524-5039

REFERENCES

Atilano, R. A., & Busey, P. (1983). Susceptibility of St. Augustinegrass germ plasm to *Piricularia griseae*. *Plant Disease*, *67*(7), 782–783. https://doi.org/10.1094/PD-67-782

Busey, P. (2003). St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze. In M. D. Casler & R. R. Duncan (Eds.), *Biology, breeding, and genetics of turfgrasses* (pp. 309–330). Hoboken, NJ: John Wiley & Sons.

Busey, P. (1995). Genetic diversity and vulnerability of St. Augustinegrass. *Crop Science*, *35*, 322–327. https://doi.org/10.2135/cropsci1995.0011183X003500020005x

Dallagnol, L. J., Rodrigues, F. A., Mielli, M. V. B., Ma, J. F., & Datnoff, L. E. (2009). Defective active silicon uptake affects some components of rice resistance to brown spot. *Phytopathology*, *99*, 116–121. https://doi.org/10.1094/PHYTO-99-1-0116

Datnoff, L., Brecht, M., Stiles, C., & Rutherford, B. (2005). The role of silicon in suppressing foliar diseases in warm-season turf. *International Turfgrass Society Research Journal*, *10*, 175–179.

Freeman, T. E. (1964). Influence of nitrogen on severity of *Pyricularia grisea* infection of St. Augustinegrass. *Phytopathology*, *54*, 1187–1189.

Genovesi, A. D., Russel, W. J., Engelke, M. C., & Burson, B. L. (2009). Interploid St. Augustinegrass [*Stenotaphrum secundatum (Walt.) Kuntze*] hybrids recovered by embryo rescue. *In Vitro Cellular & Developmental Biology—Plant*, *45*(659–666. https://doi.org/10.1007/s11627-009-9230-x

Harmon, P., Datnoff, L., Nagata, R., Brecht, M., & Stiles, C. (2005). *Gray leaf spot of St, Augustinegrass: Cultural and chemical options*. Gainesville: University of Florida, Institute of Food and Agricultural Sciences.

Kramer, C. Y. (1956). Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics*, *12*, 307–310. https://doi.org/10.2307/3001469

Ma, B., & Uddin, W. (2009). Fitness and competitive ability of an azoxystrobin-resistant G143A mutant of *Magnaporthe oryzae* from perennial ryegrass. *Plant Disease*, *93*, 1044–1049. https://doi.org/10.1094/PDIS-93-10-1044

Malca, M. I., & Owen, J. H. (1957). The gray leafspot disease of St. Augustine grass. *Plant Disease*, *41*, 871–875.

Metz, S. P., Chandra, A., Genovesi, A. D., & Campos, C. F. (2011). Comparison of screening methods to evaluate the response of St. Augustinegrass to *Magnaporthe oryzae*. *Plant Pathology*, *61*, 255–261. https://doi.org/10.1111/j.1365-3059.2011.02521.x

Milla-Lewis, S. R., Zuleta, M. C., Van Esbroeck, G. A., Quesenberry, K. H., & Kenworthy, K. E. (2013). Cytological and molecular characterization of genetic diversity in *Stenotaphrum*. *Crop Science*, *53*, 296–308. https://doi.org/10.2135/cropsci2012.04.0234

Moss, M. A., & Trevathan, L. E. (1987). Environmental conditions conducive to infection of ryegrass by *Pyricularia grisea*. *Phytopathology*, *77*, 863–866. https://doi.org/10.1094/Phyto-77-863

Mulkey, S. E. (2013). *SSR development and mapping of gray leaf spot resistance genes in St. Augustinegrass* (Master's thesis, North Carolina State University).

Saravitz, C. H., Downs, R. J., & Thomas, J. F. (2009). *Phytotron procedural manual: For controlled-environment research at the southeastern plant environment laboratory* (Technical Bulletin 244). Raleigh: North Carolina State University, North Carolina Agricultural Research Service.

Shaner, G., & Finney, R. (1977). The effect of nitrogen fertilization on the expression of slow-mildewing in Knox wheat. *Phytopathology*, *36*, 1307–1311.

Smiley, R. W., Dernoden, P. H., & Clarke, B. B. (2005). *Compendium of turfgrass diseases*. St. Paul, MN: American Phytopathological Society.

Tredway, L. P., Stevenson, K. L., & Burpee, L. L. (2003). Components of resistance to *Magnaporthe grisea* in Coyote and Coronado tall fescue. *Plant Disease*, *87*, 906–912. https://doi.org/10.1094/PDIS.2003.87.8.906

Tredway, L. P., Stevenson, K. L., & Burpee, L. L. (2005). Genetic structure of *Magnaporthe grisea* populations associated with St. Augustinegrass and tall fescue in Georgia. *Phytopathology*, *95*, 463–471. https://doi.org/10.1094/PHYTO-95-0463

Uddin, W., Serlemitsos, K., & Viji, G. (2003). A temperature and leaf wetness duration-based model for prediction of gray leaf spot of perennial ryegrass turf. *Phytopathology*, *93*, 336–343. https://doi.org/10.1094/PHYTO.2003.93.3.336

Vincelli, P., & Dixon, E. (2002). Resistance to QoI (strobilurin-like) fungicides in isolates of *Pyricularia grisea* from perennial ryegrass. *Plant Disease*, *86*, 235–240. https://doi.org/10.1094/PDIS.2002.86.3.235

Yu, Y., Mulkey, X., Ma, S. E., Arellano, B. Y., Zuleta, C., Reynolds, M. C., & Milla-Lewis, S. R. (2020). Resistance to gray leaf spot in St. Augustinegrass: Characterization and QTL mapping. *Plant Disease*. https://doi.org/10.1094/PDIS-04-20-0905-RE (in press).

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Carbajal EM, Ma B, Zuleta MC, et al. Identification of sources of resistance to gray leaf spot in *Stenotaphrum* germplasm. *Crop Science*. 2021;*61:*3069–3079. https://doi.org/10.1002/csc2.20371