ABSTRACT

CARBAJAL MELGAR, ESDRAS MANUEL. Breeding for Improved St. Augustinegrass through Ploidy Manipulation and Identification of Gray Leaf Spot-Resistant Germplasm. (Under the direction of Dr. Susana Milla-Lewis).

St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] is a warm-season grass widely grown across the southern US. Characteristics such as rapid stolon production, superior shade tolerance, and moderately low input requirements make this grass well suited for home lawns, sod production, and commercial landscapes. The genus *Stenotaphrum* includes seven species with different ploidy levels and has a base chromosome number of x=9. Because of fertility barriers across ploidy levels, diploid genotypes have been used as the primary source of genetic material for breeding efforts. While many genotypes are cross-fertile, all St. Augustinegrass cultivars are propagated vegetatively in sod production. In other warm-season grasses, the development of sterile triploid hybrids by crossing tetraploid and diploid genotypes has been successfully used as a means of ensuring varietal purity. Applying this model in St. Augustinegrass would be beneficial to sod producers and turf managers who require purity for certification and uniformity for performance, respectively. Therefore, the present study was conducted to develop colchicine-induced tetraploid lines of St. Augustinegrass. Seeds of cultivar 'Raleigh' were treated with four colchicine concentrations at four exposure times. Seedlings recovered after treatment and were screened for genome size changes using flow cytometry. A set of putative tetraploid lines was initially identified and evaluated for stomatal density, stomatal length and pollen stainability. Further analysis of putative tetraploids indicated all lines were found to be diploid with the exception of one, DSA 13005. Pollen stainability of this line was high and it produced 33 progenies through selfing, two of which were identified as putative tetraploids via flow cytometry. Average stomata lengths for DSA 13005 and its two progeny

lines, DSA 16001 and DSA 16016, were found to be significantly ($P<0.05$) larger than that of diploid control Raleigh. All three lines were corroborated as tetraploids ($2n = 4x = 36$) through chromosome counts. These lines will be used in future breeding efforts in an attempt to develop sterile triploid cultivars.

One of the most common diseases in St. Augustinegrass is gray leaf spot (GLS), caused by the fungal pathogen *Magnaporthe oryzae*. While previous studies have reported polyploid lines with resistance to GLS, no comprehensive evaluations of sources of resistance have been performed in the genus. Screening diploid and polyploid St. Augustinegrass germplasm for response to GLS will enable breeders to identify resistant parents for cultivar development. In this study, a collection of 62 genotypes of *Stenotaphrum spp.* were screened for resistance to three different *M. oryzae* sources of inoculum under controlled environmental conditions. The traits evaluated were incubation period, number of leaves with lesions, mean lesion length and derived parameters disease incidence, area under the disease progress curve (AUDPC), and area under the lesion expansion curve (AULEC). Significant differences among genotypes were identified with polyploid genotypes PI 365031, PI 290888, PI 300130, FX-10 and PI 300129 and diploid genotypes PI 410353 and PI 647924 consistently showing high levels of resistance across trials, inoculum sources and traits. 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 because of the lack of reproductive barriers with most commercial cultivars and plant introductions.

Breeding for Improved St. Augustinegrass through Ploidy Manipulation and Identification of Gray Leaf Spot-Resistant Germplasm

by
Esdras Manuel Carbajal Melgar

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Crop Science

Raleigh, North Carolina

2017

APPROVED BY:

_____	_____
Ramsey Lewis, PhD	Consuelo Arellano, PhD

_____
Susana Milla-Lewis, PhD
Chair of Advisory Committee

DEDICATION

To my mother Blanca Edith Carbajal, my sister Blanca Madelline Carbajal and my brother Mario David Perez Carbajal for supporting me and being my inspiration for all I do.

To my grandparents Maria Celia Melgar and Francisco Benjamin Pérez, whom through prayers, have always given me strength. To my great-grandmother Maria Antonia Melgar who from the heavens has been waiting for this moment.

BIOGRAPHY

Esdras Manuel Carbajal Melgar was born in Tegucigalpa, FM, Honduras. He grew up in Yorito, Yoro, Honduras with his mother Blanca Edith Carbajal Melgar, and two siblings, Mario and Blanca. Esdras completed high school in 2006 at the Instituto San Pedro in Yorito,Yoro, Honduras. During this time, he was exposed to many agronomic and ecological research projects, and fell in love with the research environment. From 2007 through 2010, Esdras atended the Universidad Nacional de Agricultura (Catacamas, Olancho, Honduras). He graduated with a B.S. degree in Natural Resources with the highest merit and was a recipient of the Trilogy Medal for academics, work ethics and discipline. During his senior year, he had the opportunity to participate in a research internship with Dr. Chris Reberg-Horton at North Carolina State University. He was mentored by Ph.D. student Aaron Fox in the area of organic cropping systems. In 2011, he came for the second time to NCSU to participate in a six month internship with the Turfgrass Breeding and Genetics program. Due to the interest on this work, his internship was extended for four years. During this time, he was exposed to diverse research and fell in love with plant breeding. In 2015, he was accepted at NCSU as a M.S. student to work on St. Augustinegrass breeding. When Esdras is not at the greenhouse playing with plants at burning temperatures, or encapsulated in his crossing room trying to get new hybrids from the crossing program, Esdras loves to eat food to build up a lot of calories that he can later burn down by teaching Zumba at the Carmichael Gym.

ACKNOWLEDGEMENTS

First, to my heavenly father El Shaddai, through this process He has been my Yireh, Rapha, Nissi and my Shalom. He has always been the Adonai in my life. I would like to extend my higher gratitude to Dr. Susana Milla-Lewis, for her investment of resources and time. Your patient and efforts, your faith in my potential and your skillfull guidance have shaped my professional and personal life. I am so grateful for the opportunity you gave me and for opening my mind to new experiences. A special recognition to my committee members Dr. Consuelo Arellano and Dr. Ramsey Lewis for taking the time to advise me through my thesis. I would like to thank people that were fundamental during the development of my investigation. I thank Dr. Brian Schwartz for taking the time counting chromosomes for the colchicine project, Dr. Joseph Chiera for assistance with microscopy for stomatal measurements, Dr. Tom Stalker for his assistance during pollen stains, Dr. Bangya Ma who made all her knowledge of pathogenic fungi available during the GLS inoculation process, Dr. Consuelo Arellano for helping me with the statistical analysis and MS. Manuel Camacho for helping me creating the graphs for the GLS project

I would also like to thank Carolina Zuleta. Since I started my life at NCSU, you have been a role model in my life. Thank you for letting me learn from you, and for solving those problems that seemed impossible to solve at that time. Thank you for being there, reminding me that the world is not square and that there is always a better way to do things. I definitely need to thank my friends at the turfgrass breeding lab: Dr. Jeffrey Dunne, Dr. Xingwang Yu, Dr. Jenny Kimball, Dr. Steve Mulkey, MS. McCamy Pruitt, Kelly Moore, Susan Gomez Ramos, Axel Gonzales, and Yessica Orosco; they had my back during this project!

A special thanks to the Family Suazo-Cardoza. For the long emotive talks that cultivated in me, the encourage and desire to pursue this degree. I want to extend my gratitude to the family Gonzalez-Ramos (Axel, Susan, Maya and Seuss). When days turned long, cold and dark, they were always there to give me support, advice, love, and of course to feed me.

Finally, thanks to my friends and family that showed their appreciation through letters, text messages and calls. I want to express a special appreciation to my girlfriend Ashly Alyssa Zsido for her love and for supporting me unconditionally in all possible ways.

TABLE OF CONTENTS

LIST OF TABLES viii
LIST OF FIGURES x
-CHAPTER I- 1
ST. AUGUSTINEGRASS INTRODUCTION 1
GENERAL OVERVIEW OF TURFGRASS 2
ORIGIN, MORPHOLOGY, PLOIDY LEVELS, AND PATHOLOGY OF ST. AUGUSTINEGRASS [*STENOTAPHRUM* SECUNDATUM (WALT.) KUNTZE] 3
POLYPLOIDY BREEDING IN ST. AUGUSTINEGRASS. 6
REFERENCES 8
-CHAPTER II- 13
DEVELOPMENT OF COLCHICINE-INDUCED TETRAPLOID ST. AUGUSTINEGRASS LINES 13
ABSTRACT 15
INTRODUCTION 16
MATERIAL AND METHODS 18
Colchicine Treatment and Seed Germination. 18
Flow Cytometry. 19
Stomata measurements. 20
Pollen Staining. 21
Cytology 21
RESULTS 23
Colchicine Treatment and Seed Germination 23
Flow cytometry 23
Stomata measurements 24
Chromosome counts 25
DISCUSSION 26
REFERENCES 29
-CHAPTER III- 39
IDENTIFICATION OF SOURCES OF RESISTANCE TO GRAY LEAF SPOT IN STENOTAPHRUM GERMPLASM 39
ABSTRACT 41

INTRODUCTION 42
MATERIAL AND METHODS 44
Plant material, establishment and maintenance 44
Fungal isolates and inocula production 45
Inoculation and incubation conditions 46
Data collection and analysis. 47
RESULTS 49
Trial I 49
Trial II 49
Trial III 50
Consistency in Performance 51
Trait correlation 51
DISCUSSION 51
Acknowledgements 54
REFERENCES 55
-APPENDICES- 70

LIST OF TABLES

CHAPTER II. Development of colchicine-induced tetraploid St. Augustinegrass lines

Table 2. 1. Germination and recovery rates, and flow cytometry results for lines generated from treatment of 'Raleigh' seeds with four colchine concentrations at four exposure times. 33

Table 2.2. Mean comparison test in stomata density of ten putative tetraploid genotypes and two controls..... 34

Table 2. 3 Final flow cytometry analysis of eight lines generated from colchicine treatment of 'Raleigh' seeds. 35

Table 2. 4. Mean comparison test in adaxial stomata lengths of eight putative tetraploid genotypes and two controls. 36

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

Table 3. 1.. *Stenotaphrum* genotypes evaluated for resistance to three sources of inocula of *Magnaporthe oryzae* under controlled environmental conditions. 58

Table 3. 2. *Magnaporthe oryzae* inoculation treatments used to screen *Stenotaphrum* germplasm for genetic resistance..... 60

Table 3. 3. Individual and combined ANOVA for evaluation trials of *Stenotaphrum* germplasm for resistance to three sources of inoculum of *Magnaporthe oryzae*. Traits evaluated included INCPER= incubation period, INCID D7 = incidence on day seven, AUDPC = area under the disease progress curve, and AULEC = area under the lesion expansion curve. 61

Table 3. 4. Virulence of Magnaporthe oryzae averaged over genotypes for evaluation of Stenotaphrum germplasm to gray leaf spot resistance. 62

Table 3. 5. Genotype by inoculum LS-means for INCPER= incubation period, INCID D7 = incidence on day seven, AUDPC = area under the disease progress curve, and AULEC = area under the lesion expansion curve in Trial III. 63

Table 3. 6. Ranks across inocula for genotypes with high and low gray leaf spot AULEC, AUDPC, incubation period (INCPER) and final incidence (INCID D7) over multiple Trials. ... 64

Table 3. 7. Means across inocula for genotypes with high and low gray leaf spot AULEC, AUDPC, incubation period (INCPER) and final incidence (INCID D7) across runs and Trials.

Genotypes highlighted in bold performed consistently among the top and were identified as highly resistant (HR). 65
Table 3. 8. Pearson correlation between genotype least squares means in traits 66
Appendix 3. 1. LS Means of genotypes across traits found in trial I, including interaction of genotype with inoculum on AULEC. 71
Appendix 3. 2. Error terms for random effects sorted by trials in all traits. 72
Appendix 3. 3. LS Means of genotypes across traits in trial II, including interaction of genotype with inoculum on final incident and AULEC. 73

LIST OF FIGURES

CHAPTER II. Development of colchicine-induced tetraploid St. Augustinegrass lines

Figure 2.1 Distribution of stomatal lengths on the abaxial surface of leaves of diploid 'Raleigh', putative tetraploid genotypes, and polyploid 'Floralawn' 37

Figure 2.2 Indicators to infer ploidy levels of five St. Augustinegrass genotypes: 38

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

Figure 3.1 Main Effects LS means for AULEC, incubation period, and final incidence of St. Augustinegrass germplasm evaluated for resistance to gray leaf spot.....67

Figure 3.2 Main Effects LS means for AUDPC, incubation period, and final incidence of St. Augustinegrass germplasm evaluated for resistance to gray leaf spot.....68

Figure 3.3 Main Effects LS means for AUDPC and final incidence based on ploidy levels of St. Augustinegrass germplasm evaluated for resistance to gray leaf spot.....69

-CHAPTER I-

ST. AUGUSTINEGRASS INTRODUCTION

GENERAL OVERVIEW OF TURFGRASS

Turfgrass, as a term of agronomy, has been disseminated within the last 70 years in the US (Waddington et al., 1992). This term had its origin when people living in the surrounded areas where greens were grazed by sheep and goats, started taking advantage of the well-knit turf that was left because of these animals' activity. Entire families enjoyed these places for recreation in the early days. As time passed by, Native Americans were involved in several sports using grass sod as a surface. Soon, the demand for grasses increased and in time they were used in parks, yards, schools, cemeteries, airport runways, highways, and even as construction material for roofs.

Two principal categories of turfgrasses have been identified based on the environmental conditions they are adapted to: Cool season turfgrasses, which include fescues (*Festuca* spp), ryegrass (*Lollium* spp.), bluegrass (*Poa* spp.), and bentgrass (*Agrostis* spp.). Warm season turfgrasses include bermudagrass [*Cynodon dactylon* (L.) Pers.], centipedegrass [*Eremochloa ophiuroides* (Munro.) Hack.], zoysiagrass (*Zoysia* spp.), and St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze]. Differentiation between both groups is that cool season grasses are long-day plants using C3 photosynthesis, while warm season grasses are short-day plants using C4 photosynthesis. Cool season grasses are well adapted to the north of the United States, while warm season grasses have been established in the south. The country is divided by two geographical regions where both winter and summer reach intense weather conditions. However, there is a transition zone between the two regions, connected from North Carolina and Virginia into the Midwest. Both groups of grasses are economically grown here, but none of them is fully adapted.

According to Gibeault and Cockerham (1985), the annual turfgrass maintenance cost for eight categories of use (including golf courses) across the 50 US states was 24.5 billion dollars in 1982 (Waddington et al., 1992). North Carolina (NC) contributed about 2.1 % to that amount. Currently, as expected, due to people's demand, turfgrass has become an enormous industry that keeps gradually expanding with the development of improved cultivars. An estimated $1.2 billion was spent on maintenance of approximately 2.14 million acres. Turfgrass in NC contributes about 5 billion dollars annually to the economy according to a state-wide survey conducted in 1999 (North Carolina Department. of Agriculture and Consumer Services, 1999).

ORIGIN, MORPHOLOGY, PLOIDY LEVELS, AND PATHOLOGY OF ST. AUGUSTINEGRASS [*STENOTAPHRUM* SECUNDATUM (WALT.) KUNTZE]

St. Augustinegrass has its place with the group of warm season-turfgrasses. Tolerance to shade (Busey, 1995; Peacock, and Dudeck, 1993) and weed infestation (Busey, 2003; Long and Bashaw, 1991) along with stoloniferous growth habit and moderately low input requirements when compared to other turfgrass species, are its best attributes. Because of these characteristics, St. Augustinegrass is considered a popular choice for lawns (Green et al., 1981). The species is known by different names in other parts of the world, and it is sometimes called carpetgrass in the southeastern US (Sauer, J.D. 1972). St. Augustinegrass is believed to be native to Africa, the West Indies, Southern Mexico and the US (Busey and Augustine 1980). The world's first known record of planting St. Augustinegrass was on November 1880, as a turf alongside an avenue at A. M. Reed's Mulberry Grove Plantation in Yukon, FL (Busey, 2003).

The genus *Stenotaphrum* comprises seven species with different ploidy levels (Sauer 1972). The base chromosome number of *S. secundatum* is x=9, with diploids (2n=2x=18),

triploids (2n=3x=27), tetraploids (2n=4x=36), hexaploids (2n=6x=54), and aneuploids (2n=28-32) reported (Long and Bashaw, 1961; Milla-Lewis at al., 2013). From the seven species, Sauer (1972) reported that *S. oostachyum* and *S.unilaterale* (both grown at elevations of 1000-1500 m), *S. helfery, S.clavigerum* and *S.micramthu* (grown in coastal regions) have been naturally scattered. Because deliberate planting is not reported, breeding purposes for these species are dismissed. The genus *S. dimidatum,* has been reported to be deliberately planted in conjunction with commercial plantations (bananas, coconuts, coffee) (Sauer, 1972). Because of its resistance to several pests such as *Pycularia grisea* (Cke.) Sacc. (Atilano and Busey, 1983), the southern chinch bug (Reinert et al., 1986; Busey, 1990), and the sting nematode (Busey et al., 1993), *S. dimidatum* has been used in interspecific crosses for breeding improvements in St. Augustinegrass (Genovesi et al., 2005). According to Sauer (1972), the origin of *S. secundatum* emanates from a fertile diploid which evolved from *S. dimidiatum* accessions from the West Indian Ocean. Polyploidy in St. Augustinegrass is present in a significant minority of specimens that differ in the characteristics of the inflorescence. These deviants are classified either as part of the Natal-Plata deme (natural habitat in the coast of Natal and adjacent parts of Mozambique and the Transkei) or as part of the Cape deme (first collected in 1791 at the Cape of Good Hope). Both classifications do not fall into the formal taxonomical ranks because they were not believed to be breeding populations (Sauer, 1972). Casler and Duncan (2003) stated that the National Plant Germplasm System had 23 foreign introductions of *Stenotaphrum* available for breeders in 2001 (USDA, ARS, National Plant Germplasm System, 2001). The potential germplasm of St. Augustinegrass comes from pastures in Oceania (Sauer, 1972), the West Indies (Busey et al, 1982), and from coastal Africa, from Kenya to the Cape of Good Hope. In the United States, The University of Florida (UF) counts with the largest St. Augustinegrass germplasm collection.

