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Assessment of bermudagrass (*Cynodon dactylon*) biotypes and bermudagrass interference with sugarcane (*Saccharum* spp. hybrids)

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ASSESSMENT OF BERMUDAGRASS (*CYNODON DACTYLON*) BIOTYPES AND
BERMUDAGRASS INTERFERENCE WITH SUGARCANE (*SACCHARUM* SPP. HYBRIDS)

A Dissertation

Submitted to the Graduate Faculty of The
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctorate of Philosophy

in

The School of Plant, Environmental and Soil Sciences

by
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ABSTRACT

Bermudagrass (*Cynodon dactylon* L. Pers.) collected from 17 Louisiana sugarcane (*Saccharum* spp. hybrids) fields and two sites outside sugarcane-growing area was evaluated for genetic diversity, growth characteristics and response to glyphosate. Random Amplified Polymorphic DNA (RAPD) genetic analysis and Jacard's similarity coefficient, a dendrogram, based on unweighted pair group mean average (UPGMA) identified two cluster groups based on presence of common alleles. Bermudagrass considered most aggressive in establishment rate based on ground cover, plant height, and biomass production included the biotypes A (St. Martinville) and Q (Port Allen) in cluster A and R (St. Gabriel) in cluster B. Biotypes J (Samuels), N (New Iberia), and T (St. Joseph) considered least aggressive were included in cluster A. Rate of establishment for biotypes J, N, and T averaged 5.3 times slower and plant height was 61% less compared with A, Q, and R. Biomass production the first year averaged 7.8 times greater for biotypes A, Q, and R compared with J, N, and T. In greenhouse and field studies, bermudagrass biotypes A, C (Baldwin), and Q in cluster A were least sensitive to glyphosate and biotypes D (Centerville) and P (Patterson) in cluster B were most sensitive to glyphosate. In a competition study, pre-sprouted single node stem cuttings of 'HoCP 96-540' sugarcane were planted in 26.5 L pots with one, two, or four bermudagrass plants, sugarcane shoot weight 56 days after planting (DAP) was reduced on average 58%; two and four bermudagrass plants reduced sugarcane root weight on average 39%. In another study, two bermudagrass plants did not negatively affect shoot population 56 DAP for the cultivars HoCP 96-540, 'L 97-128', 'L 99-226', 'HoCP 00-950', 'L 01-283', and 'L 03-371'. For L 97-128 and L 99-226, shoot weight averaged 1.7 to 3.0 times greater than the average of the other cultivars and root weight averaged 1.8 to 2.1 times greater than the average of the other cultivars. When the sugarcane cultivars were watered over a 42-day period with leachate collected from actively

growing bermudagrass, sugarcane tiller height, tiller number, shoot weight, and root weight were not negatively affected.

CHAPTER 1: INTRODUCTION

SUGARCANE INDUSTRY IN LOUISIANA

Sugarcane (*Saccharum* spp. hybrid) is grown in Louisiana, Florida, and Texas in the continental U.S. Approximately 40% of U.S. sugar obtained from sugarcane is produced in Louisiana, and Louisiana accounts for 17.4% of total U.S. sugar production (Salassi et al. 2011). In 2011, in Louisiana, 489 producers grew sugarcane on 165,000 hectares in 23 parishes (Anonymous 2011, Salassi et al. 2011). Average sugarcane yield from total acres amounted to 70.1 Mg ha⁻¹ with approximately 8,100 kg of sugar produced per harvested hectare. Sugarcane leads Louisiana's agricultural row crops in total crop market value. In 2011, the sugarcane industry contributed 2.5 to 3.0 billion dollars to the Louisiana economy.

Sugarcane typically grows in subtropical and tropical climates where the average temperature is greater than 17 C. In Louisiana, sugarcane is exposed to a winter cold period where the crops experience a dormant period. Therefore, the growing season is shorter in comparison with traditional sugarcane growing areas. Average annual rainfall at the Sugar Research Station, St. Gabriel, LA is 154.9 cm (Hendricks 2009). Abundant rainfall and warm temperatures in Louisiana contribute to the proliferation of weeds. Weeds most responsible for reducing yields in sugarcane include bermudagrass (*Cynodon dactylon* L. Pers.), johnsongrass (*Sorghum halepense* L. Pers.), morningglory (*Ipomoea* spp.), and itchgrass (*Rottboellia cochinchinensis* Lour. W. Clayton) (Hackett et al. 2011).

Bermudagrass is a serious weed problem in Louisiana sugarcane fields. The perennial nature of sugarcane and slow early season growth, combined with wide row spacing, provide a favorable environment for bermudagrass growth (Holm et al. 1977). In Louisiana, a sugarcane crop cycle, beginning in late summer, consists of three harvests over three years and a fallow period during the spring and summer of the fourth year (Richard 1993). During the fallow

period, weeds are controlled by cultivation and use of glyphosate herbicide. During the crop cycle, row tops remain undisturbed allowing bermudagrass to re-establish. Perennial weeds are especially problematic in the ratoon crops (Miller et al. 1999). Weed competition is greatest during the tillering stage of sugarcane (Blanco et al. 1984; Fadayomi and Abayomi 1988; Lencse and Griffin 1991; Millhollon 1992; Turner 1985). Season-long itchgrass competition led to a 34% reduction in millable stalk population and a 43% reduction in sugar yield (Lencse and Griffin 1991). When compared with weed free plots, heavy infestations of johnsongrass (80 to 100% infestation on rows) led to a 36% reduction in sugarcane yield and a 31% reduction in sugar yield (Ali et al. 1986).

CHARACTERISTICS OF BERMUDAGRASS

Bermudagrass is a perennial weed primarily propagated by stolon and rhizome fragmentation (Håkansson 1982). Bermudagrass rhizomes grow horizontally below ground whereas the stolons grow horizontally above ground. Bermudagrass rhizomes contain carbohydrate reserves necessary for overwintering and regrowth. Stolons support the leafy orthotropic and reproductive shoots (Dong and De Kroon 1994). Brown et al. (1985) reported that a single bermudagrass plant per row of cotton produced 25% groundcover in the first year and 75% in the second year of growth.

Application of herbicides in the sugarcane crop provide only suppression of bermudagrass, the fallow period is the ideal time to reduce bermudagrass infestation (Etheredge et al. 2009; Miller et al. 1999). Bermudagrass, a C4 plant regulated by temperature, enters a period of dormancy in temperate regions during the winter (Horowitz 1972; Overman et al. 1989). Richard (1995) reported that bermudagrass biomass increased 340% between plant cane and first stubble crops and 490% between first stubble and second stubble. When compared to a weed-free control, cane and sugar yield with bermudagrass competition was reduced an average

of 5% per year. Over a three year crop cycle when bermudagrass was removed manually by hoeing, sugarcane yield averaged 89.58 Mg ha⁻¹ compared with 85.10 Mg ha⁻¹ for the weedy control. Although bermudagrass is competitive with sugarcane early in the growing season, once sugarcane develops a dense canopy, bermudagrass growth is suppressed by shading making it noncompetitive (Horowitz 1972). Richard and Dalley (2007) reported that bermudagrass interference reduced sugar yield 8 to 32% in the plant-cane crop and an average of 9% in the first and second ratoon crops. Yield reduction was associated with reduced sugarcane stalk population and stalk height.

Bermudagrass cannot be completely controlled in sugarcane with either preemergence or postemergence herbicides (Anonymous 2013). Bermudagrass infestation, however, can be reduced when metribuzin or terbacil are applied prior to weed emergence in the spring (Richard 1993). Metribuzin at a rate of 2.7 kg a.i./ha did not negatively affect the sugarcane cultivar CP 65-357.

Bermudagrass can be effectively controlled with a combined approach of tillage and application of glyphosate during the fallow period (Anonymous 2013; Etheredge et al. 2009). Etheredge (2009) reported a decrease in bermudagrass emergence in October and November plantcane when at least one tillage treatment is substituted with a glyphosate treatment during the summer fallow period.

ALLELOPATHY

“Allelopathy refers to the beneficial or harmful effects of one plant on another plant, both crop and weed species, by the release of chemicals from plant parts by leaching, root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems” (Ferguson and Rathinasabapathi 2003). Unlike weed competition for light, water, nutrients, and space, allelopathic effects do not necessarily depend on population density of the

competitor. Allelopathy research has evolved from mere observations of plant symptoms resulting from an allelopathic plant to identifying the precise chemicals responsible for the allelopathic response. Allelo-chemicals can disrupt cell division, pollen germination, nutrient uptake, photosynthesis, and specific enzyme functions (Ferguson and Rathinasabapathi 2003). Allelo-chemicals can be present in flowers, leaves, leaf litter, leaf mulch, stems, bark, roots, soil, and soil leachates.

Research has been conducted to evaluate allelopathic characteristics of plants on targeted crops. In laboratory studies, Vasilakoglou et al. (2005) showed that bermudagrass rhizomes and foliage produce inhibitory substances that affect corn and cotton growth. In cotton, total fresh weight and root length were inhibited by bermudagrass extracts. While lab studies determine the possibility of an allelopathic effect, the effects may not be observed in the field. Allelopathic compounds disperse through soil from the suspected plant to the targeted crop and can be broken down in the soil by microbes or can attach to the soil, never encountering the targeted crop roots (Inderjit 2001).

CHARACTERISTICS OF SUGARCANE CULTIVARS

In 2011, sugarcane cultivars ‘HoCP 96-540’, ‘L 99-226’, ‘L 99-233’, ‘L 01-283’, ‘L 97-128’, ‘HoCP 00-950’, and ‘L 01-299’ were grown in Louisiana representing 43, 19, 11, 8, 6, 6, and 3% of the area planted, respectively (Gravois and Legendre 2011). Several new cultivars have been released since 2011 and area planted in these cultivars is increasing.

HoCP 96-540 was released for commercial planting in 2003. In 2011, this cultivar represented 43% of the state’s total plant-cane hectares (Gravois and Legendre 2011). Yield of cane and sugar per hectare for HoCP 96-540 is rated as excellent (Tew et al. 2005). It is a mid-season maturing cultivar. HoCP 96-540 has a moderate stalk population and medium-sized stalks. HoCP 96-540 is resistant to mosaic disease and smut and is moderately resistant to leaf

scald. Disadvantages of HoCP 96-540 is that it has poor ratooning ability and is moderately susceptible to sugarcane borer and to brown rust.

L 97-128 was released in 2004 (Gravois et al. 2008). In 2011, this cultivar represented 6% of the state's total plant-cane hectares (Gravois and Legendre 2011). L 97-128 is characterized as an excellent ratooning cultivar. This cultivar emerges early in spring and grows rapidly through the early summer. It is well adapted to mechanical harvest and early high sucrose content gives it an early maturity classification. L 97-128 is resistant to mosaic disease and is moderately resistant to leaf scald and common brown rust. However, L 97-128 is only moderately resistant to smut and is considered susceptible to sugarcane borer.

L 99-226 was released in January of 2006 as a commercial cultivar (Bischoff et al. 2009). In 2011, this cultivar represented 19% of the state's total plant-cane hectares (Gravois and Legendre 2011). It has high yield of sugar and cane per hectare. Unlike other cultivars, L 99-226 has some resistance to sugarcane borer. It is moderately resistant to mosaic disease, but is moderately susceptible to brown rust, smut, and leaf scald.

HoCP 00-950 was released as a commercial cultivar in 2007. In 2011, this cultivar represented 6% of the state's total plant-cane hectares (Gravois and Legendre 2011). It exhibits high yields of both sugar per ton of cane and sugar per hectare (Tew et al. 2009). It is an early maturing cultivar resistant to brown rust, mosaic, and leaf scald diseases. HoCP 00-950 is susceptible to the sugarcane borer.

L 01-283 was released as a commercial cultivar in 2008 (Gravois et al. 2010). In 2011, this cultivar represented 8% of the state's total plant-cane hectares (Gravois and Legendre 2011). It is an early maturing cultivar that is resistant to all major diseases that affect sugarcane, with the exception of ratoon stunting disease. L 01-283 is resistant to sugarcane borer.

L01-299 was released as a commercial cultivar in 2009 (Gravois et al. 2011). In 2011, this cultivar represented only 3% of the state's total plant cane hectares (Gravois and Legendre 2011). It is an excellent stubbling cultivar and is well adapted to mechanical harvesting. This cultivar is resistant to rust, leaf scald, and mosaic, however it is moderately susceptible to smut.

L 03-371 was released as a commercial cultivar in 2010 (Gravois et al. 2012). Expansion of this cultivar by producers will depend on its productivity. The cultivar exhibits high yields of both sugar and cane per hectare and is resistant to brown rust, smut, and mosaic virus. The cultivar is however, susceptible to the sugarcane borer.

DIVERSITY OF BERMUDAGRASS

Bermudagrass is the number one weed problem in Louisiana sugarcane fields. Chemical control options in the crop provide only bermudagrass suppression and the hope is that early emergence of sugarcane in the spring along with rapid canopy development and shading will enhance the competitiveness of the crop (Bittencourt et al. 2010). Observations within the sugarcane growing area of Louisiana indicate variation in bermudagrass growth characteristics (leaf width and biomass production), height, and aggressiveness (ability to spread by development of stolons). Anecdotally, growers report variation in control with glyphosate. Although multiple glyphosate applications are needed (Anonymous 2013), it is difficult to obtain complete control (Etheredge et al. 2009). It is possible that biotypes exist that are more competitive with sugarcane and that are less susceptible to herbicides. Wills and Bryson (1985) collected and evaluated 17 biotypes of bermudagrass from Mississippi, Arkansas, Louisiana, and Tennessee. Susceptibility of the biotypes to various herbicides was evaluated in the greenhouse and in the field. For the biotypes evaluated, Verdict (haloxyfop) provided the most consistent control (at least 87%). Control of the bermudagrass biotypes with glyphosate ranged from 38 to 87%.

OBJECTIVES

The objectives of this research are: 1) to evaluate growth characteristics, genetic diversity, and sensitivity to glyphosate of bermudagrass biotypes collected at various locations throughout Louisiana; 2) bermudagrass interference with sugarcane at planting; and 3) potential bermudagrass allelopathic effects on sugarcane.