Unfortunately, cytological studies have not been done to classify these materials into ploidy levels. This extensive germplasm source is potentially the best aid for breeders during the development of improved cultivars.

Due to the popularity of this grass among home owners, it is also grown and distributed by commercial landscape companies. However, characteristics like winter survival, turf quality (color, texture, density and uniformity), and disease resistance, are critical features for marketability purposes. Polyploid genotypes have been reported to have increased water-use efficiency (Busey, 2003), and more aggressive growth habit (Riordan et al., 1980). Furthermore, polyploids have also been found to have more resistance against several biotic stresses (Yildiz, 2013) such as lance nematodes (*Hoplolaimus galeatus*), the sting nematode (*Belonolaimus longicaudatus* Rau), the St. Augustine Decline Strain of Panicum Mosaic Virus , southern chinch bugs (*Blissus insularis* Barber), and *Magnaporthe grisea,* the causal agent of gray leaf spot (Gibling-Davis et al., 1995; Busey and Zaenker., 1992; Busey, 1990; Horn et al., 1973; Busey et al., 1993; Milla-Lewis et al., 2011).

Gray leaf spot (GLS) is one of the principal diseases in St. Augustinegrass. Conidia of *Magnaporthe grisea* are normally dispersed by wind and water splash (Smiley et al., 1996). Once the fungus is hosted in the plant, moisture conditions and temperatures between 25 and 30°C (Atilano & Busey, 1983; Uddin *et al.*, 2003; Harmon *et al.*, 2005) will incite spreading of the disease. The first symptoms appear as small brown dots, becoming necrotic areas that can reach 2 cm in length (Smiley et al., 1996). After infection, GLS can destroy large areas of turfgrass rapidly. Management includes minimizing the use of nitrogen fertilization, preventative fungicide applications (Harmon *et al.*, 2005), and use of cultivars with less susceptibility (Atilano and Busey, 1983; Jo *et al.*, 2004; Harmon *et al.*, 2005).

POLYPLOIDY BREEDING IN ST. AUGUSTINEGRASS.

Most of the work done in order to improve populations of St. Augustinegrass has been on the diploid germplasm pool (Genovesi, 2009). Theoretically, crosses between diploid and polyploid germplasm could combine the best characteristics of both groups. However, development of hybrids between ploidy levels has been hindered due to sterility issues related to unbalanced chromosome numbers (Yang et al., 2014). Development of tetraploid lines of St. Augustinegrass will generate new genetic variation to be added to the germplasms banks. Additionally, stable tetraploid plants would enable hybridization with polyploid germplasm of interest. Also, the creation of triploid sterile lines will be possible by crossing tetraploid plants with diploid genotypes.

In plant breeding, there are many chemical agents that when exposed to plant material can have an effect at the chromosome level, including duplication. Ostergreen (1944) experimented with exposing root tips of *Allium Cepa* as plant material to several chemical substances with different threshold concentrations. In his research, Ostergreen studied more than fifty substances including some of the most different groups of organic compounds. Among the substances he found with c-mitotic efficiency were: nitrous oxide, chloroform, bromoform, iodoform, acetamide, and carbamate, among others. Colchicine and oryzalin are the chemical agents that are most frequently used in agriculture to duplicate chromosome numbers in plants (Dhooghe et al., 2011). Both chemicals are mitotic inhibitors that disrupt spindle microtubules during mitosis.

Dunn and Lindstrom (2007) used oryzalin to induce polyploidy in Buddleja. They suggested that oryzalin can be used to restore fertility. Chromosome doubling using oryzalin

was reported in *Lilium* and *Nerine* (Jaap et al., 1992); haploid apple shoots in vitro (Bouvier et al,. 1994), and roses (Kermani et al., 2003).

Several studies have used colchicine to manipulate ploidy levels in turfgrass breeding. Dhooghe in 2011 offered a list of 54 experiments conducted to induce ploidy levels in plant material using colchicine. Schwartz et al. (2013a; 2013b; 2013c) used colchicine to manipulate ploidy levels in species such as zoysiagrasses (*Zoysia* Willd.), centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.], and seashore paspalum (*Paspalum vaginatum* Swartz). Quesenberry et al. (2010) produced polyploid lines using colchicine in bahiagrass (*Paspalum notatum* Flüggé).

REFERENCES

Atilano, R.A. and Busey P. 1983. Susceptibility of St. Augustinegrass germ plasm to *Pyricularia grisea.* Plant Disease 67:782-783

Bouvier L., Fillon F. R. Lespinasse Y. 1994. Oryzalin as an Efficient Agent for Chromosome Doubling of Haploid Apple Shoots *in vitro*. Plant Breeding, 113: 343–346.

Busey, P. and Augustine, B.J. 1980. Appraisal of St. Augustinegrass grasses for south Florida. Proceeding of the Florida State. Horticulture Society, 93, 99-102.

Busey, P. 1990. Polyploid Stenotaphrum germplasm: resistance to the polyploidy damaging population southern chinch bug. Crop Science 30:588-593.

Busey, P. and E.I. Zaenker. 1992. Resistance bioassay from southern chinch bug Heteroptera: *Lygaeidae excreta*. J. Econ. Entomol. 85:2032-2038.

Busey, P., R.M. Giblin-Davis, and B.J. Center. 1993. Resistance in *Stenotaphrum* to the sting nematode. Crop Sci. 33:1066-1070.

Busey, P. 1995. Genetic diversity and vulnerability of St. Augustinegrass. Crop Sci. 35:322-327.

Busey P, Broschat TK, Center BJ. 1982. Classification of St. Augustinegrass. Crop Science 22:469-473.

Busey, P. 2003. St. Augustinegrass, *Stenotaphrum secundatum* Walt Kuntze. P.309-329. In Casler, M. D. Duncan, R. R. Turfgrass Biology, Genetics and Breeding. ISBN John Wiley and Sons, Inc., Hoboken, New Yerssey.

Dhooghe E., Van K., Eeckhaut T., Leus L., Van Huylenbroeck J., 2011. Mitotic chromosome doubling of plant tissue in vitro. Plant cell tiss organ cult 104:359-373. DOI 10.1007/s11240-010-9786-5.

Dunn, B.L. and J.T. Lindstrom. 2007. Oryzalin induced chromosome doubling in Buddleja to facilitate interspecific hybridization. HortScience 42:1326–1328.

Genovesi A.D, Engelke M.C, Elias S. 2005. Interspecific Hybridization between Pembagrass (*Stenotaphrum dimidiatum*) and St. Augustinegrass (*S. secundatum*) Using Embryo Rescue.

Genovesi A.D, Jessup RW, Engelke MC, Burson BL. 2009. Interploid St. Augustinegrass [Stenotaphrum secundatum (Walt.) Kuntze] hyDebrids recovered by embryo rescue. In Vitro Cell. Dev. Biol.-Plant 45:659-666.

Green, R.L., A.E. Dudeck, L.C. Hannah, and R.L., Smith. 1981. Isoenzyme Polymorphism in St. Augustinegrass. Dep. of Ornamental Horticulture, IFAS, Univ. of Florida, Gainesville, FL 32fi11. Published with the approval of the Director of the Florida Agric. Exp. Stn. as Journal Series Paper No. 2479

Giblin-Davis, R.M. Busey, P. Center, B.J. 1995. Parasitism of hoplolaimus galeatus on diploid and polyploid St. Augustinegrasses. Journal of nematology 27(4):472-477.

Harmon, P., Datnoff, L., Nagata, R., Brecht, M., and Stiles, C. 2005. Gray leaf spot of St, Augustinegrass: cultural and chemical options. EDIS. University of Florida IFAS Extension. Accessed 2011.

Horn, G., A. Dudeck, and R. Toler. 1973. 'Floratam' St. Augustinegrass: A fast growing new variety of ornamental turf resistant to St. Augustine decline and chinch bugs. Fla. Agric. Exp. Stn. Circ. S-224.

Jaap M., Tuyl V., Meijer B., Diën V., Maria P. 1992. The use of oryzalin as an alternative for colchicine in in-vitro chromosome doubling of *Lilium* and *nerine*. Acta Hortic. 325, 625-630 DOI: 10.17660/ActaHortic.1992.325.88.

Jo YKi, Rimelspach JW, Boehm MJ, 2004. Gray Leaf Spot on turfgrass. Ohio, USA: Ohio State University.

Kermani M. J., Sarasan V., Roberts A. V., Yokoya K., Wentworth J., Sieber V. K. 2003. Oryzalin-induced chromosome doubling in Rosa and its effect on plant morphology and pollen viability. Theoretical & Applied Genetics, 107(7), 1195-1200. doi:10.1007/s00122-003-1374-1

Milla-Lewis, S.R., B. Ma, C. Arellano, M.C. Zuleta, W.C. Reynolds, and L.P. Tredway. 2011. Evaluation of St. Augustinegrass (Stenotaphrum secundatum (Walt.) Kuntze) germplasm for gray leaf spot resistance. Proc. Amer. Soc. Agron. Intl. Ann. Mtg., San Antonio, TX. Oct 16-19.

Milla-Lewis, S.R., M.C. Zuleta, G.A. Van Esbroeck, K.H. Quesenberry, and K.E. Kenworthy. 2013. Cytological and Molecular Characterization of Genetic Diversity in *Stenotaphrum* Crop Science Vol 53(1):296-308.

North Carolina Department of Agriculture and Consumer Services and National Agricultural Statistics Service U.S. Department of Agriculture. 1999. North Carolina Turfgrass Survey. North Carolina Agricultural Statistics, Raleigh, NC.

Ostergreen, G. 1944. Colchicine mitosis, chromosome contraction, narcosis and protein chain folding. Hereditas, 30: 429–467.

Peacock, C. H. and A. E. Dudeck. 1993. Response of St. Augustinegrass cultivars *Stenotaphrum secundatum* (Walt.) Kuntze] to shade. Int. Turfgrass Soc. Res. J. 7:657-663.u

Quesenberry, K.H. Dampier, J. M. Lee, Y.Y. Smith, R.L. Acuna, C.A. 2010. Doubling the chromosome number of bahiagrass via tissue culture. Euphytica 175:43-50

Sauer, J.D. 1972. Revision of *Stenotaphrum* (Gramineae: Paniceae) with attention to its historical geography. Brittonia 24: 202-222.

Schwartz, B.M., K.R. Harris-Shultz, R.N. Contreras, C.S. Hans, and S.A. Jackson. 2013a. Creation of hexaploid and octaploid zoysiagrass using colchicine and breeding. Crop Sci. 53(5):2218-2224.

Schwartz, B.M. Harris-Shultz, K.R. Contreras, R.N. Hans, C.S. Hanna, W.W. Milla-Lewis, S.R. 2013b. Creation of artificial Triploid and Tetraploid Centipedegrass using colchicine and breeding. Volume 12, 2013.

Schwartz, B.M. Contreras, R.N. Harris-Shultz, K.R. Heckart, D.L. Peake, J.B. Raymer, P.L. 2013c. Discovery and Characterization of a Turf-Type Triploid Seashore Paspalum. Hort Sci. 48(12):1424-1427.

Smiley RW, Dernoden PH, Clarke BB, 1996. Compendium of Turfgrass Diseases. St. Paul, MN, USA: The American Phytopathological Society.

Reinert J.A., P. Busey, and F. G. Bilz. 1986. Old World St. Augustinegrasses resistant to the Southern Chinch Bug (Heteroptera: Lygaeidae). J. Econ. Entomol. 79:1073-1075.

Riordan, T.P., Meier, V.D Long, J.A., and Gruis, J.T. 1980. Registration of Seville St. Augustinegrass. Crop Sci. 20: 824-825.

Uddin, W., Viji, G., and Vincelli, P. 2003. "Gray leaf spot (blast) of perennial ryegrass turf: An emerging problem for the turfgrass industry." Plant Disease 87: 880-889.

Waddington, D.V. Carrow, R.N. Shearman, R.C. (1992). Turfgrass. Monograph No. 32. Amer. Soc. of Agron., Madison, WI.

Waddington, D.V. Carrow, R.N. Shearman, R.C. (1992). Turfgrass. Monograph No. 32. Amer. Soc. of Agron., Madison, WI. In Gibeault, V.A., and S.T., Cockerham. 1985. Turfgrass water conservation. Publ.21405. Univ. of California Riverside.

Yang, Z. Shen, Z. Tetreult, H. Johnson, L. Friebe, B. Frazier, T. Huang, L. Burklew, C. Zhang, X.Q. Zhao, B. 2014. Production of Autopoliploid Lowland Switchgrass Lines through In Vitro Chromosome Doubling. Bioenergy Res 7:232-242.

Yildiz, M. 2013. Plant Responses at Different Ploidy Levels. , Current Progress in Biological Research, Dr. Marina Silva-Opps (Ed.), InTech, DOI: 10.5772/55785.

-CHAPTER II-

DEVELOPMENT OF COLCHICINE-INDUCED TETRAPLOID ST. AUGUSTINEGRASS LINES

Development of Colchicine-Induced Tetraploid St. Augustinegrass Lines

Esdras M. Carbajal[1], M. Carolina Zuleta[1], Luellen Swayzer, Brian M. Schwartz[2], M. Carolina Chavarro[3], A. Carolina Ballen-Taborda[3] and Susana R. Milla-Lewis[1]

[1]E.M. Carbajal, M.C. Zuleta, and S.R. Milla-Lewis, Dept. of Crop Science, Box 7620, N.C. State Univ., Raleigh, NC 27695-7620. [2]B.M. Schwartz and L. Swayzer, Department of Crop and Soil Sciences, University of Georgia - Tifton, Tifton, GA, [3]M.C. Chavarro, and A.C. Ballen-Taborda, Center for Applied Genetic Technologies, 111 Riverbend Rd., Athens, GA 30602

Received: _____. * Corresponding author email: susana_milla-lewis@ncsu.edu

ABSTRACT

St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] (2*n* = 2*x* = 18) is characterized by rapid stolon production which makes it well suited for sod production, home lawns and commercial landscapes. While many genotypes are cross-fertile, all St. Augustinegrass cultivars are propagated vegetatively in sod production. As a means of ensuring varietal purity, the development of sterile triploid hybrids by crossing tetraploid and diploid genotypes has been successfully used in other warm-season turfgrasses. Applying this model in St. Augustinegrass would be beneficial to sod producers and turf managers who require purity for certification and uniformity for performance, respectively. Therefore, the present study was conducted to develop colchicine-induced tetraploid lines of St. Augustinegrass. Seeds of cultivar 'Raleigh' were treated with four colchicine concentrations at four exposure times. Seedlings recovered after treatment, were screened for genome size changes using flow cytometry. A set of putative tetraploid lines was initially identified and evaluated for stomatal density, stomatal length and pollen stainability. Further analysis of putative tetraploids indicated all lines had found to be diploid with the exception of one, DSA 13005. Pollen stainability of this line was high and it produced 33 progenies through selfing, two of which were identified as putative tetraploids via flow cytometry. Average stomata lengths for DSA 13005 and its two progeny lines, DSA 16001 and DSA 16016, were found to significantly ($P<0.05$) larger than that of diploid control Raleigh. All three lines were corroborated as tetraploids (2n = 4x = 36) through chromosome counts. These lines will be used in future breeding efforts in an attempt to develop sterile triploid cultivars.

INTRODUCTION

St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] is a warm season-turfgrass, extensively distributed across the southern US, especially in coastal regions where cold temperature extremes are moderated by oceanic climatic conditions. Because of characteristics like shade tolerance, stoloniferous growth habit, and moderately low input requirements when compared to other turfgrass species, St. Augustinegrass is considered a popular choice for lawns (Green et al 1981). The genus *Stenotaphrum* comprises seven species with different ploidy levels (Sauer 1972). The base chromosome number of *S. secundatum* is x=9, with diploids (2n=2x=18), triploids (2n=3x=27), tetraploids (2n=4x=36), hexaploids (2n=6x=54), and aneuploids (2n=28-32) reported (Long and Bashaw, 1961; Milla-Lewis at all., 2013). Polyploid genotypes have been found to have more resistance against several biotic stresses (Yildiz, 2013) such as lance nematodes (*Hoplolaimus galeatus*) (Gibling-Davis et al., 1995), the sting nematode (*Belonolaimus longicaudatus* Rau) (Busey et al., 1993), the St. Augustine Decline Strain of Panicum Mosaic Virus (Horn et al., 1973), southern chinch bugs (*Blissus insularis* Barber), (Busey, 1990; Busey and Zaenker, 1992), and *Magnaporthe grisea*, (Milla-Lewis et al., 2011) the causal agent of gray leaf spot. Furthermore, polyploids have also been reported to have increased water-use efficiency (Busey, 2003), and more aggressive growth habit (Riordan et al., 1980). The majority of the work done in order to improve populations of St. Augustinegrass has been on the diploid germplasm pool (Genovesi et al., 2009). Theoretically, crosses between diploid and polyploid germplasm could combine the best characteristics of both groups. However, development of hybrids between ploidy levels has been hindered due to sterility issues related to unbalanced chromosome numbers (Yang et al., 2014). Moreover, among public

available germplasm while five ploidy levels have been reported in the genus, aneuploidy seems to be more prevalent than polyploidy and the majority of this germplasm is sterile.