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CHAPTER 2: GROWTH COMPARISONS AND GENETICS OF BERMUDAGRASS (*CYNODON DACTYLON* L. PERS.) BIOTYPES IN LOUISIANA

INTRODUCTION

Bermudagrass (*Cynodon dactylon* L. Pers.) can be a problem weed in crops and is especially troublesome in sugarcane. Holm et al. (1977) labeled bermudagrass as a “noxious” weed. Bermudagrass thrives in well-drained, fertile soil (Heath et al. 1985) where sugarcane is well adapted. Propagation of bermudagrass occurs primarily through transport of stolons and rhizomes; although some seed propagation can occur (Roche Couste 1962). *Cynodon dactylon*, a tetraploid, is more fertile than the diploid species, *Cynodon transvaalensis* (Duble 2010). Dewey (1966) found that self-fertility of crested wheatgrass (*Agropyron cristatum*) was higher in hexaploid populations and that vigor decreased as ploidy number decreased. The genus *Cynodon* has the ability to cross-pollinate and is highly self-incompatible, therefore, out-crossing is common (Burton 1947). Sexual reproduction of a species leads to the transfer of genes and the adaptation of a species to a particular environment. In Louisiana sugarcane fields, plant height, growth rate, leaf width, and internode length of bermudagrass can vary considerably. Plant populations that vary phenotypically are considered to be biotypes. The Weed Science Society of America defines a biotype as “a population within a species with distinct genetic variation” (Vencill 2002). An ecotype is a subspecies within a population that is adapted to a particular set of environmental conditions (McWhorter 1971; Millhollon and Burner 1993). In reality, reference to ecotype and biotype are used inter-changeably.

Bryson (1990) evaluated growth habits of 17 bermudagrass biotypes collected in cotton (*Gossypium hirsutum* L.) fields in Mississippi, Arkansas, Louisiana, and Tennessee. Differences in growth characteristics among the biotypes were observed when present in cotton that was planted solid compared with a skip-row pattern. Guertal and Walker (2013) reported differences in growth, appearance, and resiliency of 12 bermudagrass ecotypes of hybrid bermudagrass when

exposed to different mowing heights. Anderson (2002) evaluated 11 clonal bermudagrass plants for freeze tolerance. Of the clones evaluated, nine were tetraploid with varying cold tolerance and two were sterile triploid hybrids with freeze tolerance ranging from -7.2 to -10.5 C in one experiment and -6.6 to -10.0 C in the second experiment. Roquette et al. (2011) reported that from samples collected from two original bermudagrass plantings, nine genetically similar ecotypes were identified with differences in plant height, leaf width, and leaf coarseness.

Silva and Snaydon (1995) reported that soil pH was a factor in selection of bermudagrass based on chromosome number. Of the 480 plants sampled from 32 locations, 80% had a chromosome number of $2n=36$ while 20% possessed a chromosome number of $2n=18$. Diploid populations were present in very acidic areas while only tetraploid populations were present at non-acid sites; both tetraploid and diploid populations were found where soil pH was neutral.

Amplified fragment length polymorphism (AFLP) analysis of 27 bermudagrass genotypes revealed high genetic diversity (Zhang et al. 1999). The 27 genotypes were separated into 3 clusters on the Unweighted Pair Group Mean Average (UPGMA) tree. Of the four *Cynodon dactylon* accessions, Tifton 10 and Tiflawn were clustered in Group A while T90 and T110 were placed in Group B, suggesting wide genetic diversity. Hybrids tested were divided into two distinct groups of the UPGMA tree despite the fact that most were interspecific hybrids between *Cynodon dactylon* and *Cynodon transvaalensis*. Genetically heterozygous plants are highly variable since bermudagrass is cross-pollinated. Results also unexpectedly showed mutants to have larger dissimilarity coefficients, such as was the case for Tifway 2 and Tifway.

Variation among ecotypes can also occur in johnsongrass (*Sorghum halepense* L. Pers.). McWhorter and Jordan (1976) compared growth characteristics of six johnsongrass ecotypes from six states that were characterized as susceptible or resistant to the herbicide dalapon. The ecotypes varied in regard to average height, fresh and dry weight, and rhizome production. It was

suggested that susceptibility of the ecotypes to dalapon may be related to differences in growth characteristics. The time required from emergence to flowering also varied among the ecotypes and was correlated to the latitude of the original site of collection.

The objectives of this research were to compare growth characteristics of bermudagrass biotypes collected in Louisiana to include growth rate, plant height, leaf width and internode length, and dry matter production, as well as potential seedhead production and sensitivity to frost. Genetic diversity among the biotypes was evaluated using RAPD analysis.

MATERIALS AND METHODS

In August and September 2010, 20 bermudagrass biotypes were collected; 12 from outfield sugarcane variety trial locations (5 heavy and 7 light soils) used by the Louisiana sugarcane breeding programs (LSU Agcenter Sugar Research Station, USDA Agricultural Research Service, and the American Sugarcane League); five from sugarcane farms in Louisiana; and three from LSU AgCenter Research Stations (Table 2.1). At each location bermudagrass plants were removed and placed in 11.4 L pots for use as “mother plants” for propagation. On March 28, 2011, two to three inch stem sections from stolons were collected from each “mother plant” and planted into 5 cm pots containing a 2:1 river silt and Jiffy Mix Plus¹ mixture in the greenhouse. Pots were watered and fertilized weekly with Miracle-Gro² water-soluble 24-8-16 fertilizer solution. On May 23, 2011, plants were transplanted into the field with a Cancienne Silt Loam soil at the Central Research Station, Ben Hur Research Farm, Baton Rouge, LA in plots 1.5 x 1.5 m in size. Two plants were planted in the center of each plot 60 cm from one another. The experimental design was a randomized complete block with four

¹ A sterile soil mix with an optimal blend of sphagnum and vermiculite with MagAmp slow release fertilizer (7-40-6). Jiffy Products of America, Inc., 600 Industrial Parkway, Norwalk, OH 44857.

² An all purpose water soluble fertilizer. Scotts Miracle-Gro Products, Inc. 14111 Scottslawn Road, Marysville, OH 43041.

replications. Alleys between plots were 1.5 meter wide and were sprayed with glyphosate using a hooded sprayer to prevent bermudagrass encroachment from adjacent plots.

Table 2.1. Bermudagrass biotypes collected in Louisiana for comparison of growth characteristics and genetics.^a

Biotype	Grower	Farm	Location	Parish
Outfield sites ^b				
A	Lawrence Levert	St. John	St. Martinville	St. Martin
B	Ronald Hebert	Ronald Hebert	Jeanerette	Iberia
C	Brett Allain	Allain	Baldwin	St. Mary
D	Wilson Judice	Frank Martin	Centerville/Calumet	St. Mary
E	Pete Lanaux	Lanaux	Lucy	St. John the Baptist
F	Brian Graugnard	Bon Secour	Vacherie	St. James
G	Joel Landry	Glenwood	Napoleonville	Assumption
H	Howard Robichaux	Mary	Raceland	Lafourche
I	Danny Naquin	Magnolia	Schriever	Terrebonne
J	Joe Beard III	Brunswick	Samuels	Point Coupee
K	Todd Andre	Alma	Allon	Point Coupee
L	Al Landry	Landry Farm	Plaquemine	Iberville
Off-Station nursery site ^b				
M	Blake Newton	Bunkie	Bunkie	Avoyelles
Other sites ^b				
N	Ronnie Gonsoulin	Airport Road	New Iberia	Iberia
O	Ronald Hebert	Bayside	Jeanerette	Iberia
P	Mike Cremaldi	Cremaldi Farms	Patterson	St. Mary
Q	Kerny Gros	Barrowza Plantation	Port Allen	West Baton Rouge
R	LSU AgCenter	Sugar Research Station	St. Gabriel	Iberville
S	LSU AgCenter	Dean Lee Research Station	Alexandria	Rapides
T	LSU Agcenter	Northeast Research Station	St. Joseph	Tensas

^a Actively growing bermudagrass collected at each site was potted and stem node cuttings from each site were used for planting in the field study. Biotype O was not evaluated in the field study because of the inability to re-establish and the overall lack of vigor.

^b Outfield sites consisted of locations where sugarcane variety trials are conducted. The off-station nursery is a site also used for sugarcane variety trials. Other sites included sugarcane farms where bermudagrass concerns have been expressed as well as three LSU AgCenter Research Stations, one where sugarcane is grown and the other two where non-sugarcane crops are grown.

Plots were irrigated as needed to promote establishment. Nitrogen fertilizer was applied on July 1, 2011 using ammonium nitrate³ (34-0-0) at a rate 46 kg N/ha based on

³ Red Fox Fertilizer. 356 E. Inez Road. Dothan, AL 36301.

recommendations for newly planted bermudagrass pastures (Twidwell 2009). Because of difficulty in propagation from node cutting and poor establishment in the field, biotype O was omitted from the study. Fertilizer was not applied to test plots in 2012.

Percent bermudagrass ground cover was assessed on July 16, July 26, August 5, and August 18, 2011 [54, 64, 74, and 87 days after planting (DAP)] based on a scale of 0 to 100%, where 100% = total area of 1.5 x 1.5 m plot covered with plant foliage. On August 25, 2011, internode length, leaf width, plant height, and above ground biomass were measured. A push mower with a 53 cm cutting width and a bag attachment was used to collect biomass from the center area of each plot. Biomass was transferred to a cloth bag and dried for three days at 60 C and weight was recorded. Bermudagrass seedhead production was recorded on November 7, 2011 and April 25, 2012, using a visual rating scale of 1 (20% or less), 2 (30 to 70%), or 3 (80% or more) based on percentage of the plot area having seedheads present. Susceptibility of bermudagrass biotypes to damage from frost was visually rated for percent green foliage approximately every 15 days from December 1 through March 1 for 2011-2012 and 2012-2013 using a scale of 0-100%, where 0 = no green foliage and 100 = total area of plot with green foliage.

DNA analysis of the 19 bermudagrass biotypes was conducted at the Sugarcane Genetics Lab located in the School of Plant, Environmental, and Soil Sciences at Louisiana State University. Leaf samples from 19 biotypes collected from “mother plants” were ground to powder in liquid Nitrogen. Total genomic DNA was extracted using Plant DNeasy mini kit⁴ according to the manufacturer’s protocol. Concentrations of extracted DNA were estimated by

⁴ A kit used for DNA isolation from plant tissue. QIAGEN Inc. 28159 Stanford Avenue, Valencia, CA 91355.

Nanodrop 1000 spectrophotometer⁵ at 260 nm of UV wavelength. DNA was stored at -20 C until further use.

Genotyping of 19 bermudagrass biotypes was performed as described by Baisakh et al. (2006). The DNA amplification was carried out with 24 RAPD primers (Table 2.2; www.operon.com) on a programmable MyiQ-Thermal Cycler⁶ using the profile: one cycle of 2 min at 94 C, 45 cycles of 1 min at 94 C, 1 min at 36 C, and 2 min at 72 C, followed by one cycle of 7 min at 72 C. The final volume of the PCR reaction was 25 µl containing 5 µl of 5x PCR reaction buffer⁷, 2.5 µl of 25 mM MgCl₂, 200 µM of each dNTP, 2.5 U *GoTaq flexi* DNA polymerase⁸, and 50 µg/µl of primer. Three µl of 5x loading dye was added to the PCR amplification product and 25 µl of PCR product was electrophoresed on 2% agarose gel in 1x TBE buffer at 100 V for 3 h, stained with ethidium bromide, and visualized and documented under UV light in a KODAK Gel Logic 100⁹. Four hundred nanograms of a DNA size ladder¹⁰ (Hi-Lo DNA marker; www.mnmolecular.com) was loaded onto the gel along with the PCR products.

⁵ A full-spectrum spectrophotometer that measures 1 ul samples with high accuracy and reproducibility. NanoDrop products. 3411 Silverside Rd, Bancroft Building, Wilmington, DE 19810.

⁶ A Real-Time PCR Detection System. Bio-Rad Laboratories. 4000 Alfred Nobel Drive, Hercules, CA 94547.

⁷ An aqueous solution used to control pH in a reaction. PromegaCorporations. 2800 Woods Hollow Rd., Madison, WI 53711.

⁸ A buffer containing two dyes that separate during electrophoresis to show migration progress and increase sample density. PromegaCorporations. 2800 Woods Hollow Rd., Madison, WI 53711.

⁹ An imaging system designed for gel documentation and analysis. Eastman Kodak Company. 4 Science Park, New Haven, CT 06511.

¹⁰ A set of standards that are used to identify the approximate size of a molecule run on a gel during electrophoresis. Minnesota Molecular. 3109 West 50th Street, # 104, Minneapolis, MN 55410.

Table 2.2. RAPD primers used for genetic analysis of the bermudagrass biotypes.

Operon Primer ^a	Sequence 5'-3'
OPA1	CAGGCCCTTC
OPA2	TGCCGAGCTG
OPA3	AGTCAGCCAC
OPA4	AATCGGGCTG
OPA5	AGGGGTCTTG
OPA6	GGTCCCTGAC
OPA7	GAAACGGGTG
OPA8	GTGACGTAGG
OPA9	GGGTAACGCC
OPA10	GTGATCGCAG
OPA11	CAATCGCCGT
OPA13	CAGCACCCAC
OPA14	TCTGTGCTGG
OPA15	TTCCGAACCC
OPA16	AGCCAGCGAA
OPA17	GACCGCTTGT
OPA18	AGGTGACCGT
OPA19	CAAACGTCGG
OPA20	GTTGCGATCC
OPB3	CATCCCCCTG
OPB4	GGACTGGAGT
OPB6	TGCTCTGCCC
OPB8	GTCCACACGG
OPB10	CTGCTGGGAC

^a www.operon.com

Genetic diversity among the bermudagrass biotypes was assessed as described by Suman et al. (2012). Amplified bands were scored manually as 1 for presence and 0 for absence in all 19 biotypes. Only clear and unambiguous DNA fragments were scored. Pair-wise genetic similarity among individual biotypes was analyzed using the Jaccard's similarity coefficient of the SIMQUAL module (Jaccard 1908) of the Numerical Taxonomy System (NTSYSpc) Version 2.2 software (Rohlf 2005). The resulting matrix was employed for clustering analysis to create a dendrogram (tree) based on the UPGMA (unweighted pair group mean average) with the SAHN module, a program designed to perform the sequential, agglomerative, hierarchal, and nested clustering methods (Sneath and Sokal 1973).