An important factor to determine sterility in plants relates to balanced numbers of chromosome sets. Even numbers of chromosome sets results in fertile plants, while both aneuploidy and odd numbers of chromosome sets result in sterile plants. Sterility is a problem when the importance of the crop is rooted in seed production, such as when the commercial product is the actual seed (grains, legumes, etc.) or when seeds are the only viable option for commercial propagation (turfgrasses and others ornamental plants). On the other hand, sterility can be used as a tool in plant breeding to reduce/eliminate the number of seeds in edible fruits (f.e. watermelon (Gray and Elmstrom., 1991), grapes (Scorza et al., 1996)). Sterility can also be used to guarantee genetic purity for vegetative propagated species, for example in sod production. In bermudagrass, crossing tetraploid genotypes (*Cynodon dactylon* (L.) Pers.) with diploid genotypes (*C. transvaalensis* Burtt-Davy*)* to produce sterile triploid hybrids has been a common breeding methodology to ensure varietal purity in turf stands (as in sod production or golf courses), as a means of intellectual property protection, and also to combine traits of interest from the two species. This model has had significant impact on the evolution of the turfgrass industry (Hanna and Anderson, 2008) as cultivars that do not produce seedlings are beneficial to both sod producers and turf managers who require purity for certification and uniformity for performance, respectively.

Several studies have used colchicine to manipulate ploidy levels in turfgrass breeding. In zoysiagrasses (*Zoysia* Willd.) (Schwartz et al, 2013a) and in centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.] (Schwartz et al., 2013b), colchine was successfully used to develop polyploid lines in an attempt to generate useful morphological variation. In seashore Paspalum

(*Paspalum vaginatum* Swartz), colchicine was used to create artificial tetraploid plants that could be crossed with diploid genotypes to generate sterile triploid plants. One stable triploid plant was identified, but there was no certainty on its true origin (Schwartz et al., 2013c). In bahiagrass (*Paspalum notatum* Flüggé), colchicine was used to produce tetraploid plants that could be hybridized with apomictic tetraploids (Quesenberry et al., 2010). Native adapted switchgrass (*Panicum virgatum* L.) in North America is divided in two ecotypes; lowland types which are tetraploid (2n = 4X = 36) and upland ones which are octoploid (2n = 8X = 72). To exploit heterosis, colchicine was used to create autooctoploid lines from the tetraploid lowlands types that could be crossed with octoploid upland types. From thirteen autooctaploid plants, one was successfully crossed to an upland plant and the hybrid was confirmed with molecular marker analysis (Yang et al., 2014). No such studies have been conducted in St. Augustinegrass although development of stable tetraploid lines would of interest for creation of sterile triploid hybrids, to be used in crosses with polyploid germplasm of interest and to increase genetic variation. Therefore, the objectives of the present study were to develop colchicine-induced St. Augustinegrass lines.

MATERIAL AND METHODS

Colchicine Treatment and Seed Germination.

In 2013, over 1500 mature 'Raleigh' St. Augustinegrass seeds were collected from the greenhouse and exposed to four colchicine concentrations and four exposure times. Concentrations included 0.025%, 0.05%, 0.1%, and 0.2% colchicine w/v. Exposure times included 5, 24, 48, and 72 hours. A non-treated control was added. The experiment consisted of three replications and seventeen treatments (including the non-treated control) with 30 seeds

each. All seeds were disinfected using alcohol 70%, Clorox 50% and sterilized water. Seeds were induced to germination by being submerged into water for 24 hours before treatment. Each treatment involved soaking 30 seeds in 5.0 mL of colchicine solutions in 100x15 mm petri dishes (with germination paper on the bottom). Petri dishes were stored in darkness at 21°C. After each treatment was completed, seeds/seedlings were transferred to MS media to induce germination and root system development. After 30 days, seedlings were transferred to soil and taken to the NCSU greenhouses (Raleigh, NC). An analysis of Variance (ANOVA) was performed to test the effect of colchicine concentration and exposure times on the germination rates of St. Augustinegrass seeds. To compare germination rates from treatments to a single control, multiple comparison tests of Dunett was performed.

Flow Cytometry.

Flow cytometry analysis was used to evaluate all recovered plants for changes in 2C nuclear DNA content as compared to DNA content of untreated Raleigh (2n = 2x = 18; Milla-Lewis et al., 2013). Additionally, corn (*Zea mays L.*) was used as a standard. Nuclear DNA content of the genotypes was determined using fresh leaf tissue taken from greenhouse-grown plants. The nuclear 2C DNA content for corn was assumed to be 5.43 pg. (Milla-Lewis et al., 2013). Approximately 1 cm^2 of fresh tissue from newly emerging leaves of treated plants and corn were placed in 47 mm petri dishes (Millipore SAS,67120 Molsheim, france). 600 µL of nuclei extraction buffer (CyStain PI Absolute P, Sysmex Partec GmbH, Münster, Germany) were added and leave tissue was chopped with single edge industrial blades for approximately 45 seconds. The solution was then filtered through a 30 µm filter into a 5-mL polystyrene round-bottom tube. Nuclei solution was stained with a Cystain PI Absolute P Staining solution (Partec

GmbH, Münster, Germany) and incubated at 21°C for a minimum of 20 min. In order to accelerate the screening process, bulk samples of four St. Augustinegrass plants and corn were analyzed at once. In bulked samples, if a single Gap1 (G_1) peak was observed, all plants were characterized as diploids. Individual analyses were performed for groups where a second peak indicated a G1 phase for a tetraploid plant. Flow cytometry analysis was performed at the North Carolina School of Veterinary Medicine (Raleigh, NC). A FACSCalibur flow cytometer (Becton-Dickinson Biosciences, San Jose, CA) was used. It was conducted with a 15-mW argon laser (excitation at 488 nm) and propidium iodide fluorescence (FLA-2) was detected. For screening purposes, the DNA peak data were generally based on the fluorescence of between 2,000 and 4,000 scanned particles. DNA content was calculated dividing the sample histogram peak of the sample, by the standard histogram peak and then multiplying the result by the DNA content of the standard. Ploidy levels were obtained by comparing mean DNA of each sample to that of Raleigh (Milla-Lewis et al., 2013).

Stomata measurements.

Based on flow cytometry results, stomata measurements were taken from ten putative tetraploid and three diploid plants according to the protocol of Schwartz et al. (2013b). Additionally, Raleigh ($2n = 2x = 18$) and Floralawn (2n=4x=c.32) were used as non-treated controls. Clear fingernail polish was spread uniformly on the abaxial (underside) and adaxial (surface) side of five different leaves per genotype. After one minute, the polish was pealed from the leaf surface, placed under a slip on a microscope slide and studied under a light Nikon Biophot VBS microscope with 280X magnification. The software Image J (Schneider et al., 2012) was used to capture and manipulate the sample for measurements. The impressions made

were used to measure stomata density (stomata/mm^2) and stomata length averages (μm) for each genotype. Five samples per leaf for a total of 25 samples were measured per each putative colchicine doubled plant. An ANOVA was performed for both stomata length and stomata density to test for differences among genotypes. When significant ($\alpha = 0.05$), Fisher's protected LSD values were used to determinate significant differences among entries.

Pollen Staining.

Pollen viability was analyzed using the acetocarmine staining method of Hesse et al. (2009). Fresh pollen was taken from the putative artificial tetraploid plants and two controls, Raleigh and FX-10 (2n = 4x = 30). Grains of pollen were placed into a drop of acetocarmine, and observed under light microscopy for detection of the nucleus and the sperm nuclei. Pollen turned into red color was identified as fertile (Hesse et al., 2009).

Cytology

Chromosome counts of flow cytometry confirmed tetraploids, Raleigh, and Floralawn were conducted as described by Schwartz et al. (2013 b).The apical meristem from the distal ~2 cm of actively growing roots was excised from plants growing in potting mix. The roots were rinsed in cold water before being treated with nitrous oxide for 75 to 90 min (Kato, 1999). Root tips were immediately fixed in 3:1 ethanol: acetic acid and left at room temperature for up to one month before generating chromosome spreads (Kato et al., 2004; Gill et al., 2009; Findley et al., 2010). Chromosomes were stained and mounted with Vectashield with DAPI (H-1200, Vector Labs) and viewed using a Zeiss AxioImager M2 epifluorescence microscope (Carl Zeiss Microscopy GmbH). Images were captured at 1000X magnification and analyzed using the

attached Zeiss AxioCam MRc camera and analyzed with Zeiss Axiovision Release 4.8 software. Chromosome spreads of at least 15 cells for each genotype were counted to determine final chromosome number.

RESULTS

Colchicine Treatment and Seed Germination

The number of seeds that germinated from all colchicine treatments was 1,031 from which 405 plants (39%) survived (Table 2.1). Seed germination rates for all treatments ranged from 36% (C2T2 and C2T5) to 83% (C2T3 and C3T5). As expected, the control treatment (no colchicine) had the highest germination rate at 89%. The average seed germination for all treatments was 67%. Significant differences in germination were found among treatments at p=0.05. The control treatment had significantly higher germination rates than treatments C2T2, C2T4, C2T5, C3T4, C4T2, C5T2, and C5T5.

Flow cytometry

Based on flow cytometry results (Table 2.1), the 80 plants regenerated from the control treatment had a 2C nuclear DNA content that corresponded to that of diploid 'Raleigh' ($2n = 2x = 18$). From 405 plants that were analyzed, an initial 10 (2.5%) had a 2C content doubled that of Raleigh and were considered putative tetraploids. One putative colchicine-induced tetraploid was found in each of the treatments 0.025 % colchicine for 5 hours (C2T2), 0.025 % for 48 hours (C2T4), and 0.1 % for 24 hours (C4T3). The most successful treatment was 0.05% colchicine for 24 hours (C3T3) with seven putative colchicine- induced tetraploid plants identified. Putative tetraploids were maintained in the greenhouse for further analysis. In 2015, colchicine-induced putative tetraploids were re-checked for ploidy level using flow cytometry. From the original 10 identified in 2014, 12 were found to have a 2C nuclear DNA content that corresponded with diploid Raleigh. Lines DSA 13005 (C2T4) and DSA 13013 (C3T3) were found to still have doubled the 2C content of Raleigh. From treatment using colchicine at 0.05% during 24 hours

(C3T3), line DSA 13006 showed reduplication, meaning that its DNA-histogram showed nuclei with different ploidy levels during the G1 phase of the cell cycle.

In 2016, different stolon samples presenting thickness increment were taken from putative tetraploid plants DSA 13005, DSA 13013 and DSA 13015 and flow cytometry was performed once more. For DSA 13005, out of 17 samples ten were tetraploid and the remaining seven were diploid. For DSA 13013, ten samples were taken and all were found to be diploids. For DSA 13015, from ten samples taken four showed reduplication in the flow cytometry histogram and the remaining samples were diploids. These results showed that only one unique line, DSA 13005, from the treatment of colchicine at 0.025 % during 48 hours (C2T4), remained a tetraploid.

Pollen viability was evaluated in line DSA 13005 and controls Raleigh and Floralawn. Results showed no visual differences in pollen stainability (data not shown), between DSA 13005 and the controls. The fertility of genotype DSA 13005 was confirmed when in December of 2015 it flowered and produced seed. Seeds were harvested and germinated and 33 offspring were recovered. Based on flow cytometry results, out of 33 offspring, plants DSA 16001 and DSA 160016 were found to be tetraploid (Table 2.3).

Stomata measurements

Stomata density and stomata length were measured on both the abaxial and adaxial surfaces of the leaf. Ten putative plants originally identified after treatment and two controls, were measured for stomata density (Table 2.2). Results in the abaxial side of the leaf showed significant differences between putative-tetraploid lines and Floralawn, and between controls Raleigh and Floralawn. Not differences were found between putative-tetraploid lines and

Raleigh. Stomata density in the adaxial side shows statically differences among three lines and Raleigh. Eight colchicine-induced tetraploid lines were screened for variation in stomata measurements. Six putative tetraploid plants, including two spring from DSA 13005 (DSA 13005, DSA 13010, DSA 13013, DSA 13015, and DSA 16001 DSA 16016), two diploid plants (DSA 16030 and DSA 16031) and two controls (diploid Raleigh and tetraploid Floralawn) were included for stomatal analysis. Stomata length in the abaxial side shows differences between controls and differences between putative lines and Floralawn (Results not shown). Results from stomata length, in the adaxial side showed genotypes were found to be statically different ($P<0.0001$). Stomata length of DSA 16001, are significantly ($P<0.0001$) bigger than the other genotypes. Lines DSA 16016 and DSA 13005 show similar stomata length, smaller stomata than DSA 16001, but bigger lengths than the others genotypes. Results from this study indicate stomata length on the adaxial surface of leaves, as the most accurate measurement parameter that can be used to infer ploidy level in St. Augustinegrass.

Chromosome counts

In 2014, initial chromosome counts were performed on only two genotypes (DSA13005 and DSA13013) to confirm results from flow cytometry. Both lines were found to contain 36 chromosomes thereby confirming their tetraploid status. In 2017, chromosome counts were performed in all remaining putative tetraploid lines. DSA 13005 and its two progeny lines, DSA16001 and DSA 16016, were found to be $2n=4x=36$ (Figure 2.2D).

DISCUSSION

The objective of this study was to create tetraploid lines of St. Augustinegrass to target the creation of sterile triploid lines, but also to generate genetic variation and facilitate interspecific breeding with polyploid *Stenotaphrum* germplasm. The best treatment producing putative-tetraploid plant was using colchine at 0.05% during 24 hours (Table 2.1). Ten putative colchine-induced tetraploid plants were initially identified. Only one line remained tetraploid, line DSA 13005 from treatment C2T4 (Table 2.1). Low success rates in the use of colchicine for induction of polyploidy have been previously observed in other grasses. In zoysiagrass, Schwartz et al. (2013a) developed five putative plants using six colchicine concentrations from 0.025% to 0.1%. In seashore paspalum, Schwartz et al. (2013c) were able to develop a triploid line that remained stable using colchicine concentrations of 0.1%. Levan and Ostergren (1943) indicated that plant material treated with c-mitotic substances can in many cases suffer from the poisonous effects of the chemicals. Higher concentrations can rapidly kill the roots while diluted concentrations can cause inhibition of growth and cell division. Furthermore, Schwartz (2013a) indicated that the effectiveness of colchicine treatments depends on whether mitosis is arrested at the right stage. Both observations could explain the low success rate when developing polyploid lines. Dermen (1940) described chimeras as plants in which, after colchicine treatment, only a portion becomes polyploid while the other part remains diploid. The formations of chimeras as a result of colchicine use have been previously observed in zoysiagrass (Schwartz et al. 2013a) and centipedegrass (Schwartz et al. 2013b). In the present study, the loss of most putative tetraploids (Table 2.1) and the presence of both diploid and tetraploid genotypes among offspring of artificial tetraploid DSA 13005 were indicative of the presence of chimeras as a result of colchicine treatment. Another reason for the poor germination and survival rates observed in our

study can be attributed to the seed harvesting process. In this study, the use of forceps to harvest the seeds might have caused damage to the embryo affecting the performance of the seedlings and eventually causing death. Quesenberry et al. (2010) doubled the number of chromosomes of bahiagrass using colchicine and reported a 24% success rate using 313-900 µM. Application of this protocol in future research might help improve success rates in St. Augustinegrass.

Doubling chromosome numbers in plants increases the size of vegetative cells and concomitantly tends to result in bigger plant structures. This process is described by Levan (1938) and by Ostergren (1944) as the c-tumor. Plant material exposed to colchicine can present swelling which is caused by a change in the growth direction of the cell; instead of growing longitudinally, the growth is presented in all directions. Evidence of tumefaction was observed at the morphological level in our study. Artificial tetraploid plants DSA13005, DSA16001 and DSA16016 had increased leaf and internode sizes when compared with the diploid control (Figure 2.2A). It was previously shown that stomatal lengths can be used as a predictor of ploidy level (Schwartz et al., 2013a; Schwartz et al., 2013b). Stomatal lengths were measured and significant ($P<0.001$) differences were observed among genotypes. Putative tetraploid lines DSA16001, DSA13005 and DSA16016, possessed significantly larger stomatal lengths than those of diploid Raleigh and similar lengths to those of tetraploid (aneuploid, 2n= c.32) Floralawn (Table 2.4 and Figure 2.1). These results provide additional evidence that tumefaction is a good predictor of ploidy levels in turfgrass. While stomata lengths were good indicators of changes in ploidy level, this was not found to be the case for density. Stomata density of tetraploid lines was not significantly different from that of Raleigh (Table 2.2).

In this study, a tetraploid line was developed using colchicine treatments and two F1 lines from auto-pollination of this line were identified as tetraploid. These new genotypes might be

used in St. Augustinegrass breeding to: 1. Hybridize these lines with polyploid germplasm of interest to create lines with improved biotic stress tolerance, and 2. Develop triploid sterile plants via hybridization with diploid materials. Furthermore, future evaluation of these polyploids for their performance as turf will be used in determining the value of the use of colchicine as a breeding method in St. Augustinegrass improvement.

Acknowledgements:

The authors wish to thank Dr. Joe Chiera of the NCSU Environmental Facility for assistance with microscopy for stomatal length measurement. The assistance of Dr. Tom Stalker with pollen stains is also appreciated. 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.

REFERENCES

Busey, P. 1990. Polyploid *Stenotaphrum* germplasm: resistance to the polyploidy damaging population southern chinch bug. Crop Science 30:588-593.

Busey, P. and E.I. Zaenker. 1992. Resistance bioassay from southern chinch bug Heteroptera: Lygaeidae excreta. J. Econ. Entomol. 85:2032-2038.

Busey, P., R.M. Giblin-Davis, and B.J. Center. 1993. Resistance in *Stenotaphrum* to the sting nematode. Crop Sci. 33:1066-1070.