Data are presented individually by biotype and as an average for biotype groups. Assignment to biotype groups was determined by plotting mean values for each parameter and separating biotypes into groups based on similarity in response. Data for individual biotypes and for biotype groups were subjected to the Proc Mixed Procedure in SAS (SAS Institute 2012) with replications and years (depending on the variable) considered random effects. Data for percent ground cover determined only in 2011 (establishment year) were subjected to repeated measure analysis with unstructured covariance. Plant height, leaf width, and internode length data collected in 2011 were analyzed with replications as random effects. Because 2011 was the establishment year and because bermudagrass was well established in 2012, dry weight, seedhead production, and percent green foliage data collected both years were analyzed separately by year. Percent green foliage data were further subjected to repeated measure analysis with unstructured covariance. For each parameter, least square means were calculated and mean separation was performed at $P \leq 0.05$. Letter groupings were converted using the PDMIX800 macro in SAS (Saxton 1998).

RESULTS AND DISCUSSION

For bermudagrass percent ground cover a significant biotype group x DAP interaction was observed. For those biotypes that established most rapidly (Group 1; biotypes A, Q, and R), ground cover 54 DAP in 2011 was 52% and increased to 94% 74 DAP (Table 2.3). Ground cover for this biotype group did not change from 74 to 87 DAP. For the intermediate group (Group 2; biotypes B, C, D, E, F, G, H, I, K, L, M, P, and S), bermudagrass ground cover was only 13% 54 DAP and ground cover increased with each successive rating date. At 87 DAP, ground cover for the Group 2 biotypes was 71% and equivalent to ground cover for the Group 1 biotypes 64 DAP. Ground cover was equivalent for the Group 3 biotypes (J, N, and T) at 54, 64,

and 74 DAP (5 to 16%) and coverage averaged 31% 87 DAP, equivalent to that for the Group 2 biotypes 74 DAP.

Table 2.3. Bermudagrass percent ground cover 54 to 87 days after planting (DAP) for biotypes separated into three groups based on similarity in rate of establishment.^a

Biotype group ^b	DAP / Groundcover (%)			
	54	64	74	87
1	52 c ^c	74 b	94 a	97 a
2	13 g	22 ef	43 cd	71 b
3	5 g	7 fg	16 fg	31 de

^a See Table 2.1 for information on bermudagrass biotypes. Two established bermudagrass plants of each biotype in 5 x 5 cm pots were transplanted in the center of each 1.5 x 1.5 m plot 30 cm from one another on May 23, 2011. Ground cover was based on bermudagrass coverage of the entire plot.

^b Group 1 represented by biotypes A, Q, and R; Group 2 included biotypes B, C, D, E, F, G, H, I, K, L, M, P, and S; Group 3 included biotypes J, N, and T (see Table 2.1 for information on bermudagrass biotypes and Table 2.4 for individual biotype response averaged across DAP).

^c Means in columns and rows followed by the same letter are not significantly different ($P \leq 0.05$).

For Group 1, ground cover for the biotypes ranged from 75 to 84%; from 29 to 45% for Group 2; and from 12 to 18% for Group 3 (Table 2.4). Averaged across biotypes within each group, ground cover was 79, 37, and 15% for Groups 1, 2, and 3, respectively. Data show that biotypes varied considerably in their ability to establish with some biotypes very aggressive while others were only minimally aggressive.

Data for bermudagrass internode length measured in August 2011 (following bermudagrass planting in May) are shown in Table 2.4. Based on groupings averaged across replications, internode length was 68 and 78 mm for biotypes S and Q, respectively, for Group 1 and ranged from 43 to 56 mm for Group 2 biotypes (A, B, C, E, K, N, P, and R) and from 26 to 41 mm for Group 3 biotypes (D, F, G, H, I, J, L, M, and T). Averaged across biotypes within each group, internode length was 73, 50, and 35 mm for Groups 1, 2, and 3, respectively, and average internode length for the three groups was significantly different.

Table 2.4. Bermudagrass ground cover, internode length, leaf width, and plant height for 19 bermudagrass biotypes presented individually and by grouping of biotypes.^a

Bermudagrass biotype-group	Groundcover (%) ^b	Bermudagrass biotype/group	Internode length (mm) ^b	Bermudagrass biotype/group	Leaf width (mm) ^b	Bermudagrass biotype/group	Plant height (mm) ^b
A - Group 1	75 abc ^c	Q - Group 1	78 a	A - Group 1	3.6 ab	Q - Group 1	325 a
Q - Group 1	79 ab	S - Group 1	68 ab	J - Group 1	3.6 ab	R - Group 1	285 ab
R - Group 1	84 a	Group 1 avg.	73 A	K - Group 1	3.5 bc	Group 1 avg.	305 A ^b
Group 1 avg.	79 A ^d	A - Group 2	50 bcdef	Q - Group 1	3.6 ab	A - Group 2	234 abc
B - Group 2	39 de	B - Group 2	51 bcde	S - Group 1	4.2 a	B - Group 2	206 abcd
C - Group 2	43 cd	C - Group 2	56 abc	Group 1 avg.	3.7 A	E - Group 2	236 abc
D - Group 2	45 bcd	E - Group 2	55 abcd	B - Group 2	3.2 bc	S - Group 2	208 abcd
E - Group 2	40 d	K - Group 2	43 cdef	C - Group 2	3.1 bc	Group 2 avg.	221 B
F - Group 2	40 d	N - Group 2	49 bcdef	D - Group 2	3.0 bc	C - Group 3	148 bcd
G - Group 2	38 d	P - Group 2	50 bcde	E - Group 2	3.0 bc	D - Group 3	141 cd
H - Group 2	37 d	R - Group 2	47 bcdef	F - Group 2	3.1 bc	F - Group 3	125 cd
I - Group 2	31 de	Group 2 avg.	50 B	H - Group 2	3.1 bc	G - Group 3	120 cd
K - Group 2	29 de	D - Group 3	38 cdef	I - Group 2	3.1 bc	H - Group 3	125 cd
L - Group 2	37 d	F - Group 3	29 ef	N - Group 2	3.1 bc	K - Group 3	161 bcd
M - Group 2	35 de	G - Group 3	39 cdef	R - Group 2	3.2 bc	L - Group 3	120 cd
P - Group 2	36 d	H - Group 3	41 cdef	Group 2 avg.	3.0 B	M - Group 3	141 cd
S - Group 2	36 d	I - Group 3	33 cdef	G - Group 3	2.8 c	N - Group 3	144 cd
Group 2 avg.	37 B	J - Group 3	39 cdef	L - Group 3	2.8 c	Group 3 avg.	136 C
J - Group 3	15 f	L - Group 3	37 cdef	M - Group 3	2.8 c	I - Group 4	95 d
N - Group 3	18 ef	M - Group 3	31 def	P - Group 3	2.9 bc	J - Group 4	105 cd
T - Group 3	12 f	T - Group 3	26 f	T - Group 3	2.9 bc	P - Group 4	118 cd
Group 3 avg.	15 C	Group 3 avg.	35 C	Group 3 avg.	2.8 C	T - Group 4	77d
						Group 4 avg.	99D

^a See Table 2.1 for information on bermudagrass biotypes. Grouping of the biotypes was based on similarity in response.