Busey, P. 2003. St. Augustinegrass, *Stenotaphrum secundatum* Walt Kuntze. P.309-329. In Casler, M. D. Duncan, R. R. Turfgrass Biology, Genetics and Breeding. ISBN John Wiley and Sons, Inc., Hoboken, New Yerssey.

Dermen, H. Botanical Review. (1940) 6: 599. doi:10.1007/BF02919557

Findley, S., S. Cannon, K. Varala, J. Du, and J. Ma. 2010. A fluorescence in situ hybridization system for karyotyping soybean. Genetics 185: 727-744.

Genovesi, A.D, Jessup RW, Engelke MC, Burson BL. 2009. Interploid St. Augustinegrass [Stenotaphrum secundatum (Walt.) Kuntze] hybrids recovered by embryo rescue. In Vitro Cell. Dev. Biol.-Plant 45:659-666.

Giblin-Davis, R.M. Busey, P. Center, B.J. 1995. Parasitism of *Hoplolaimus galeatus* on diploid and polyploid St. augustinegrasses. Journal of nematology 27(4):472-477.

Gill, N., S. Findley, J.G. Walling, C. Hans, and J. Ma. 2009. Molecular and chromosomal evidence for allopolyploidy in soybean. Plant Physiology 151**:** 1167–1174.

Gray, D.J. Elmstrom G.W. 1991. Process for the accelerated production of triploid seeds for seedless watermelon cultivars. US 5007198 A

Green, R.L., A.E. Dudeck, L.C. Hannah, and R.L., Smith. 1981. Isoenzyme Polymorphism in St. Augustinegrass. Dep. of Ornamental Horticulture, IFAS, Univ. of Florida, Gainesville, FL 32fi11. Published with the approval of the Director of the Florida Agric. Exp. Stn. as Journal Series Paper No. 2479.

Hanna, W.W. and W.F. Anderson. 2008. Development and impact of vegetative propagation in forage and turf bermudagrasses. Agron. J. 100:S-103-S-107.

Horn, G., A. Dudeck, and R. Toler. 1973. 'Floratam' St. Augustinegrass: A fast growing new variety of ornamental turf resistant to St. Augustine decline and chinch bugs. Fla. Agric. Exp. Stn. Circ. S-224.

Hesse, M. Halbritter, H. Weber, M. Buchner, R. Frosch-Radivo, A.S. Zetter, R. 2009. Pollen Terminology: An illustrated handbook. Chapter 51

Kato, A. 1999. Air drying method using nitrous oxide for chromosome counting in maize. Biotechnic & Histochemistry 74**:** 160–166.

Kato, A., Lamb, J.C., and Birchler, J.A. 2004. Chromosome painting using repetitive DNA sequences as probes for somatic chromosome identification in maize. Proceedings of the National Academy of Sciences of the United States of America 101**:** 13554–13559.

Levan, A. (1938). The effect of colchicine on root mitoses in allium. Hereditas, 24: 471–486.

Levan, A. and Ostergreen, G. 1943. The mechanism of c-mitotic actin on the naphthalene series. Hereditas, 29: 381–442.

Long, J. A., and E.C. Bashaw. 1961. Microsporogenesis and chromosome numbers in St. Augustinegrass. Crop Sci. 1:41-43.

Milla-Lewis, S.R., B. Ma, C. Arellano, M.C. Zuleta, W.C. Reynolds, and L.P. Tredway. 2011. Evaluation of St. Augustinegrass (Stenotaphrum secundatum (Walt.) Kuntze) germplasm for

gray leaf spot resistance. Proc. Amer. Soc. Agron. Intl. Ann. Mtg., San Antonio, TX. Oct 16-19.

Milla-Lewis, S.R., M.C. Zuleta, G.A. Van Esbroeck, K.H. Quesenberry, and K.E. Kenworthy. 2013. Cytological and Molecular Characterization of Genetic Diversity in *Stenotaphrum* Crop Science Vol 53(1):296-308.

Ostergreen, G. 1944. Colchicine mitosis, chromosome contraction, narcosis and protein chain folding. Hereditas, 30: 429–467.

Quesenberry, K.H. Dampier, J. M. Lee, Y.Y. Smith, R.L. and Acuna, C.A. 2010. Doubling the chromosome number of bahiagrass via tissue culture. Euphytica 175:43-50

Riordan, T.P., Meier, V.D Long, J.A., and Gruis, J.T. 1980. Registration of Seville St. Augustinegrass. Crop Sci. 20: 824-825.

Sauer, J.D. 1972. Revision of *Stenotaphrum* (Gramineae: Paniceae) with attention to its historical geography. Brittonia 24: 202-222.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9, 671-675.

Schwartz, B.M., Harris-Shultz, K.R., Contreras, R.N., Hans, C.S., and Jackson, S.A. 2013a. Creation of hexaploid and octaploid zoysiagrass using colchicine and breeding. Crop Sci. 53(5):2218-2224.

Schwartz, B.M., Harris-Shultz, K.R., Contreras, R.N., Hans, C.S., Hanna, W.W., and Milla-Lewis, S.R. 2013b. Creation of artificial Triploid and Tetraploid Centipedegrass using colchicine and breeding. Volume 12, 2013.

Schwartz, B.M., Contreras, R.N., Harris-Shultz, K.R., Heckart, D.L., Peake, J.B., Raymer, P.L. 2013c. Discovery and Characterization of a Turf-Type Triploid Seashore Paspalum. Hort Sci. 48(12):1424-1427.

Scorza R. and Cordts, J.M. Gray, D.J. Gonsalves, D. Emershad, R.L. and Ramming, D.W. 1996. Producing Transgenic 'Thompson Seedless' Grape (*Vitis vinifera* L.) Plants. Amer. Soc. Hort. Sci. 121(4):616–619.

Yang, Z. Shen, Z. Tetreult, H. Johnson, L. Friebe, B. Frazier, T. Huang, L. Burklew, C. Zhang, X.Q. and Zhao, B. 2014. Production of Autopoliploid Lowland Switchgrass Lines Through In Vitro Chromosome Doubling. Bioenergy Res 7:232-242.

Yildiz, M. 2013. Plant Responses at Different Ploidy Levels. , Current Progress in Biological Research, Dr. Marina Silva-Opps (Ed.), InTech, DOI: 10.5772/55785.

Table 2. 1. Germination and recovery rates, and flow cytometry results for lines generated from treatment of 'Raleigh' seeds with four colchine concentrations at four exposure times.

Tretment		Germination		Transplanted		Initial Flow Cytometry Determination	ID of Putative Tetraploids	Flow cytometry Confirmation	
Colchicine (%)	Time (Hrs.)	No.	Rate (%)	No.	Rate (%)			7/16/2015	3/9/2016
0	0	80	89	10	14				
0.025	5	32	36	25	78	Tetraploid	DSA 13001	Diploid	Diploid
0.025	24	75	83	56	75				
0.025	48	59	66	23	39	Tetraploid	DSA 13005	Tetraploid	Tetraploid
0.025	72	32	36	4	13				
0.05	5	62	69	46	74				
0.05	24	74	82	65	88	Tetraploid	DSA 13006	Reduplication	Diploid
						Tetraploid	DSA 13008	Diploid	
						Tetraploid	DSA 13009	Diploid	
						Tetraploid	DSA 13010	Diploid	
						Tetraploid	DSA 13012	Diploid	
						Tetraploid	DSA 13013	Tetraploid	Diploid
						Tetraploid	DSA 13014	Diploid	
0.05	48	53	59	10	10				
0.05	72	75	83	1	1				
0.1	5	49	54	38	38				
0.1	24	71	79	50	48	Tetraploid	DSA 13015	Diploid	
0.1	48	67	74	6	9				
0.1	72	69	77	0	0				
0.2	5	52	58	34	65				
0.2	24	64	71	35	56				
0.2	48	70	78	1	1				
0.2	72	47	52	1	2				
TOTAL		1031	67	405	39	10		2	1

Table 2.2. Mean comparison test in stomata density of ten putative tetraploid genotypes and two controls

Entry	N	Mean† Abaxial MSE 77.04		Mean† Adaxial MSE196.14	
Raleigh	5	35.8	ABDC	87.4	CDE
Floralawn	5	19.4	E	65.8	F
DSA13001	5	34.2	BDC	97.4	ABCD
DSA13005	5	32.0	DC	88.0	CDE
DSA13006	5	44.0	AB	76.2	EF
DSA13008	5	37.6	ABC	108.6	AB
DSA13009	5	36.6	ABC	97.0	ABCD
DSA13010	5	33.4	BDC	88.0	CDE
DSA13012	5	41.6	ABC	80.4	DEF
DSA13013	5	32.2	DC	85.2	CDE
DSA13014	5	45.6	A	106.0	AB
DSA13015	5	46.4	A	112.8	A

† Means followed by the same letter are not significantly different according to Fisher's protected LSD values.

Table 2. 3 Final flow cytometry analysis of eight lines generated from colchicine treatment of 'Raleigh' seeds.

Identity	**Source†**	**Flow Cytometry Determination**
DSA 13005	C2T4 Treatment	Tetraploid
DSA 13010	C3T3 Treatment	Diploid
DSA 13013	C3T3 Treatment	Diploid
DSA 13015	C4T3 Treatment	Diploid
DSA 16001	DSA 13005 progeny	Tetraploid
DSA 16016	DSA 13005 progeny	Tetraploid
DSA 16030	DSA 13005 progeny	Diploid
DSA 16031	DSA 13005 progeny	Diploid
Raleigh	Control	Diploid
Floralawn	Control	Tetraploid Aneuploid

† C1-C5 refer to colchicine concentrations of 0, 0.025, 0.05, 0.10, and 0.20% and T1-T5 refer to exposure time of 0, 5, 24, 48, and 72 hours.

Table 2. 4. Mean comparison test in adaxial stomata lengths of eight putative tetraploid genotypes and two controls.

Entry	Source	N	Mean† MSE 8.96	
Floralawn	Control	25	39.58	C
Raleigh	Control	25	31.04	E
DSA13005	C2T4 Treatment	25	42.81	B
DSA13010	C3T3 Treatment	25	29.43	EF
DSA13013	C3T3 Treatment	25	32.77	D
DSA13015	C4T3 Treatment	25	28.62	F
DSA16001	DSA 13005 progeny	25	45.18	A
DSA16016	DSA 13005 progeny	25	42.20	B
DSA16030	DSA 13005 progeny	25	32.84	D
DSA16031	DSA 13005 progeny	25	28.84	F

† Means followed by the same letter are not significantly different according to Fisher's protected LSD values.

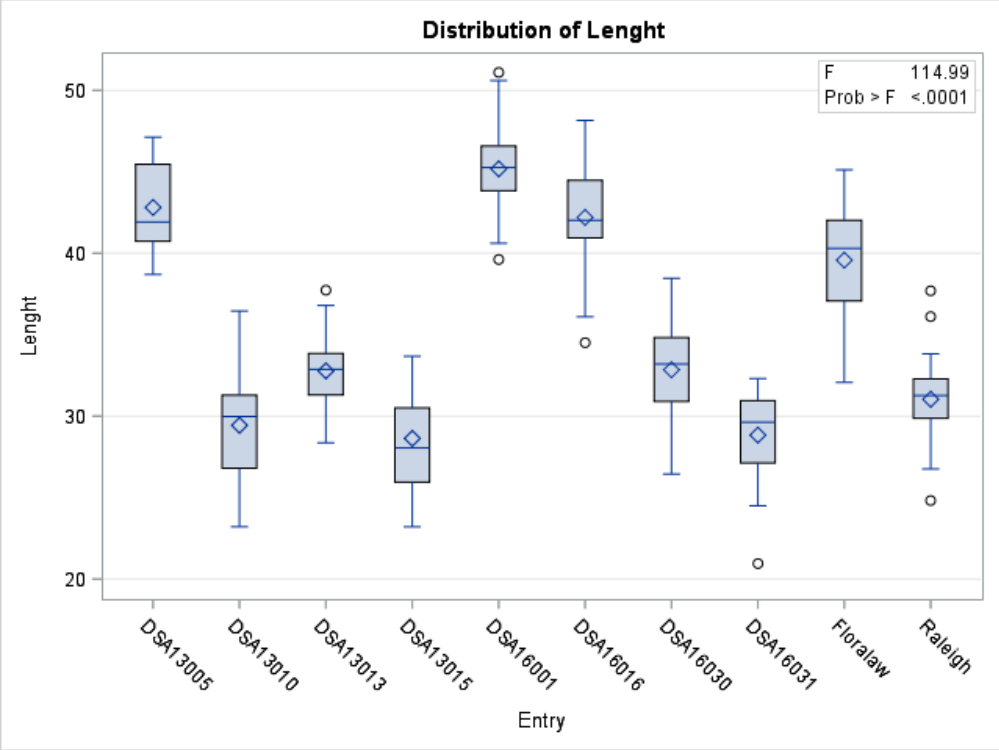

Figure 2.1.Distribution of stomatal lengths for the abaxial surface of five leaves of diploid 'Raleigh', putative tetraploid genotypes, and polyploid 'Floralawn'.

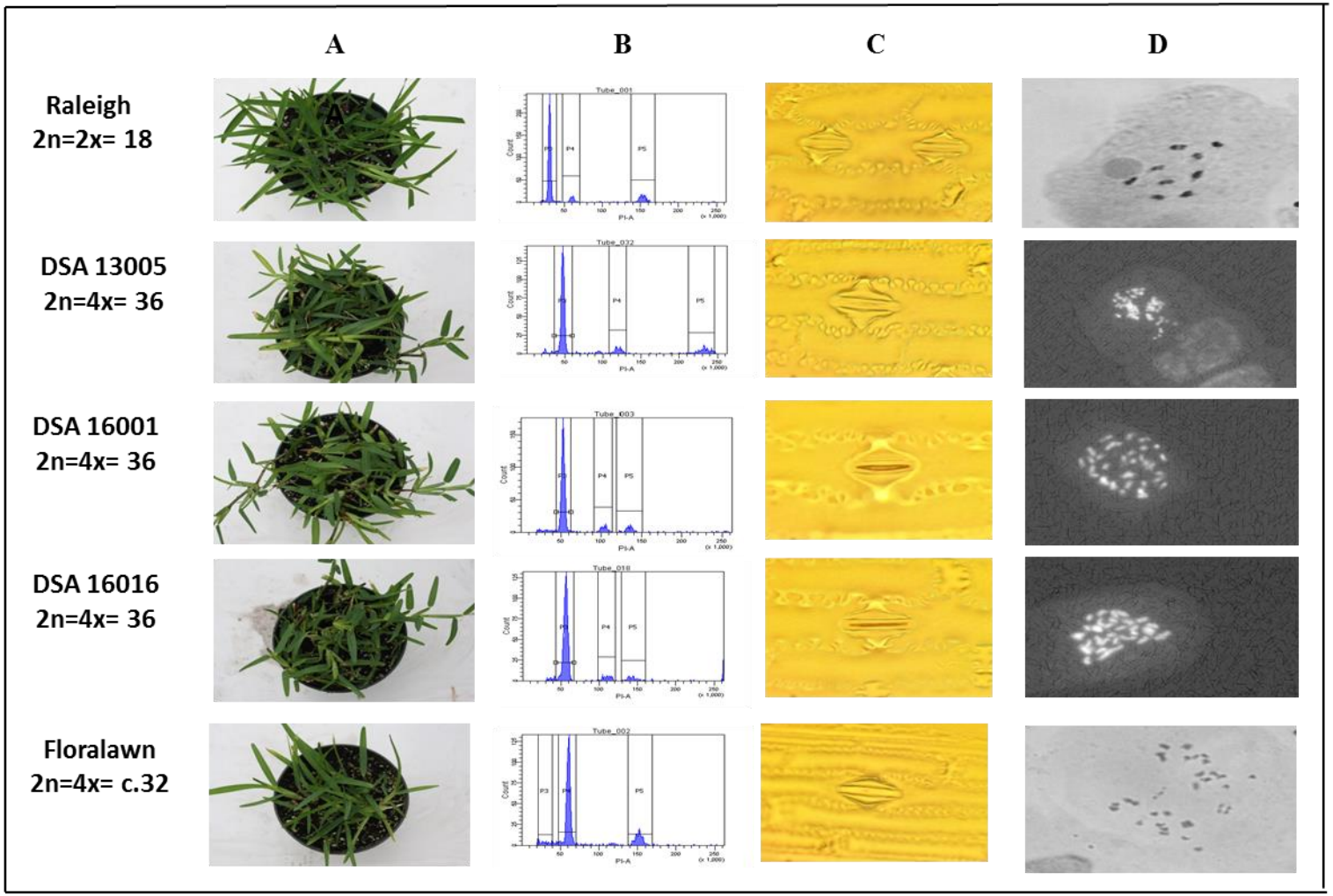

Figure 2.2. Indicators to infer ploidy levels of five St. Augustinegrass genotypes. A. Morphological appearance, B. Flow cytometry histograms showing 2C peaks for DSA 13005, DSA 16001 and DSA 16016 corresponding with that of Floralawn, C. Photomicrographs of leaf stomata at 420X magnification demonstrating that tetraploid colchicine-induced genotypes possess significantly larger stomatal lengths than those of Raleigh, and D. Photomicrographs of root tip spreads at 1000X magnification confirming the tetraploid nature of DSA 13005, DSA 16001 and DSA 16016.

-CHAPTER III-

IDENTIFICATION OF SOURCES OF RESISTANCE TO GRAY LEAF SPOT IN STENOTAPHRUM GERMPLASM

Identification of Sources of Resistance to Gray leaf spot in *Stenotaphrum* germplasm

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

[1]E. M. Carbajal, M. C. Zuleta, W.C. Reynolds, and S.R. Milla-Lewis, Dept. of Crop Science, Box 7620, [2]B. Ma, and L.P. Tredway, Dept. of Plant Pathology, Box 7616, and [3]C. Arellano, Dept. of Statistics, Box 8203, N.C. State Univ., Raleigh, NC 27695. Received: _____.