^b Two established bermudagrass plants of each biotype in 5 x 5 cm pots were transplanted in the center of each 1.5 x 1.5 m plot 30 cm from one another on May 23, 2011. Ground cover was based on bermudagrass coverage of the entire plot and was averaged across ratings made 54, 64, 74, and 87 days after planting. Internode length, leaf width, and plant height data were collected on August 25, 2011 (94 days after planting).

^c Biotype means within each column followed by the same lower case letter are not significantly different ($P \leq 0.05$).

^d Each growth parameter group average means (averaged across biotypes) followed by the same upper case letter are not significantly different ($P \leq 0.05$).

Bermudagrass leaf width in 2011 ranged from 3.5 to 4.2 mm for biotypes in Group 1 (A, J, K, Q, and S), 3.0 to 3.2 mm for Group 2 (B, C, D, E, F, H, I, N, and R), and 2.8 to 2.9 mm for Group 3 (G, L, M, P, and T) (Table 2.4). Averaged across biotypes within each group, leaf width was greatest for Group 1 (3.7 mm) and least for Group 3 (2.8 mm). Based on similarities among biotypes, four groups were identified for plant height (Table 2.4). Bermudagrass plant height for Group 1 biotypes Q and R averaged 325 and 285 mm, respectively. For Group 2 biotypes (A, B, E, and S), plant height ranged from 206 to 236 mm and averaged 221 mm. Plant height for Group 3 biotypes (C, D, F, G, H, K, L, M, and N) ranged from 120 to 161 mm and averaged 136 mm. For the Group 4 biotypes, plant height ranged from 77 mm for biotype T to 118 mm for biotype P. Average plant height for this group was 99 mm. Other research has documented differences in growth characteristics of bermudagrass biotypes (Bryson 1990; Guertal and Walker 2013; Rouquette et al. 2011).

For the bermudagrass biotypes evaluated, biotype Q appeared in Group 1 for ground cover (79%), internode length (78 mm), leaf width (3.6 mm), and plant height (325 mm) (Table 2.4). Other biotypes that showed the ability to establish rapidly were biotype R which was in Group 1 for ground cover (84%) and plant height (285 mm) and in Group 2 for leaf width (3.2 mm) and biotype A which was included in Group 1 for ground cover (75%) and leaf width (3.6 mm) and in Group 2 for plant height (234 mm). Of the bermudagrass biotypes evaluated, biotypes A, Q, and R, based on the growth parameters evaluated, were most aggressive in their ability to establish and would be expected to be highly competitive with crops such as sugarcane. In contrast, the biotypes expected to be least aggressive were included in Groups 3 and 4. Ground cover was 15% for biotype J and 12% for biotype T. Internode length was 39 mm for biotype J and 26 mm for biotype T. Biotype J plant height was 105 mm and 77 mm for biotype T. Another biotype that was slow to establish was biotype N (18% ground cover).

The ability of bermudagrass to establish would directly affect bermudagrass biomass production. Dry weight for the bermudagrass biotypes was determined 94 days after planting in 2011 (Table 2.5) and three groups were identified. Bermudagrass dry weight for Group 1 biotypes (A, Q, and R) ranged from 216 to 223 g/plot with a group average of 219 g/plot. For the Group 2 biotypes (B, C, E, F, M, and S), dry weight averaged 116 g/plot and was 47% less than for Group 1 biotypes. Dry weight for the Group 3 biotypes ranged from 18 to 76 g/plot and averaged 51 g/plot; 56% less than for the Group 2 biotypes.

Dry weight biomass was also determined in April of 2012, 11 months after planting. The dry weight increase from 2011 to 2012 for all biotypes was expected and can be attributed to the time period allowed for the biotypes to fully establish. In 2012, bermudagrass dry weight for Group 1 biotypes (A, B, Q, and S) ranged from 400 to 438 g/plot and averaged 413 g/plot (Table 2.5). Biotypes A and Q were also identified in Group 1 (most productive) for dry weight biomass in 2011. For the Group 2 biotypes (E, F, G, H, I, K, L, M, N, P, and R) dry weight averaged 283 g/plot and was 32% less compared with Group 1 biotypes. Dry weight for the Group 3 biotypes (C, D, J, and T) ranged from 171 to 200 g/plot and averaged 186 g/plot, 34% less than for Group 2 biotypes. Biotypes J and T were also identified in Group 3 (least productive) in 2011 and 2012. Differences among the bermudagrass biotypes in regard to seedhead production were also assessed. Seedhead production for the biotypes in Group 1 (A, B, P, and S) for November 2011 and in Group 1 (G, H, I, and P) for April 2012 averaged 2.9 on a rating scale where 3 equalled 80% or more of the plot having seedheads present (Table 2.5). Biotypes in Group 4 (E, F, N, R, and T) in 2011 and (A, C, E, N, R, and T) in 2012 averaged 1.1 or 1.2 where 1 equalled presence of 20% or less seedhead per plot. Of interest is that biotype A was included in Group 1 in 2011, but was in Group 4 in 2012. Biotypes consistent over years in seedhead production included biotype P (Group 1 in 2011 and 2012) and biotypes E, N, R, and T (Group 4 in 2011 and 2012).

Table 2.5. Bermudagrass dry weight and seedhead production for 19 bermudagrass biotypes presented individually and by grouping of biotypes.^a

Bermudagrass biotype/group	Dry weight (g) 8/25/2011	Bermudagrass biotype/group	Dry weight (g) 4/25/2012	Bermudagrass biotype/group	Seedhead emergence ^b 11/7/11	Bermudagrass biotype/group	Seedhead Emergence 4/25/12
A – Group 1	219 a ^c	A – Group 1	438 a	A – Group 1	2.8 ab ^c	G – Group 1	2.5 ab
Q – Group 1	223 a	B – Group 1	407 ab	B – Group 1	2.8 ab	H – Group 1	3.0 a
R – Group 1	216 ab	Q – Group 1	400 abc	P – Group 1	3.0 a	I – Group 1	3.0 a
Group 1 avg.	219 A ^d	S – Group 1	407 ab	S – Group 1	3.0 a	P – Group 1	3.0 a
		Group 1 avg.	413 A	Group 1 avg.	2.9 A	Group 1 avg.	2.9 A
B – Group 2	111 bcd			I – Group 2	2.3 abcd	B – Group 2	2.0 abcd
C – Group 2	98 cd	E – Group 2	286 abcd	K – Group 2	2.3 abcd	K – Group 2	2.0 abcd
E – Group 2	141 abc	F – Group 2	241 abcd	M – Group 2	2.3 abcd	L – Group 2	2.3 abc
F – Group 2	108 cd	G – Group 2	299 abcd	Q – Group 2	2.5 abc	M – Group 2	2.0 abcd
M – Group 2	97 cd	H – Group 2	349 abcd	Group 2 avg.	2.4 B	Q – Group 2	2.3 abc
S – Group 2	141 abc	I – Group 2	261 abcd			S – Group 2	2.0 abcd
Group 2 avg.	116 B	K – Group 2	338 abcd	C – Group 3	1.8 abcd	Group 2 avg.	2.1 B
		L – Group 2	265 abcd	D – Group 3	1.5 bcd		
D – Group 3	76 cd	M – Group 2	239 abcd	G – Group 3	1.8 abcd	D – Group 3	1.5 bcd
G – Group 3	73 cd	N – Group 2	259 abcd	H – Group 3	1.8 abcd	F – Group 3	1.8 bcd
H – Group 3	47 cd	P – Group 2	295 abcd	J – Group 3	1.8 abcd	J – Group 3	1.5 bcd
I – Group 3	44 cd	R – Group 2	286 abcd	L – Group 3	1.5 bcd	Group 3 avg.	1.6 C
J – Group 3	31 d	Group 2 avg.	283 B	Group 3 avg.	1.7 C		
K – Group 3	75 cd			E – Group 4	1.0 d	A – Group 4	1.0 d ¹
L – Group 3	59 cd	C – Group 3	171 d	F – Group 4	1.0 d	C – Group 4	1.3 cd
N – Group 3	35 cd	D – Group 3	200 bcd	N – Group 4	1.3 cd	E – Group 4	1.3 cd
P – Group 3	55 cd	J – Group 3	179 cd	R – Group 4	1.0 d	N – Group 4	1.3 cd
T – Group 3	18 d	T – Group 3	196 bcd	T – Group 4	1.3 cd	R – Group 4	1.0 d
Group 3 avg.	51 C	Group 3 avg.	186 C	Group 4 avg.	1.1 D	T – Group 4	1.0 d
						Group 4 avg.	1.2 D

^a See Table 2.1 for information on bermudagrass biotypes. Grouping of the biotypes was based on similarity in response.

^b Seedhead emergence was determined from visual ratings based on a scale of 1 to 3 with 1 = 20% or less of the plot with seedheads present, 2 = 30-70% of the plot with seedheads, 3 = 80% or more of the plot with seedheads.

^c Biotype means within each column followed by the same lower case letter are not significantly different ($P \leq 0.05$).

^d For each growth parameter group average means (averaged across biotypes) followed by the same upper case letter are not significantly different ($P \leq 0.05$).

Beginning December 1 in 2011 and 2012 and continuing until March 1, 2012 and 2013, bermudagrass biotypes were visually rated approximately every 15 days for percent green foliage to evaluate response to temperature changes as plants entered the winter dormant period and as plants initiated new growth in spring. In Louisiana, bermudagrass, like sugarcane, enters a winter dormant period following the first killing frost and re-emerges in the spring as soil temperature rises. Sugarcane germination and growth is closely related to temperature. Although optimum germination of sugarcane buds and root development occurs at 30 to 35 C (Ingamells 1989), sugarcane bud germination is poor below 20 C (Smit 2010). In contrast, optimum regrowth of bermudagrass from stolons and rhizomes occurs at 20 C (Satorre et al. 1996), but rhizome buds do not sprout below 10 C (Horowitz 1972; Satorre et al. 1996). These findings suggest that bermudagrass is less sensitive to cool temperatures compared with sugarcane and the earlier emergence of bermudagrass in the spring would enhance its ability to compete with the crop. Based on the differences in growth observed among the bermudagrass biotypes, it would be plausible to expect there to also be differences in cold tolerance.

The first freeze (ambient temperature of 0 C or less) occurred on November 11 in 2011 and by February 15 in 2012 a freeze was recorded for a total of 16 days (Table 2.6). For the second year of the study the first freeze occurred on November 25 of 2012 and only three additional freeze days were recorded by February 15. A freeze was not recorded after February 15 in 2012, but in 2013 a freeze occurred on March 2, 3, and 27.

For the December 2011 through March 2012 time period, bermudagrass biotypes were separated into three groups based on similarities in percent green foliage on the December 1 rating date. On December 1, biotypes ranging from 92 to 99% (A, J, K, N, Q, S, and T) were assigned Group 1 while biotypes ranging from 82 to 89% (B, C, E, F, H, L, P, and R) and 63 to 78% (D, G, I, and M) were assigned to Groups 2 and 3, respectively (Table 2.7). These group

designations were maintained for all subsequent percent green foliage evaluations. For the Group 1 biotypes, percent green foliage averaged 95% on December 1, 2011 72% on December 15, and 11% on January 1, 2012 but was only 1% on January 15 (Table 2.8). Percent green foliage on December 1 for the Group 2 biotypes averaged 85% which was less for the Group 1 biotypes but was greater than the 73% average observed for the Group 3 biotypes. On December 15, percent green foliage for the Group 2 and 3 biotypes averaged 30 and 10%, respectively. A freeze was observed 4 days during the November 1, 2011 through December 1, 2012 time period (Table 2.6). December 2 to December 15, 2011 noted 4 freeze days.

Table 2.6. Minimum and maximum air and soil temperature from November through March when percent green foliage data for the bermudagrass biotypes were collected.^a

Time period	2011-2012		2012-2013	
	Air temperature	Soil temperature	Air temperature	Soil temperature
	minimum / maximum (C)	minimum / maximum (C)	minimum / maximum (C)	minimum / maximum (C)
Nov 1 – Nov 15	0 (1) / 27.8	11.7 / 23.3	2.2 (0) / 28.3	10.0 / 26.7
Nov 16 – Dec 1	-0.6 (3) / 25.6	9.4 / 23.3	0 (1) / 25.0	10.0 / 19.4
Dec 2 – Dec 15	-3.3 (4) / 24.4	7.8 / 20.6	0.6 (0) / 25.6	7.2 / 21.7
Dec 16 – Jan 1	0 (1) / 23.3	8.9 / 20.6	-1.7 (2) / 25.0	6.1 / 19.4
Jan 2 – Jan 15	-3.9 (5) / 23.9	6.7 / 19.4	1.1 (0) / 21.7	6.1 / 21.7
Jan 16 – Feb 1	1.7 (0) / 25.0	10.6 / 22.2	0 (1) / 25.0	6.1 / 20.0
Feb 2 – Feb 15	-2.2 (2) / 23.3	8.3 / 20.6	2.2 (0) / 23.9	10.6 / 18.9
Feb 16 – Mar 1	3.9 (0) / 25.6	12.2 / 21.7	0.6 (0) / 21.7	9.4 / 16.7
Mar 2 – Mar 15	2.8 (0) / 27.2	13.3 / 22.2	-2.8 (2) / 25.6	7.2 / 17.8
Mar 16 – Apr 1	9.4 (0) / 28.3	16.7 / 23.9	-1.1 (1) / 27.2	10.6 / 20.0

^a Bermudagrass green foliage data collected every 15 days beginning November 15 through March 1. Green foliage data presented in Tables 2.8 and 2.9.

^b Data in parentheses represent the number of days during the time period when minimum air temperature was 0 C or less.