* Corresponding author email: susana_milla-lewis@ncsu.edu

ABSTRACT

St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] is a popular warm-season turfgrass in the Southern US. Gray leaf spot (GLS), caused by the fungal pathogen *Magnaporthe oryzae*, is one of the major diseases in St. Augustinegrass. While previous studies have reported polyploid lines with resistance to GLS, no comprehensive evaluation of sources of resistance have been performed in the genus. Screening diploid and polyploid St. Augustinegrass germplasm for response to GLS will enable breeders to identify resistant parents for cultivar development. In this study, a collection of 62 genotypes of *Stenotaphrum spp.* were screened for resistance to three different *M. oryzae* sources of inoculum under controlled environmental conditions. The traits evaluated were incubation period, number of leaves with lesions, mean lesion length and derived parameters disease incidence, area under the disease progress curve (AUDPC), and area under the lesion expansion curve (AULEC). Significant differences among genotypes were identified with polyploid genotypes PI 365031, PI 290888, PI 300129, PI 300130, and cultivar FX-10 and diploid genotype PI 410353 consistently showing high levels of resistance across trials, inoculum sources and traits. 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 because of the lack of reproductive barriers with most commercial cultivars and plant introductions.

KEYWORDS: *Stenotaphrum*, genetic resistance, gray leaf spot, *Magnaporthe oryzae*

INTRODUCTION

Gray leaf spot (GLS) is a turfgrass disease caused by *Magnaporthe oryzae* (T.T. Hebert) Yaegashi & Udagawa (anamorph: *Pyricularia grisea* (Cooke) Sacc.). Favorable environmental conditions with extended periods of leaf wetness (Deputy and Brosman, 2008), warm temperatures and high humidity levels accelerate symptom development (Harmon et al., 2005) limiting the efficacy of any cultural or chemical practice available to control the disease. Previous studies indicate that the fungus infects equally urban and golf course lawns, being more severe in the latter (Douhan et al., 2011). Likewise, regenerating sod fields and recently established lawns are also severely affected due to additional pressure being applied during a recovery period (Atilano and Busey, 1983). Despite GLS being reported in North America as early as 1957 in St. Augustinegrass (Malca and Owen, 1957) and later in other grasses including annual ryegrass in 1972 (Bain et al., 1972; Carver et al., 1972), perennial ryegrass in 1991 (Uddin et al., 1999) and more recently in kikuyugrass (Wong et al., 2005), currently there are no or limited resistant materials available which results in a common and aggressive disease among both cold and warm season grasses.

St. Augustinegrass is a warm-season grass widely distributed worldwide in tropical and subtropical regions. It is well adapted to shady, humid and sandy environments which make this grass a good candidate for residential lawns and coastal areas (Busey 1995). Gray leaf spot of St. Augustinegrass is a common disease and tends to be an issue in both sod production and landscape stands. The disease develops 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 (Smiley et al. 1996). Under favorable conditions, the disease develops rapidly, and entire grass swards can be killed within a few days. Cultural management

practices often do not provide adequate GLS control due to rapid disease development. Chemical control not only causes environmental and safety concerns, but also encourages fungicide resistance (Harmon et al. 2005). Development of cultivars with genetic resistance to the fungus would be a potential alternative for managing the disease (Atilano and Busey, 1983; Harmon et al. 2005).

Although GLS was first reported in St. Augustinegrass back in 1957 (Malca and Owen, 1957) it was not until 1964 when a small number of genotypes were evaluated for GLS resistance (Freeman 1964) and not until the 1980s when a more significant number of genotypes including African and Caribbean plant introductions, golf coast selections, hybrids and mutants was evaluated (Atilano and Busey, 1983). The study identified *S. dimidatum* accessions PI 289729 and PI 365032 as highly resistant (Atilano and Busey, 1983). Later in 2005, a GLS evaluation reported the polyploid cultivar FX-10 as the most resistant St. Augustinegrass genotype (Harmon et al., 2005). Metz et al. (2011) reported polyploids FX-10 (Busey 2003), TAES 5382 and TAES 5384 along with interploids DALSA 0605 and DALSA 0607 to be highly resistant (HR) and resistant (R).

Since first reported, most St. Augustinegrass population developments have been done in the diploid gene pool, with only a few polyploid cultivars available. Interploid breeding in St. Augustinegrass is limited because of sexual hybridization barriers among different ploidy levels and also to some extent because of restricted germplasm availability. Thus, examples of successful introgression of traits of interest from polyploid genotypes into commercial cultivars are just a few with FX-10 being one example. Early attempts to use conventional breeding methods between genotypes of different ploidy level resulted in failure (Busey, 2003), until more

recently when in-vitro embryo rescue techniques were used to successfully develop interploid populations (Genovesi et al, 2009).

Given the difficulties in exploiting the genetic variation present among *Stenotaphrum* polyploids and the lack of a comprehensive evaluation of levels of GLS resistance in the species, the objectives of the present study were to 1) evaluate a diverse collection of *Stenotaphrum* germplasm for resistance to different isolates of *M. oryzae,* and 2) determine if resistance to GLS exists in diploid germplasm and how it compares to that of polyploid materials.

MATERIAL AND METHODS

Plant material, establishment and maintenance. Sixty-two St. Augustinegrass genotypes were included in this study (Table 3.1): 21 commercial cultivars, 20 plant introductions from the National Plant Germplasm System (NPGS) (Griffin, GA) and 21 accessions from the North Carolina State University turfgrass breeding program collection. Genotypes were vegetatively propagated by planting five stolons into 16 oz. styrofoam cups (Dart Container Corp., Mason, MI) filled with Profile® Greens Grade™ (Profile Products LLC, Buffalo Grove, IL). Plants were maintained in a growth chamber at the Southeastern Plant Environment Laboratory (NCSU Phytotron) under 12-h days at 30°C and 75% RH and 12-h nights (no light) at 24°C and 100% RH for approximately three months until pots reached 100% coverage. During this period, plants were watered daily with a standard nutrient solution (Saravitz 2009) and mowed weekly to a height of 7 cm.

Due to space limitations in the growth chamber, the 62 genotypes were divided in two trials (Table 3.1). Additionally, a selection of the best and worst performers from each trial was included in a third trial to compare responses. The diploid cultivar Sunclipse and hexaploid plant

introduction 365031 were included as the susceptible and resistant controls, respectively, in each trial and run. Each trial was run twice and Chlorothalonil (DACONIL ULTREX® TURF CARE®, Syngenta Crop Protection, Greensboro, NC) was applied at a rate of 3.2 fl oz/1,000 sq ft two times ten days 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 allowed to recover for 10 weeks before reinoculation.

Fungal isolates and inocula production. Three inocula sources of *M. oryzae* were used in this study (Table 3.2). Each inoculum consisted of two individual *M. oryzae* isolates: isolates LWS1 and LWC3 were collected from NCSU's Lake Wheeler Turf Field Lab (Raleigh, NC), SRS1 and SRS3 were collected from the Sandhills Research Station (Jackson Springs, NC), and 1173 and 1345 were collected from Georgia. Conidial suspensions were prepared individually for each isolate and then the two isolates from the same source were mixed to form three types of inocula. Conidia were produced based on the method developed by Ma and Uddin with modifications (2009). Briefly, pieces of dry filter paper stored at - 80°C containing *M. 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 amended with tall fescue extract (20 g of Difco agar and 20 g of Gerber oatmeal in 1 liter of diluted tall fescue tea) for greater sporulation. Tall fescue extract was prepared by boiling 20 g of fresh tall fescue leaf blades in 1 liter water for 10 min and then, the fescue extract was diluted twofold by adding an equal volume of distilled water. Plates were sealed with Parafilm M All-Purpose Laboratory Film (Bemis, Oshkosh, WI) and incubated at 26°C with 12 h day/night cycles of fluorescent light (76 μE m–2

s–1) for an additional 5 to 6 days until the growth of *M. oryzae* was approximately 50 mm in diameter. The parafilm was then removed and the culture plates were placed upside down and incubated for an additional 10 days 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 using a hemacytometer.

Inoculation and incubation conditions. A split-plot experimental design with three replications was established, where main plots were determined by the three types of inocula and the subplots were represented by the St. Augustinegrass genotypes. Each block contained a total of 96 pots sprayed with a paarticular inoculum, distributed in a set of 12 containers holding 8 pots each, for a total of 32 genotypes distributed in three replications (Reps). Before inoculation, the total number of leaves in each cup was counted and genotypes were randomly arranged in four ~~twelve~~ 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 Co., Franklin Park, IL) powered with compressed CO_2 at 2.2 kg m^{-2}. Following inoculation, the plastic containers were covered and incubation conditions for the first 24 hours were set at 24°C, 100% relative humidity (RH), and no light. Subsequently, the growth chamber was programmed for 12 hours of light at 30°C and 75% RH and 12 hours of darkness at 24°C and with RH between 70-90%. 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 with lids to maintain leaf wetness and humidity overnight. Additional mist was constantly applied during the first 48 hours after inoculation and every night until the

end of the experiment using three Herrmidifier 707-U series atomizing humidifiers (Trion Inc., Sanford, NC).

Data collection and analysis. Two days after inoculation, symptoms were evaluated daily for a total of five days. The number of leaves with lesions was recorded and used to estimate disease incidence. Disease incidence was reported as number of leaves with lesions (LWL) over the total number of leaves per cup counted within 24 hours prior to inoculation. Mean lesion length (LL) was determined by measuring the length of at least five lesions arbitrarily selected per cup using a digital fractional caliper (General Tools and Instruments, New York, NY). Incubation period was recorded as the day when two or more lesions were present in a cup. Based on LWL and LL, derived traits disease progress rate, 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 cup from the five observations of disease incidence and mean lesion length, respectively. AUDPC and AULEC were calculated from the disease incidence and mean lesion length data, respectively, using the formula $\sum_i^n[(y_i + y_{i+1})/2](t_{i+1} - t_i)$ developed by Shaner and Finney (1977), where $_i$ =rating day, y_i is disease incidence or mean lesion length on the $_i^{th}$ rating date, and t_i is the day of the $_i^{th}$ rating.

The PROC MIXED procedure (SAS v. 9.2, SAS Institute, Cary, NC) 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 and their interaction were considered fixed-effects. Trial, run nested within trial, rep nested within trial and run and their interactions with fixed effects were considered random effects. Kenward-Roger criteria was

used to improve estimation of standard errors and degrees of freedom in 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 0.05 (Kramer 1956). In order to detect correlations among the different traits evaluated Pearson's coefficients were calculated using the CORR procedure in SAS 9.5 (SAS v. 9.5, SAS Institute, Cary, NC).

RESULTS

Given the number of genotypes evaluated and size of the environmental chambers used for the study, the evaluation had to be 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. While seven parameters were evaluated and analyzed, only incubation period, final incidence, AUDPC, and AULEC are presented here.

Trial I. In this trial, significant differences among genotypes were identified for all traits evaluated except incubation period (Table 3.3). Meanwhile, significant differences among inocula sources were detected only for final incidence. The interaction effect between genotype and inoculum source was significant only for AULEC. Plant introduction 365031 was found to be the most resistant genotype across traits (Appendix 3.1). However, plant introductions 289729, 291595, 300129, 300130, and cultivar FX-10 were not significantly different from PI 365031 for any of the traits. Cultivars Classic, Mercedes, and Jade were consistently the worst performers. Inoculum LWS was the most virulent across runs, achieving the highest scores across traits. Inocula SRS and ISO 3 obtained rank two and three, respectively (Table 3.4). Covariance parameter estimates are presented in Appendix 3.2.

Trial II. In general, disease pressure was higher for Trial II as compared to Trial I. In Trial II, disease developed faster as reflected by lower numbers for incubation period and reached higher levels which was evidenced by higher values for incidence, AULEC and AUDPC. While

genotypes were found to be significantly different for all traits, inoculum was not a source of variation for any of the traits (Table 3.3). There was a significant interaction effect between genotype and inoculum for final incidence and AULEC. As in Trial I, PI 365031 was among the top performers across traits. Genotypes PI 410353 and PI 410364 had similar or better performance than PI 360531 for all variables measured (Appendix 3.3). In Trial II differences in virulence among inocula were not as marked as in Trial I. However, LWS generated the highest levels of disease obtaining the highest values for all traits except AULEC (Table 3.4). Covariance parameter estimates are presented in Appendix 3.2.

Trial III. In Trial III, levels of disease were similar than those in Trial I. ANOVA results showed significant differences among genotypes for all traits, but no differences among inocula (Table 3.3). The interaction effect between both factors was found to be significant for incubation period only. Plant introduction 365031 had once again the best overall performance across traits. However, plant introductions 300129, 300130, 410353 and cultivar FX-10 had comparable performances and were not significantly different from this check for any of the traits with the exception of incubation period for PI 410353 with inoculum SRS (Table 3.5). While not the worst, cultivar Sunclipse remained a poor performer. In terms of inoculum virulence (Table 3.4), in this Trial ISO 3 ranked first, followed by LWS and SRS. Data collected showed a similar range of values as Trial I. Covariance parameter estimates are presented in Appendix 3.2.

Consistency in Performance. Genotypes with consistent performance across trials and traits could be identified (Tables 3.6 and 3.7). The resistant check, PI 365031, ranked in the top 5 for 10 out of 12 trial by trait combinations (Table 3.6). Plant introductions 300129, 300130 and 410353 we also consistently among the top. Isolate LWS ranked first in terms of virulence across traits (Table 3.4). Plots of AULEC vs Incubation period and final incidence (Figure 3.1), AUDPC vs Incubation period and final incidence (Figure 3.2) revealed a good spread in disease response. A group of polyploid genotypes (PI 365031, PI 300129, PI 300130, PI 291594, and PI 289729) with consistent performance across these traits was identified. Moreover, the plot of AULEC vs AUDPC by ploidy level (Figure 3.3) identified diploid PI 410353 among this group.

Trait correlation. Pearson's correlation coefficients were calculated between each pair of traits. Results (Table 3.8) indicated that AUDPC, AULEC and final incidence were highly correlated ($p<0.0001$) among themselves. On the other hand, incubation period had a significant association ($p=0.0002$) with AUDPC and final incidence only.

DISCUSSION

Significant differences in incubation period, final disease incidence, AUDPC, and AULEC were observed among *Stenotaphrum* genotypes evaluated in this study. These results confirmed, as has been previously reported in other studies (Atilano and Busey, 1983, Metz et al. 2011), that diverse germplasm collections of St. Augustinegrass contain differences in response to biotic stresses such as gray leaf spot. Previous studies have reported high levels of GLS resistance in *S. dimidatum* plant introductions PI 365031 and PI 289729 (Atilano and 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 PI 365031. Additionally, while PI 647924, PI 410357, PI 410360, and PI 410364 had higher levels of disease than PI 410353 in a few trials, they still maintained consistently good performance across trials for most traits and were classified as moderately resistant. The high levels of resistance in PI 410353 have been corroborated in a later study (Mulkey, 2013).

The use of growth chambers for disease evaluations is beneficial in reducing space requirements and the 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 (Meltz 2011). Our work supports previous reports (Metz et al. 2011, Mulkey 2013) that the use of growth chambers is an effective way to evaluate large numbers of St. Augustinegrass genotypes for response to *M. oryzae*.

Primary traits (incubation period, final disease incidence, final lesion length) and derived traits (AUDPC, AULEC) were consistent at identifying the most resistant genotypes. While incidence tended to be efficient in terms of combining multiple traits such as incubation period, infection efficiency and lesion expansion into one (Tredway et al. 2003), 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 traits were found to be highly ($p<0.001$) correlated indicating evaluation of one should be effective at also assessing

response of the other two. As in different studies (Mulkey, 2013; Tredway et al., 2003: Dallagnol et al., 2009), AULEC and AUDPC were useful criteria for uniform selection of resistant genotypes. In trial three, polyploid genotypes 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 plants PI 410353 and PI 410357 were consistently low indicating these genotypes are also highly 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 one day difference detected between highly resistance PI 365031 and susceptible Seville might translate into the latter going over more disease cycles than the former over the same time period.

Even though the severity of the inocula was assumed to be low (Iso 3), medium (LWS) and high (SRS), results indicated similar levels of virulence for all inocula on the genotypes evaluated. Similar results were presented by Metz et al. (2011) when different *M. oryzae* isolates used separately and in combination were found to exhibit similar virulence and 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) also identified similar virulence among isolates of *M. grisea* to tall fescue cultivars even for isolates from different lineages. In our study, the significant interaction of genotype and inoculum identified for most traits was mostly given by differences in rank among genotypes with intermediate performance. Highly resistant genotypes identified here demonstrated consistency in disease response across runs, trials, traits and inocula. While polyploids PI 365031

and PI289729 had been previously identified as resistant materials, this is the first report of high level 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 because of the lack of reproductive barriers with most commercial cultivars and plant introductions.

Acknowledgements: The authors wish to thank personnel at the NCSU Environmental Facility for assistance with maintenance of the plants and set up 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.

REFERENCES

Atilano, R. A., and Busey, P. 1983. Susceptibility of St. Augustinegrass germ plasm to *Piricularia griseae*. Plant Disease 67 (7): 782-783.

Bain, D. C., Patel, B. M., and Patel, M. V. 1972. Blast of ryegrass in Mississippi. Plant Dis. Rep. 56: 210.

Busey, P. 2003. St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze. In Biology, Breeding, and Genetics of Turfgrasses, by P. Busey, edited by and R. R. Duncan M. D. Casler, 309-330. Hoboken, NJ: John Wiley & Sons, Inc.,.

Busey, P. 1995. Genetic diversity and vulnerability of St. Augustinegrass. Crop Science 35: 322-327.

Carbone D, Kalaljev A. 1932. Ricercha sulla vaccinaxione della plante. Phytopathol. Z 5:91-98

Carver, R. B., Rush, M. C., and Linderberg, G. D. 1972. An epiphytopic of ryegrass blast in Lousiana. Plant Dis. 56: 157-159.

Dallagnol, L. J., Rodrigues, F. A., Mielli, M. V. B., Ma, J. F., and Datnoff, L. E. 2009. Defective active silicon uptake affects some components of rice resistance to brown spot. Phytopathology, 99: 116‑121.

Deputy, J.T., and Brosman, J., 2008. St. Augustinegrass. Turf Management TM-3. College of tropical agriculture and human resources. University of Hawai at Manoa, Feb.

Douhan, G. W., De la Cerda, K. A., Huryn, K., Greer, C. A., and Wong, F. P. 2011. Contrasting genetic structure between *Magnaporthe grisea* populations associated with the golf course turfgrasses *Lolium perenne* (perennial ryegrass) and *Pennisetum clandestinum* (kikuyugrass). Phytopathology 101 (1): 85-91.

Freeman, T. E. 1964. Influence of nitrogen on severity of *Piricularia grisea* infection of St. Augustinegrass." Phytopathology 1187-1189.

Genovesi, A.D., Russel, W.J., Engelke, M.C., and Burson, B.L. 2009. Interploid St. Augustinegrass [*Stenotaphrum secundatum (Walt.) Kuntze*] hybrids recovered by embryo rescue. In Vitro Cell. Dev. Biol. - Plant 659-666.

Harmon, P., Datnoff, L., Nagata, R., Brecht, M., and Stiles, C. 2005. Gray leaf spot of St, Augustinegrass: cultural and chemical options. EDIS. University of Florida IFAS Extension. Accessed 2011.

Kramer, C. Y. 1956. Extension of Multiple Range Tests to Group Means with Unequal Numbers of Replications. Biometrics 307-310.

Ma, B., and Uddin, W. 2009. Fitness and competitive ability of an azoxystrobin-resistant G143A mutant of *Magnaporthe oryzae* from perennial ryegrass. Plant Disease 93: 1044-1049.

Malca, M. I., and 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., and Campos C. F. 2011. Comparison of screening methods to evaluate the response of St. Augustine grass to *Magnaporthe oryzae*. Plant Pathology.

Mulkey, S.E. 2013. SSR Development and Mapping of Gray Leaf Spot Resistance Genes in St. Augustinegrass. Master thesis. North Carolina State University.

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

Shaner, G. and Finney, R. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing in knox wheat. Phytopathology 36: 1307-1311.

Shearman, R. C. 2006. Fifty Years of Splendor in the Grass. Crop Science 46: 2219-2229.

Smiley, R.W., Dernoden, P.H., Clarke, B.B., 1996. Compendium of Turfgrass Diseases. St. Paul, MN, USA: The American Phytopathological Society.

Ryals J, Uknes S, Ward E. 1994. Systemic acquired resistance. Plant Physiol. 104:1 109-12.

Tredway, L. P., Stevenson, K. L., and Burpee, L. L. 2005. Genetic Structure of *Magnaporthe grisea* populations associated with St. Augustinegrass and Tall Fescue in Georgia. Phytopathology 95 (5): 463-471.

Tredway, L. P., Stevenson, K. L., and Burpee, L. L. 2003. Components of resistance to *Magnaporthe grisea* in Coyote and Coronado tall fescue. Plant Dis. 87:906-912.

Uddin, W., Soika, M. D., Moorman, F. E., and Viji, G. 1999. A serious outbreak of blast disease (gray leaf spot) of perennial ryegrass in golf course fairways in Pennsylvania. Plant Disease 83: 783.

Wong, F. P., Gelernter, W., and Stowell, L. 2005. First report of *Piricularia grisea* causing gray leaf spot on Kikuyugrass (*Pennisetum clandestinum*) in the United States. Plant Disease 89: 433.

Table 3. 1.. *Stenotaphrum* genotypes evaluated for resistance to three sources of inocula of *Magnaporthe oryzae* under controlled environmental conditions.

No.	Identity	Type	Ploidy[x]	Species	Source[y]	Trial Evaluated		
1	106SVT3	Collection	Diploid	*S. secundatum*	NCSU		II	III
2	200Elm4	Collection	Diploid	*S. secundatum*	NCSU		II	
3	904AT4	Collection	Diploid	*S. secundatum*	NCSU		II	III
4	Amerishade	Cultivar	Diploid	*S. secundatum*	UF	I		
5	Bitterblue	Cultivar	Triploid	*S. secundatum*	UF	I		III
6	C1	Collection	Diploid	*S. secundatum*	NCSU		II	III
7	C2	Collection	Diploid	*S. secundatum*	NCSU		II	
8	C3	Collection	Diploid	*S. secundatum*	NCSU		II	
9	C4	Collection	Diploid	*S. secundatum*	NCSU		II	
10	C6	Collection	Diploid	*S. secundatum*	NCSU		II	
11	C7	Collection	Diploid	*S. secundatum*	NCSU		II	
12	Captiva	Cultivar	Diploid	*S. secundatum*	UF	I		
13	Classic	Cultivar	Diploid	*S. secundatum*	UF	I		III
14	Clem	Collection	Diploid	*S. secundatum*	NCSU		II	
15	Co2	Collection	Diploid	*S. secundatum*	NCSU		II	
16	Craig	Collection	Diploid	*S. secundatum*	NCSU		II	
17	Delmar	Cultivar	Diploid	*S. secundatum*	UF	I		
18	Deltashade	Cultivar	Diploid	*S. secundatum*	UF	I		
19	Eclipse	Cultivar	Diploid	*S. secundatum*	MSU	I		
20	Floralawn	Cultivar	Aneuploid	*S. secundatum*	UF	I		III
21	Floratam	Cultivar	Aneuploid	*S. secundatum*	UF	I		
22	Floratine	Cultivar	Triploid	*S. secundatum*	UF	I		
23	Floraverde	Cultivar	Diploid	*S. secundatum*	UF	I		
24	FX-10	Cultivar	Aneuploid	*S. secundatum*	UF	I		III
25	GF2	Collection	Diploid	*S. secundatum*	NCSU		II	III
26	Jade	Cultivar	Diploid	*S. secundatum*	UF	I		
27	Jones	Collection	Diploid	*S. secundatum*	NCSU		II	
28	Mercedes	Cultivar	Diploid	*S. secundatum*	UF	I		
29	Palmetto	Cultivar	Diploid	*S. secundatum*	UF	I		
31	PI 289729	Plant Introduction	Tetraploid	*S. dimidatum*	NPGS	I		III
32	PI 290888	Plant Introduction	Aneuploid	*S. secundatum*	NPGS	I		III
33	PI 291594	Plant Introduction	Triploid	*S. secundatum*	NPGS	I		III
34	PI 300129	Plant Introduction	Triploid	*S. secundatum*	NPGS	I		III
35	PI 300130	Plant Introduction	Aneuploid	*S. secundatum*	NPGS	I		III

[x] ploidy levels according to Milla-Lewis et al. 2013

[y] 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

Table 3.1. *(continued from the previous page)*

No.	Identity	Type	Ploidy[x]	Species	Source	Trial Evaluated		
36	PI 365031	Plant Introduction	Hexaploid	*S. dimidatum*	NPGS	I	II	III
37	PI 410353	Plant Introduction	Diploid	*S. secundatum*	NPGS		II	III
38	PI 410355	Plant Introduction	Diploid	*S. secundatum*	NPGS		II	
39	PI 410357	Plant Introduction	Diploid	*S. secundatum*	NPGS		II	III
40	PI 410360	Plant Introduction	Diploid	*S. secundatum*	NPGS		II	III
41	PI 410361	Plant Introduction	Diploid	*S. secundatum*	NPGS	I		III
42	PI 410363	Plant Introduction	Diploid	*S. secundatum*	NPGS	I		III
43	PI 410364	Plant Introduction	Diploid	*S. secundatum*	NPGS		II	III
44	PI 414079	Plant Introduction	Diploid	*S. secundatum*	NPGS		II	
45	PI 509038	Plant Introduction	Diploid	*S. secundatum*	NPGS		II	III
46	PI 509039	Plant Introduction	Diploid	*S. secundatum*	NPGS		II	III
47	PI 600734	Plant Introduction	Diploid	*S. secundatum*	Seed		II	III
48	PI 647924	Plant Introduction	Diploid	*S. secundatum*	NPGS		II	III
49	PI 647925	Plant Introduction	Diploid	*S. secundatum*	NPGS	I		
50	Polaris	Cultivar	Diploid	*S. secundatum*	MSU	I		
51	Raleigh	Cultivar	Diploid	*S. secundatum*	UF	I		
52	Ray	Collection	Diploid	*S. secundatum*	NCSU		II	
53	Rebok	Collection	Diploid	*S. secundatum*	NCSU		II	
54	Sapphire	Cultivar	Diploid	*S. secundatum*	UF	I		
55	Seville	Cultivar	Diploid	*S. secundatum*	UF	I		III
56	Sunclipse	Cultivar	Diploid	*S. secundatum*	UF	I	II	III
57	SV20	Collection	Diploid	*S. secundatum*	NCSU		II	III
58	T638	Collection	Diploid	*S. secundatum*	NCSU		II	
59	T644	Collection	Diploid	*S. secundatum*	NCSU		II	
60	T672	Collection	Diploid	*S. secundatum*	NCSU		II	
61	TX Common	Cultivar	Diploid	*S. secundatum*	UF	I		III

[x] ploidy levels according to Milla-Lewis et al. 2013

[y] 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, G

Table 3. 2. *Magnaporthe oryzae* inoculation treatments used to screen *Stenotaphrum* germplasm for genetic resistance.

Inoculum [x]	Isolate	Origin	Source	Host origin
1	LWS2	Lake Wheeler Turfgrass Field Laboratory (Raleigh, NC)	North Carolina State University	St. Augustinegrass
	LWC3	Lake Wheeler Turfgrass Field Laboratory (Raleigh, NC)	North Carolina State University	St. Augustinegrass
2	SRS1	Sandhills Research Station (Jackson Springs, NC)	North Carolina State University	St. Augustinegrass
	SRS3	Sandhills Research Station (Jackson Springs, NC)	North Carolina State University	St. Augustinegrass
3	1173	Lanier, GA	North Carolina State University	St. Augustinegrass
	1345	Taylor, GA	North Carolina State University	St. Augustinegrass

[x] All inocula were prepared by mixing the two isolates at a ratio of 50 to 50.

Table 3. 3. Individual and combined ANOVA for evaluation trials of *Stenotaphrum* germplasm for resistance to three sources of inoculum of *Magnaporthe oryzae*. Traits evaluated included INCPER= incubation period, INCID D7 = incidence on day seven, AUDPC = area under the disease progress curve, and AULEC = area under the lesion expansion curve.

		INCPER			INCID D7			Sq Rt AUDPC[x]			AULEC		
SOURCE	**Num. DF**	**Den.DF**	**F Value**		**Den.DF**	**F Value**		**Den.DF**	**F Value**		**Den.DF**	**F Value**	
Trial I													
Inoculum	2	2	7.98	NS[y]	10.5	12.09	**	2.01	14.5	NS	2	1.28	NS
Genotype	30	30	1.26	NS	29.7	6.67	***	29.5	5.47	***	29.9	14.58	***
Gen × Inoc	60	60	0.98	NS	60.8	1.24	NS	60.2	1.49	NS	58.1	2.18	**
Trial II													
Inoculum	2	2	0.19	NS	2	0.39	NS	2	0.07	NS	2	0.54	NS
Genotype	31	31	2.54	**	31	4.06	***	31	3.29	***	93	6.54	***
Gen × Inoc	62	446	0.99	NS	62	1.57	*	62	1.39	NS	93	1.91	**
Trial III													
Inoculum	2	2	0.1	NS	2	0	NS	2	0.01	NS	2.97	0.17	NS
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	0.9	NS	54	1.38	NS	54.4	1.36	NS

[x] square root transformed area under the disease progress curve

[y] NS, *, **, and *** denote non-significant, significant at 0.05, significant at 0.01, and significant at 0.001, respectively.

Table 3. 4. Virulence of Magnaporthe oryzae averaged over genotypes for evaluation of Stenotaphrum germplasm to gray leaf spot resistance.

	ISO 3	**LWS**	**SRS**
INC PER			
Trial I	3.47	2.82	4.55
Trial II	3.46	3.50	3.41
Trial III	4.52	4.77	3.11
INCID D7			
Trial I	21.93	30.53	24.31
Trial II	78.05	79.82	74.75
Trial III	29.62	30.24	29.11
AUDPC			
Trial I	6.32	8.13	6.72
Trial II	12.81	13.02	13.05
Trial III	6.81	6.49	6.66
AULEC			
Trial I	4.75	5.20	4.81
Trial II	9.10	8.42	7.50
Trial III	4.29	3.69	3.96
RANK			
Trial I	3	1	2
Trial II	2	1	3
Trial III	1	2	3

Table 3. 5. Genotype by inoculum LS-means for INCPER= incubation period, INCID D7 = incidence on day seven, AUDPC = area under the disease progress curve, and AULEC = area under the lesion expansion curve in Trial III.

	INCPER						INCID D7						Sq Rt AUDPC[x]						AULEC					
Genotype	**Iso 3**		**LWS**		**SRS**		**Iso 3**		**LWS**		**SRS**		**Iso 3**		**LWS**		**SRS**		**Iso 3**		**LWS**		**SRS**	
106SVT3	4.17	B	4.17	A	4.33	BCD	37.09	A	33.28	A	23.62	A	7.29	ABC	7.04	A	5.73	A-E	4.1	A-F[y]	4.39	A-D	4.05	A-D
904AT4	4.5	B	4.67	A	4.33	BCD	38.62	A	54.77	A	44.26	A	7.92	ABC	8.91	A	8.1	A-E	5.24	A-E	6.11	AB	5.11	A-D
Bitterblue	4.67	AB	5	A	4.33	BCD	13.07	A	13.24	A	30.09	A	5.25	ABC	4.66	A	7.44	A-E	2.83	A-F	2.41	A-D	5.01	A-D
C1	4.67	AB	4.5	A	5	A-D	31.91	A	32.5	A	29.81	A	6.99	ABC	6.35	A	6.59	A-E	4.7	A-F	3.99	A-D	3.63	A-D
Classic	4.33	B	4.83	A	4.17	CD	49.64	A	50.12	A	41.49	A	9.01	AB	8.29	A	8.1	A-E	5.97	A-D	4.77	A-D	5.43	A-D
Floralawn	4.5		4.67	A	4.17	CD	13.81	A	28.38	A	45.97	A	5.38	ABC	6.6	A	8.45	A-E	4.24	A-F	3.95	A-D	6.2	AB
FX-10	5.17	AB	4.67	A	6.67	AB	14.91	A	9.82	A	3.9	A	5.09	ABC	4.27	A	1.65	DE	2.3	C-F	1.7	BCD	1.13	CD
GF2	4.17	B	4.67	A	4.5	BCD	59.65	A	47.91	A	47.35	A	10.04	A	7.93	A	8.7	A-D	4.95	A-F	4.97	A-D	4.49	A-D
PI 289729	4.5	B	4.25	A	4.33	BCD	24.83	A	19.1	A	23.44	A	6.65	ABC	5.77	A	6.2	A-E	4.64	A-F	4.09	A-D	4.04	A-D
PI 290888	2.92	B	3.5	A	3.33	D	5.21	A	17.05	A	20.2	A	4.62	ABC	5.87	A	7.74	A-E	0.56	EF	0.69	D	1.06	CD
PI 291594	3.5	B	5.17	A	3.67	D	24.47	A	22.13	A	36.03	A	7.48	ABC	5.76	A	8.68	A-D	4.68	A-F	2.34	A-D	5.63	ABC
PI 300129	5	AB	5.67	A	5.67	A-D	7.7	A	6.51	A	6.04	A	3.78	ABC	2.92	A	2.92	B-E	2.38	B-F	2.07	A-D	2.21	BCD
PI 300130	5.33	AB	5.67	A	6.33	ABC	3.12	A	7.88	A	3.68	A	2.6	BC	3.11	A	1.85	CDE	1.65	DEF	1.45	BCD	1.31	CD
PI 365031	5	AB	5.67	A	7	A	6.24	A	5.75	A	3.38	A	3.74	ABC	3.04	A	1.4	E	3.08	A-F	1.61	BCD	0.74	D
PI 410353	7	A	4	A	4.17	CD	4.76	A	11.28	A	9.26	A	1.54	C	5.39	A	4.54	A-E	0.33	F	1.03	CD	1.89	BCD
PI 410357	4.67	AB	4.83	A	4.17	CD	29.79	A	25.99	A	14.77	A	7.57	ABC	6.07	A	5.47	A-E	3.01	A-F	3	A-D	3.13	A-D
PI 410360	3.5	B	4.17	A	4	CD	24.71	A	34	A	16.81	A	7.81	ABC	8.22	A	5.44	A-E	5.65	A-E	3.97	A-D	3.11	A-D
PI 410361	4	B	4.83	A	3.67	D	36.25	A	35.74	A	49.47	A	8.25	ABC	8.24	A	9.88	AB	5.11	A-F	3.04	A-D	5.27	A-D
PI 410363	3.67	B	4.83	A	4.33	BCD	49.8	A	47	A	15.09	A	9.35	AB	7.39	A	5.4	A-E	5.75	A-E	4.75	A-D	2.22	BCD
PI 410364	4	B	5	A	4	CD	17.29	A	21.49	A	32.72	A	6.17	ABC	5.82	A	7.58	A-E	3.91	A-F	3.15	A-D	5.46	A-D
PI 509038	4.83	AB	4.86	A	4.5	BCD	55.06	A	51.87	A	64.43	A	9.28	AB	8.7	A	10.63	A	6.65	ABC	6.58	A	7.08	A
PI 509039	4.17	B	4.67	A	4.67	A-D	58.18	A	42.74	A	58.69	A	10.35	A	7.83	A	10.12	A	7.29	A	5.68	ABC	6.4	AB
PI 600734	4	B	4.5	A	4	CD	50.09	A	46.74	A	38.57	A	9.25	AB	8.57	A	8.98	ABC	7.13	AB	5.7	ABC	5.72	ABC
PI 647924	4.83	AB	5.33	A	4.17	CD	17.43	A	19.32	A	23.21	A	5.06	ABC	4.91	A	6.21	A-E	4.1	A-F	3.31	A-D	3.01	A-D
Seville	4.5	B	4.5	A	4.67	A-D	45.3	A	45.07	A	39.46	A	8.72	AB	7.94	A	7.87	A-E	5.5	A-E	4.78	A-D	4.52	A-D
Sunclipse	5	AB	5.17	A	4.33	BCD	36.33	A	34.89	A	20.25	A	7.22	ABC	7.12	A	6.21	A-E	5.24	A-E	5.39	A-D	3.89	A-D
SV20	4.67	AB	4.5	A	4.83	A-D	29.45	A	33.24	A	33.74	A	6.3	ABC	6.91	A	6.55	A-E	3.3	A-F	3.55	A-D	4.13	A-D
TX Common	5.17	AB	5.17	A	4	CD	44.59	A	49	A	39.22	A	8.04	ABC	7.96	A	8.03	A-E	5.84	A-D	4.9	A-D	4.93	A-D

[x] Square root transformed area under the disease progress curve

[y] Within a column, means followed by the same letter are not significantly different.

Table 3. 6. Ranks across inocula for genotypes with high and low gray leaf spot AULEC, AUDPC, incubation period (INCPER) and final incidence (INCID D7) over multiple Trials.

	INCPER			INCID D7			Sq Rt AUDPC[x]			AULEC			
Genotype	**Rank TI**	**Rank TII**	**Rank TIII**	**Rank TI**	**Rank TII**	**Rank TIII**	**Rank TI**	**Rank TII**	**Rank TIII**	**Rank TI**	**Rank TII**	**Rank TIII**	**Rank**
PI_290888	9	.	28	9	.	6	10	.	8	7	.	1	9
PI_410353	.	13	5	.	4	4	.	8	5	.	2	2	5
PI_300130	3	.	2	4	.	1	3	.	1	3	.	3	1
FX-10	7	.	3	6	.	5	6	.	4	6	.	4	4
PI_365031	1	8	1	2	1	2	1	1	2	1	10	5	3
PI_300129	4	.	4	1	.	3	2	.	3	4	.	6	2
PI_410357	.	15	14	.	8	10	.	15	10	.	4	7	11
Bitterblue	11	.	12	8	.	7	8	.	7	8	.	8	8
PI_647924	.	9	8	.	5	8	.	3	6	.	1	9	6
SV20	.	11	12	.	7	18	.	4	12	.	6	10	10
C1	.	7	10	.	9	17	.	5	13	.	8	11	10
PI_410364	.	11	21	.	3	11	.	11	11	.	5	12	12
106SVT3	.	7	23	.	11	16	.	7	14	.	9	13	16
PI_291594	5	.	26	5	.	13	5	.	18	5	.	14	13
PI_410363	14	.	22	13	.	19	12	.	19	11	.	15	21
PI_410360	.	11	27	.	2	12	.	6	17	.	3	16	14
PI_289729	2	.	20	3	.	9	4	.	9	2	.	17	7
PI_410361	15	.	25	12	.	20	15	.	24	12	.	18	24
Floralawn	7	.	18	7	.	14	7	.	15	9	.	19	15
GF2	.	2	18	.	15	26	.	14	25	.	11	20	22
Sunclipse	12	4	6	10	6	15	9	2	16	15	7	21	19
Seville	10	.	14	14	.	21	13	.	21	10	.	22	20
TX_Common	13	.	8	11	.	22	11	.	20	13	.	23	17
Classic	8	.	18	15	.	25	14	.	23	14	.	24	25
904AT4	.	2	16	.	13	24	.	9	22	.	12	25	18
PI_600734	.	14	25	.	10	23	.	13	26	.	13	26	27
PI_509039	.	4	16	.	14	27	.	12	27	.	15	27	26
PI_509038	.	4	9	.	12	28	.	10	28	.	14	28	23

[x] Square root transformed area under the disease progress curve

[y] Within a column, means followed by the same letter are not significantly different.

Table 3. 7. Means across inocula for genotypes with high and low gray leaf spot AULEC, AUDPC, incubation period (INCPER) and final incidence (INCID D7) across runs and Trials. Genotypes highlighted in bold performed consistently among the top and were identified as highly resistant (HR).

	INC PER						INCID D7						Sq Rt AUDPC[x]						AULEC					
	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			3.56	A	4.22	AB			79.15	AB	31.33	A-F			12.12	AB	6.69	A-F			7.13	D-G	4.18	A-E
904AT4			3.67	A	4.50	AB			81.37	AB	45.89	ABC			12.53	AB	8.31	AB			8.23	D-G	5.49	ABC
Bitterblue	3.11	AB			4.67	AB	19.50	A			18.80	B-F	6.53	A-E			5.78	A-F	4.93	A-D			3.41	A-E
Captiva	2.84	B					32.34	A					7.85	A-D					5.38	A-D				
Classic	3.28	AB			4.44	AB	38.87	A			47.08	ABC	8.70	AB			8.47	A	6.26	AB			5.39	ABC
Floralawn	3.61	AB			4.44	AB	17.84	A			29.39	A-F	5.79	A-E			6.81	A-F	4.95	A-D			4.80	A-E
FX-10	**3.61**	**AB**			**5.50**	**A**	**13.06**	**A**			**9.54**	**DEF**	**4.88**	**A-E**			**3.67**	**C-F**	**3.31**	**A-E**			**1.71**	**CDE**
Jade	2.67	B					38.17	A					8.97	A					7.03	A				
PI 289729	**4.06**	**AB**			**4.36**	**AB**	**9.55**	**A**			**22.58**	**A-F**	**4.27**	**B-E**			**6.21**	**A-F**	**0.62**	**E**			**4.25**	**A-E**
PI 290888	3.22	AB			3.28	B	25.40	A			14.66	C-F	7.63	A-D			6.11	A-F	3.48	A-E			0.79	E
PI 291594	3.67	AB			4.11	AB	11.23	A			27.55	A-F	4.54	A-E			7.31	A-E	2.19	B-E			4.22	A-E
PI 300129	**3.72**	**AB**			**5.44**	**AB**	**6.90**	**A**			**6.75**	**EF**	**3.40**	**DE**			**3.21**	**DEF**	**1.98**	**CDE**			**2.22**	**B-E**
PI 300130	**3.89**	**AB**			**5.78**	**A**	**10.23**	**A**			**4.90**	**F**	**4.07**	**CDE**			**2.52**	**F**	**1.78**	**DE**			**1.47**	**CDE**
PI 365031	**5.22**	**A**	**3.44**	**A**	**5.89**	**A**	**6.93**	**A**	**53.66**	**B**	**5.13**	**F**	**2.55**	**E**	**10.29**	**B**	**2.73**	**EF**	**0.30**	**E**	**7.16**	**D-G**	**1.81**	**CDE**
PI 410353			**3.17**	**A**	**5.06**	**AB**			**62.06**	**AB**	**8.43**	**EF**			**12.43**	**AB**	**3.82**	**B-F**			**5.45**	**G**	**1.08**	**DE**
PI 410357			**3.06**	**A**	**4.56**	**AB**			**72.27**	**AB**	**23.52**	**A-F**			**13.92**	**AB**	**6.37**	**A-F**			**5.59**	**G**	**3.05**	**A-E**
PI 410360			**3.28**	**A**	**3.89**	**AB**			**58.09**	**AB**	**25.17**	**A-F**			**12.03**	**AB**	**7.15**	**A-E**			**5.56**	**G**	**4.19**	**A-E**
PI 410361	2.39	B			4.17	AB	32.38	A			40.49	A-E	8.96	A			8.79	A	5.90	A-D			4.47	A-E
PI 410363	2.86	B			4.28	AB	33.66	A			37.30	A-F	7.97	A-D			7.38	A-D	5.85	A-D			4.24	A-E
PI 410364			**3.28**	**A**	**4.33**	**AB**			**61.39**	**AB**	**23.84**	**A-F**			**12.71**	**AB**	**6.52**	**A-F**			**6.26**	**FG**	**4.17**	**A-E**
PI 509038			3.61	A	4.70	AB			80.13	AB	57.12	A			12.61	AB	9.54	A			9.89	A-F	6.77	A
PI 509039			3.61	A	4.5	AB			87.64	AB	53.20	AB			12.92	AB	9.43	A			10.96	A-E	6.45	A
PI 600734			3.11	A	4.17	AB			74.01	AB	45.14	ABC			13.55	AB	8.93	A			8.28	C-G	6.18	AB
PI 647924			3.33	A	4.78	AB			62.36	AB	19.99	B-F			11.48	B	5.39	A-F			5.33	G	3.47	A-E
Seville	3.17	AB			4.56	AB	34.36	A			43.27	A-D	8.42	ABC			8.18	ABC	5.67	A-D			4.93	A-E
Sunclipse	3.06	B	3.61	A	4.83	AB	30.40	A	64.24	AB	30.49	A-F	7.42	A-D	11.11	B	6.85	A-F	6.50	A	6.72	FG	4.84	A-E
SV20			3.28	A	4.67	AB			67.71	AB	32.14	A-F			11.76	B	6.59	A-F			6.32	FG	3.66	A-E
TX Common							31.98	A			44.27	ABC	7.90	A-D			8.01	ABC	6.18	AB			5.22	A-D

[x] Square root transformed area under the disease progress curve

[y] Within a column, means followed by the same letter are not significantly different.

Table 3. 8. Pearson correlation between genotype least squares means in traits

Pearson Correlation Coefficients, N = 28 Prob > |r| under H0: Rho=0				
	AUDPC	**AULEC**	**D7INC**	**INCPER**
AUDPC	1	0.88066	0.9543	-0.65602
		<.0001	<.0001	0.0002
AULEC	0.88066	1	0.91231	-0.34738
	<.0001		<.0001	0.0701
D7INC	0.9543	0.91231	1	-0.43383
	<.0001	<.0001		0.0211
INCPER	-0.65602	-0.34738	-0.43383	1
	0.0002	0.0701	0.0211	

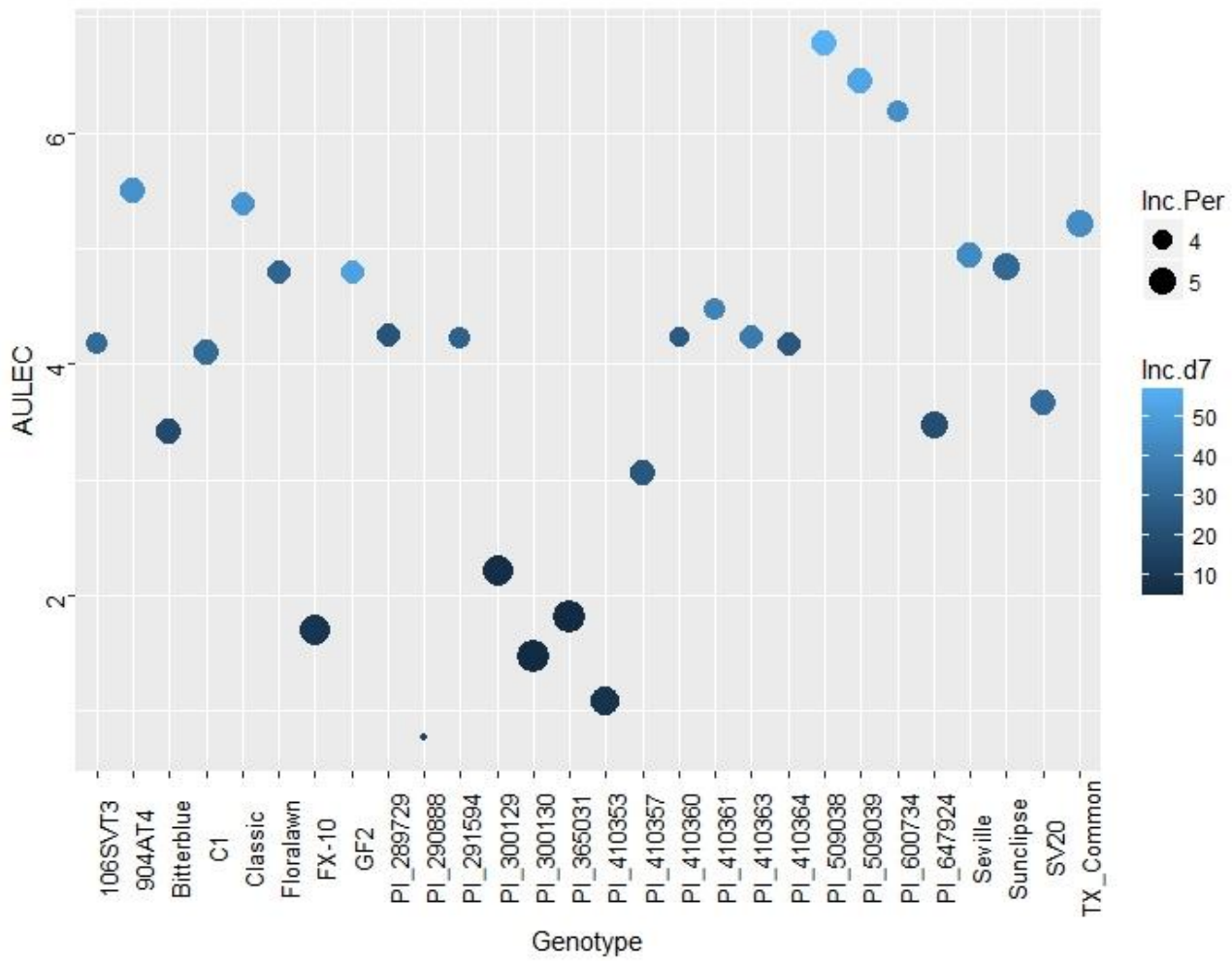

Figure 3. 1. Main Effects LS means for AUDPC, incubation period, and final incidence of St. Augustinegrass germplasm evaluated for resistance to gray leaf spot.

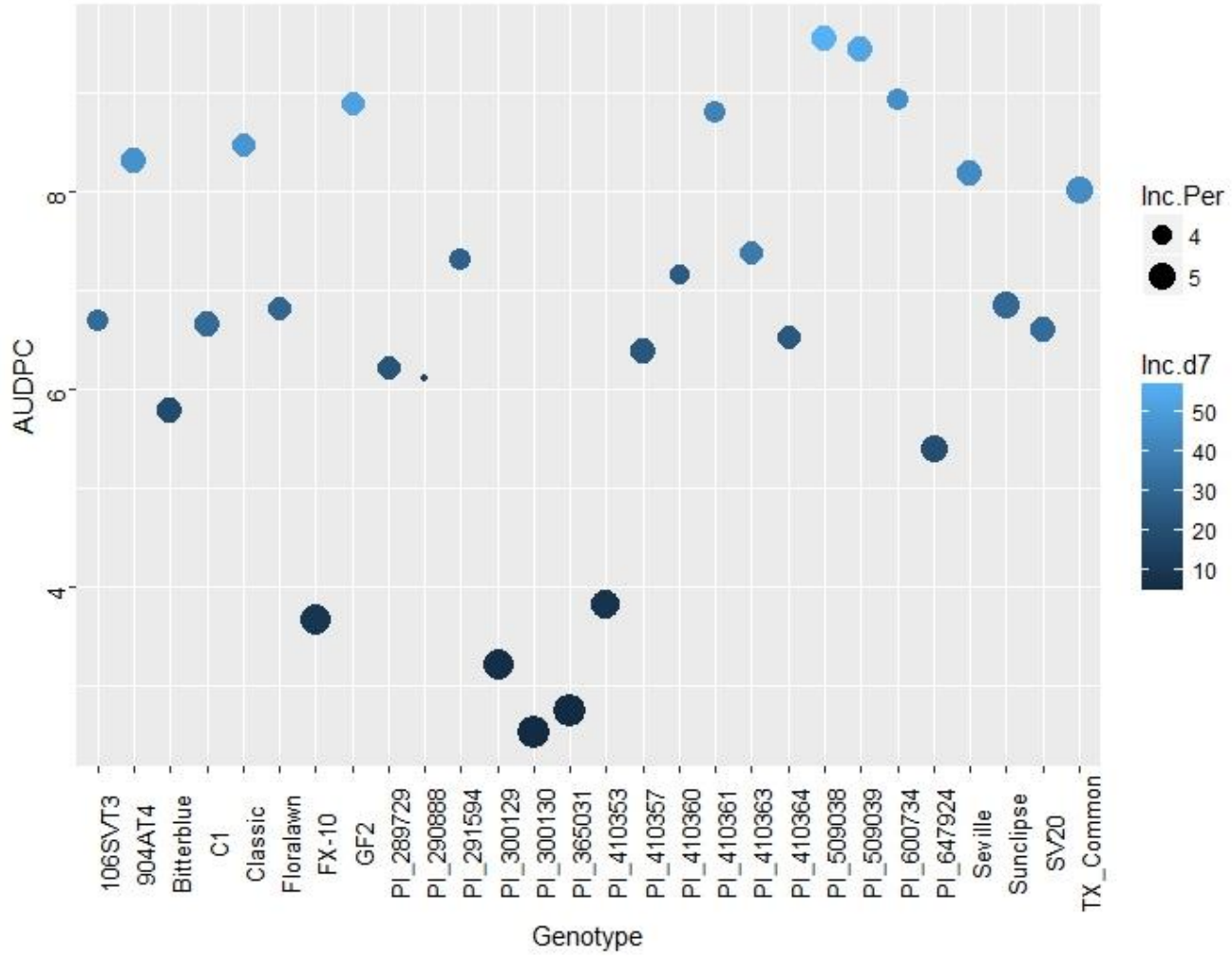

Figure 3. 2. Main Effects LS means for AUDPC, incubation period, and final incidence of St. Augustinegrass germplasm evaluated for resistance to gray leaf spot.

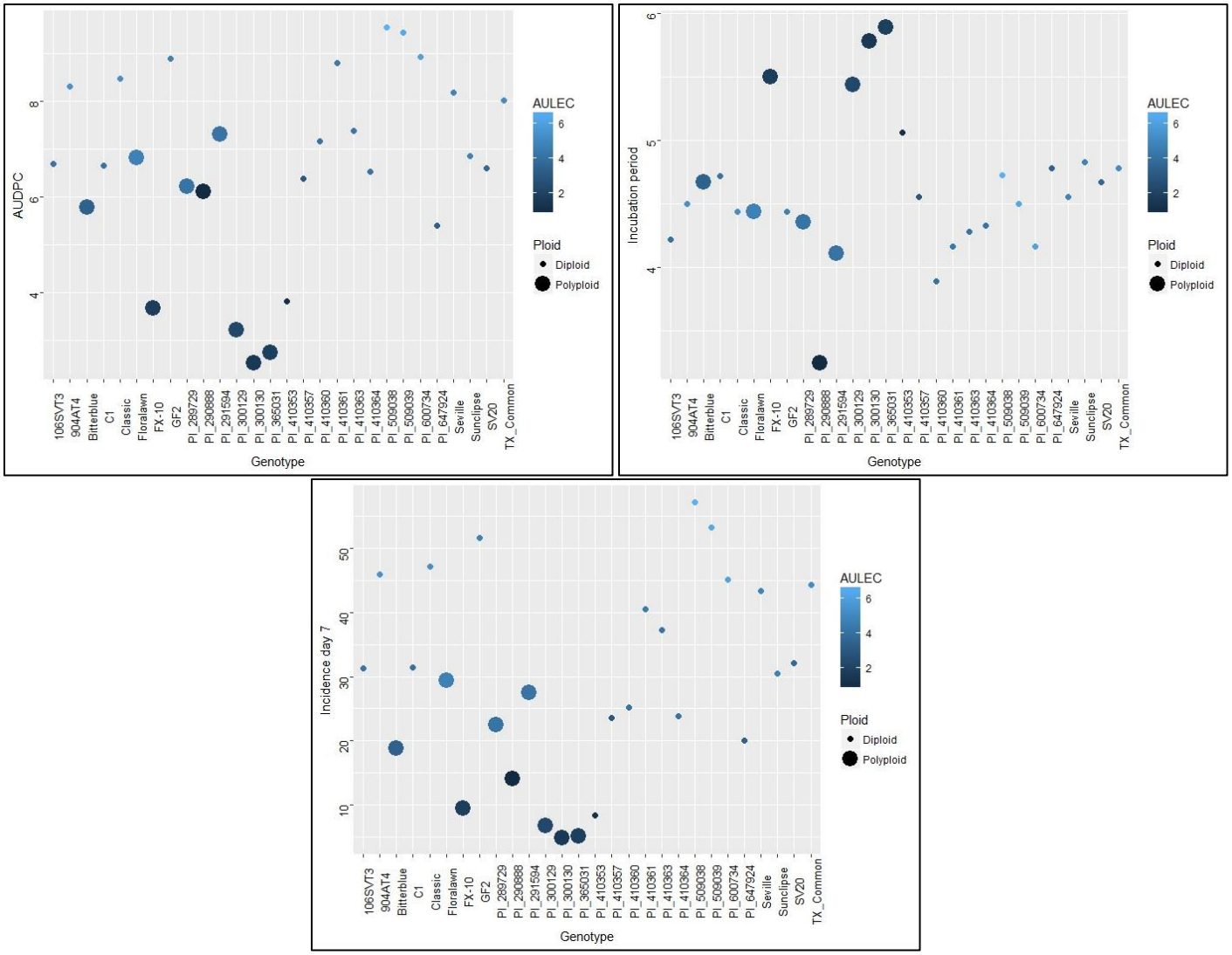

Figure 3. 3. Main Effects LS means for traits in trail III, based on ploidy levels of St. Augustinegrass germplasm evaluated for resistance to gray leaf spot.

-APPENDICES-

Appendix 3. 1. LS Means of genotypes across traits found in trial I, including interaction of genotype with inoculum on AULEC.

	INCPER			INCIDENT D7			Sq Rt AUDPC[x]			AULEC								
Genotype	Estimate		Rank	Estimate		Rank	Estimate		Rank	Est. Iso_3		Rank	Est. LWS		Rank	Est. SRS		Rank
Amerishade	2.22	A	1	22.5	A-F	9	7.44	A-D	15	5.4	A-E	15	6.48	AB	23	5.32	A-F	12
Bitterblue	3.11	A	19	19.5	A-F	8	6.53	A-E	8	5.32	A-E	14	3.92	B-E	7	5.62	A-E	17
Captiva	2.84	A	10	31.98	ABC	23	7.85	A-D	20	5.4	A-E	16	5.77	A-D	16	4.99	A-F	10
Classic	3.28	A	23	38.87	A	31	8.7	AB	27	6.71	AB	27	6.26	AB	21	5.89	A-E	23
Delmar	2.78	A	8	36.78	A	29	9.07	A	31	6.19	ABC	25	6.95	AB	28	6.03	ABC	25
Deltashade	3.06	A	16	31.36	A-D	21	7.8	A-D	18	5.52	A-E	17	6.84	AB	26	5.06	A-F	11
Eclipse	2.78	A	9	30.15	A-E	19	7.81	A-D	19	5.59	A-E	20	5.71	A-D	15	5.53	A-E	14
Floralawn	3.61	A	25	17.84	A-F	7	5.79	A-E	7	2.92	A-G	7	4.84	A-D	9	7.1	A	30
Floratam	3.11	A	20	24.65	A-F	12	7.18	A-D	12	3.83	A-G	8	5.23	A-D	12	6.88	A	29
Floratine	2.94	A	12	23.98	A-F	11	7.03	A-E	9	3.96	A-G	9	5.16	A-D	11	5.59	A-E	16
Floraverde	2.94	A	13	25.09	A-F	13	7.36	A-D	13	7.2	A	29	6.69	AB	25	5.55	A-E	15
FX-10	3.61	A	26	13.06	B-F	6	4.88	A-E	6	4.25	A-G	10	4.26	A-E	8	1.42	EFG	3
Jade	2.67	A	4	38.17	A	30	8.97	A	30	6.83	A	28	8.44	A	30	5.83	A-D	19
Mercedes	2.44	A	3	32.54	AB	25	8.94	A	28	7.21	A	30	6.86	AB	27	5.87	ABC	21
Palmetto	3.39	A	24	28.9	A-E	16	7.17	A-D	11	5.56	A-E	19	5.78	ABC	17	5.35	A-F	13
PI 289729	4.06	A	30	9.72	EF	3	4.27	B-E	4	0.45	FG	2	0.41	E	2	1	FG	2
PI 290888	3.22	A	22	25.4	A-F	14	7.63	A-D	17	1.97	C-G	5	3.91	B-E	6	4.49	A-G	9
PI 291594	3.67	A	27	11.23	C-F	5	4.54	A-E	5	2.41	B-G	6	1.94	CDE	4	2.23	B-G	6
PI 300129	3.72	A	28	6.9	F	1	3.4	DE	2	1.44	EFG	3	2.99	B-E	5	1.51	D-G	4
PI 300130	3.89	A	29	10.23	DEF	4	4.07	CDE	3	1.86	D-G	4	1.42	DE	3	2.06	C-G	5
PI 365031	5.22	A	31	6.93	F	2	2.55	E	1	0.27	G	1	0.1	E	1	0.53	G	1
PI 410361	2.39	A	2	32.38	ABC	24	8.96	A	29	5.59	A-E	21	6.22	ABC	20	5.92	ABC	24
PI 410363	2.87	A	11	33.3	AB	26	7.97	A-D	23	5.07	A-F	12	6.63	AB	24	5.75	A-D	18
PI 647925	2.72	A	7	23.79	A-F	10	7.11	A-E	10	5.53	A-E	18	5.13	A-D	10	4.12	A-G	7
Polaris	2.94	A	14	33.47	AB	27	8.17	ABC	24	6.1	A-D	24	6.36	AB	22	5.85	ABC	20
Raleigh	3.06	A	17	29.68	A-E	17	7.44	A-D	16	5.82	A-D	22	6.01	ABC	18	4.34	A-G	8
Sapphire	2.67	A	6	30.02	A-E	18	8.47	ABC	26	4.25	A-G	11	5.25	A-D	13	6.5	AB	28
Seville	3.17	A	21	34.36	A	28	8.42	ABC	25	5.2	A-E	13	5.42	A-D	14	6.4	AB	27
Sunclipse	3.06	A	18	30.4	A-E	20	7.42	A-D	14	6.08	A-D	23	7.08	AB	29	6.34	ABC	26
TX_Common	3	A	15	31.98	ABC	22	7.9	A-D	21	6.62	AB	26	6.05	ABC	19	5.87	ABC	22

[x] Square root transformed area under the disease progress curve

[y] Within a column, means followed by the same letter are not significantly different.

Appendix 3. 2. Error terms for random effects sorted by trials in all traits.

	INCPER			INCID D7			AUDPC			AULEC		
Cov Parm	**TRIAL I**	**TRIAL II**	**TRIAL III**	**TRIAL I**	**TRIAL II**	**TRIAL III**	**TRIAL I**	**TRIAL II**	**TRIAL III**	**TRIAL I**	**TRIAL II**	**TRIAL III**
run	1.1912	0.005586	0.2648	211.89	226.15	279.32	9.8002	3.6553	5.7478	0.7665	0.01107	0
rep(run)	0	0	0	66.7635	14.1033	0	1.611	0.1689	0	0.2827	0.3254	0
run*inoc	0.0094	0.01811	0.3207	0	23.2517	144.98	0.04987	0.3282	3.9696	0.03448	2.0869	0.9664
rep*inoc(run)	0.0012	0	0.07956	5.9846	22.9055	14.2745	0.1066	0.3437	0.4965	0.1017	0.3655	0.2252
run*gen	0.3433	0.009328	0.01398	8.9197	16.8394	58.0648	0.6177	0.2492	0.6953	0.1522	4.93E-18	0.1434
rep*gen(run)	0.06757	0	0	12.9961	0	9.4337	0.3138	0	0.1031	0.1607	0	0
run*gen*inoc	0.2872	0	0	4.8586	19.7954	73.7817	0.2043	0.5259	0.499	0.1812	1.9403	0.3454
Residual	0.7154	0.242	0.9847	101.11	278.89	179.3	2.8087	3.8644	3.8414	1.5617	9.7362	1.9639

Appendix 3. 3. LS Means of genotypes across traits in trial II, including interaction of genotype with inoculum on final incident and AULEC.

	INC PER			AUDPC			INCIDENT DAY 7									AULEC					
Genotype	Estimate		Rank	Estimate		Rank	Est. Iso_3		Rank	Est. LWS		Rank	Est. SRS		Rank	Est. Iso_3		Rank	Est. LWS		Rank	Est. SRS		Rank
106SVT3	3.56	AB	17	12.12	AB	8	72.64	AB	11	89.39	A	24	75.43	AB	17	6.3	D	8	8.96	AB	22	6.13	A	6
200Elm4	3.61	AB	21	14.11	AB	30	94.78	A	30	95.31	A	29	81.76	AB	22	17.74	AB	31	11.98	AB	28	9.08	A	27
904AT4	3.67	AB	28	12.53	AB	11	90.37	AB	26	80.45	A	14	73.3	AB	16	8.8	BCD	19	8.88	AB	20	7.02	A	13
C1	3.56	AB	18	11.96	AB	6	62.65	AB	5	82.79	A	17	72.75	AB	15	6.69	D	11	6.86	AB	11	6.69	A	10
C2	3.33	AB	9	12.70	AB	13	74.55	AB	12	81.98	A	15	78.74	AB	19	7.01	CD	13	6.62	B	9	6.85	A	11
C3	3.50	AB	15	13.01	AB	17	87.19	AB	23	75.21	A	9	88.37	A	30	9.22	BCD	20	7.41	AB	15	7.73	A	20
C4	3.39	AB	11	13.54	AB	22	80.13	AB	18	83.13	A	18	85.36	A	26	9.23	BCD	21	7.6	AB	16	9.29	A	29
C6	3.56	AB	19	12.33	AB	9	83.31	AB	21	80.1	A	12	71.92	AB	13	8.08	BCD	17	6.75	B	10	7.44	A	17
C7	3.39	AB	12	13.19	AB	18	80.84	AB	19	80.28	A	13	85.81	A	27	6.64	D	9	8.88	AB	21	6.9	A	12
Clem	3.61	AB	22	14.02	AB	28	87.53	AB	24	91.92	A	27	83.8	A	25	17.06	ABC	30	14.22	AB	31	7.87	A	21
Co2	3.89	A	32	12.90	AB	15	78.78	AB	15	85.36	A	20	68.71	AB	9	10	BCD	23	9.46	AB	25	8.15	A	24
Craig	3.50	AB	16	13.42	AB	21	90.82	AB	27	92.62	A	28	70.68	AB	11	9.91	BCD	22	16.94	A	32	6.42	A	9
GF2	3.67	AB	29	13.88	AB	25	72.61	AB	10	98.21	A	30	93.12	A	32	8.14	BCD	18	7.4	AB	14	7.88	A	22
Jones	3.72	AB	31	13.41	AB	20	79.97	AB	17	91.22	A	25	83.52	AB	24	10.48	BCD	24	12.78	AB	30	7.11	A	15
PI 365031	3.44	AB	14	10.30	B	1	40.41	B	1	57.95	A	4	62.62	AB	3	4.23	D	1	7.92	AB	18	9.34	A	30
PI 410353	3.17	AB	5	12.43	AB	10	61.44	AB	4	52.07	A	3	72.66	AB	14	5.82	D	5	4.42	B	1	6.12	A	5
PI 410355	3.06	B	1	16.56	A	32	95.54	A	31	99.07	A	32	79.2	AB	20	6.68	D	10	6.17	B	7	6.28	A	7
PI 410357	3.06	B	2	13.92	AB	26	75.19	AB	13	78.16	A	10	63.47	AB	4	4.98	D	3	4.67	B	2	7.1	A	14
PI 410360	3.28	AB	6	12.03	AB	7	72.40	AB	9	69.95	A	8	31.94	B	1	7.36	CD	15	5.86	B	6	3.46	A	1
PI 410364	3.28	AB	7	12.71	AB	14	65.01	AB	6	50.8	A	2	68.36	AB	7	5.69	D	4	5.22	B	3	7.88	A	23
PI 414079	3.06	B	3	13.74	AB	24	75.70	AB	14	88.41	A	23	70.66	AB	10	7.21	CD	14	7.15	AB	12	5.73	A	3
PI 509038	3.61	AB	23	12.61	AB	12	83.60	AB	22	88.36	A	22	68.43	AB	8	12.29	A-D	27	9.17	AB	23	8.19	A	25
PI 509039	3.61	AB	24	12.92	AB	16	93.41	A	29	82.09	A	16	87.41	A	29	11.23	A-D	26	9.17	AB	24	12.48	A	32
PI 600734	3.11	AB	4	13.55	AB	23	71.38	AB	8	62.04	A	6	88.6	A	31	8.02	BCD	16	7.79	AB	17	9.04	A	26
PI 647924	3.33	AB	10	11.48	B	4	79.80	AB	16	50.39	A	1	56.9	AB	2	5.95	D	7	5.42	B	4	4.62	A	2
Ray	3.67	AB	30	11.22	B	3	70.64	AB	7	57.97	A	5	75.55	AB	18	5.94	D	6	5.78	B	5	6.31	A	8
Rebok	3.61	AB	25	14.13	AB	31	95.98	A	32	98.86	A	31	82.94	AB	23	20.85	A	32	12.66	AB	29	7.63	A	18
Sunclipse	3.61	AB	26	11.11	B	2	60.53	AB	3	68.51	A	7	63.69	AB	5	6.72	D	12	7.38	AB	13	6.06	A	4
SV20	3.28	AB	8	11.76	B	5	57.48	AB	2	78.34	A	11	67.31	AB	6	4.69	D	2	6.59	B	8	7.67	A	19
T638	3.56	AB	20	13.24	AB	19	82.76	AB	20	91.26	A	26	70.92	AB	12	10.97	A-D	25	10.01	AB	26	7.29	A	16
T644	3.39	AB	13	14.09	AB	29	92.03	AB	28	87.94	A	21	86.82	A	28	12.82	A-D	28	10.91	AB	27	9.21	A	28
T672	3.61	AB	27	13.93	AB	27	88.03	AB	25	84.19	A	19	81.23	AB	21	14.33	A-D	29	8.52	AB	19	11.11	A	31

[x] Square root transformed area under the disease progress curve

[y] Within a column, means followed by the same letter are not significantly different.