Percentage bermudagrass green foliage on December 15, 2011 for the Group 1 biotypes (72%) suggest that they are less sensitive to frost damage compared with bermudagrass biotypes in Group 2 and 3 (no more than 30% green foliage) (Table 2.8). Between January 2 and February 15, 2012, seven days were noted where minimum ambient temperature was 0 C or less (Table 2.6). On January 1 and January 15, 2012, percent green foliage was no more than 11%. New growth of bermudagrass was present on February 1 and percent green foliage for the three

Table 2.7. Initial ratings of 19 biotypes for percent green foliage used to assign grouping of biotypes based on similarity in response for 2011 and 2012.^a

Biotypes	Bermudagrass green foliage (%)	
	December 1, 2011	December 1, 2012
A	94 (1) ^a	87 (1)
B	84 (2)	75 (2)
C	84 (2)	63 (2)
D	63 (3)	68 (2)
E	88 (2)	87 (1)
F	82 (2)	50 (3)
G	78 (3)	78 (2)
H	86 (2)	76 (2)
I	75 (3)	74 (2)
J	92 (1)	70 (2)
K	92 (1)	88 (1)
L	87 (2)	80 (1)
M	77 (3)	68 (2)
N	94 (1)	75 (2)
P	83 (2)	47 (3)
Q	99 (1)	75 (2)
R	89 (2)	77 (2)
S	99 (1)	86 (1)
T	98 (1)	40 (3)

^a See Table 2.1 for information on bermudagrass biotypes.

^b Means for each year were used to separate biotypes into groups. Values in parentheses represent the assigned Group (1, 2, or 3).

Table 2.8. Percent green foliage assessments every 15 days from December 1, 2011 through March 1, 2012 for bermudagrass biotypes separated into three groups.^a

Group ^b	Bermudagrass green foliage (%)						
	Dec 1, 2011	Dec 15, 2011	Jan 1, 2012	Jan 15, 2012	Feb 1, 2012	Feb 15, 2012	Mar 1, 2012
1	95 a ^c	72 de	11 j	1 k	48 g	73 e	96 a
2	85 cd	30 hi	4 k	1 k	51 fg	73 e	93 ab
3	73 e	10 ijk	2 k	0 k	48 gh	64 ef	87 bc

^a Bermudagrass biotypes planted May 23, 2011. Grouping of the biotypes was based on similarity in response for the December 1, 2011 rating (see Table 2.7). Green foliage based on 0 - 100%, where 0 = no green foliage and 100 = total area of plot with green foliage.

^b Group 1 represented by biotypes A, J, K, N, Q, S, and T; Group 2 included biotypes B, C, E, F, H, L, P, and R; Group 3 included biotypes D, G, I, and M (see Table 2.1 for information on bermudagrass biotypes).

^c Means in columns and rows followed by the same letter are not significantly different ($P \leq 0.05$).

biotype groups was equivalent and ranged from 48 to 51%. Percent green foliage was also equivalent for the three groups on February 15 (64 to 73%) and on March 1, percent green

foliage for Group 3 biotypes averaged 87%; less than for the Group 1 biotypes (96%) but equal to the Group 2 biotypes (93%). A freeze was not observed after February 15, 2012 (Table 2.6).

Averaged across the December, 2011 through March, 2012 time period, percent green foliage for the seven biotypes in Group 1 ranged from 51% for biotype T to 65% for biotype S with an average of 56% (Table 2.9). For the Group 2 biotypes, average percent green foliage was 47% and less than for the Group 1 biotypes. Percent green foliage for the Group 3 biotypes ranged from 34 to 44% and averaged 41%, less than for the other groups.

For the December 2012 through March 2013 time period, bermudagrass biotypes were separated into three groups based on similarities in percent green foliage at the December 1 rating date. On December 1, biotypes ranging from 80 to 88% (A, E, K, L, and S) were assigned Group 1 while biotypes ranging from 63 to 78% (B, C, D, G, H, I, J, M, N, Q, and R) and 40 to 50% (F, P, and T) were assigned to Groups 2 and 3, respectively (Table 2.7). As noted for the previous year, the group designations were maintained for the remaining percent green foliage evaluations. For the Group 1 biotypes percent green foliage averaged 86% on December 1, 2012 and 51% on December 15 (Table 2.10). For comparison, percent green foliage for the Group 2 and 3 biotypes averaged 73 and 46%, respectively, on December 1 and 28 and 11%, respectively, between November 1 and December 15, 2012, a freeze was noted for only one day (Table 2.6). The greater percentage of green foliage for the Group 1 biotypes compared with the Group 2 and 3 biotypes between December 1 and 15 (Table 2.10) suggest that Group 1 biotypes are less sensitive to frost damage. Green foliage percentage on January 1 was 18% for the Group 1 biotypes and was greater than for the Group 2 and 3 biotypes (no more than 6%) (Table 2.10). During the December 16 and January 1, 2013 time period two freeze days were noted (Table 2.6). For January 15 and February 1, percentage green foliage was no more than 6% for any of the biotype groups.

Table 2.9. Bermudagrass percent green foliage for 19 bermudagrass biotypes averaged for assessments every 15 days from December 1, 2011 through March 1, 2012 and from December 1, 2012 to March 1, 2013 and presented individually and by grouping of biotypes.^a

Bermudagrass biotype/group	Green foliage (%) 2011/2012	Bermudagrass biotype/group	Green foliage (%) 2012/2013
A – Group 1	55 abcd ^b	A – Group 1	35 bcd
J – Group 1	54 abcde	E – Group 1	51 a
K – Group 1	54 abcde	K – Group 1	32 bcde
N – Group 1	56 abc	L – Group 1	29 cdef
Q – Group 1	60 ab	S – Group 1	43 ab
S – Group 1	65 a	Group 1 average	38 A
T – Group 1	51 bcdef		
Group 1 average	56 A ^c	B – Group 2	31 cde
		C – Group 2	18 fg
B – Group 2	43 efg	D – Group 2	31 cde
C – Group 2	47 cdef	G – Group 2	35 bcd
E – Group 2	48 cdef	H – Group 2	35 bcd
F – Group 2	47 cdef	I – Group 2	26 cdef
H – Group 2	51 bcdef	J – Group 2	24 cdef
L – Group 2	47 cdef	M – Group 2	29 cdef
P – Group 2	48 cdef	N – Group 2	24 cdef
R – Group 2	54 abcde	Q – Group 2	35 bcd
Group 2 average	48 B	R – Group 2	27 cdef
		Group 2 average	29 B
D – Group 3	34 g	F – Group 3	24 cdef
G – Group 3	44 defg	P – Group 3	21 ef
I – Group 3	41 fg	T – Group 3	9 g
M – Group 3	44 defg	Group 3 average	18 C
Group 3 average	41 C		

^a See Table 2.1 for information on bermudagrass biotypes. Bermudagrass biotypes planted May 23, 2011. Grouping of the biotypes was based on similarity in response for the December 1, 2011 and December 1, 2012 rating (see Table 2.7). Green foliage based on 0 - 100%, where 0 = no green foliage and 100 = total area of plot with green foliage.

^b Biotype means within each column followed by the same lower case letter are not significantly different ($P \leq 0.05$).

^c For each growth parameter group average means (averaged across several biotypes) followed by the same upper case letter are not significantly different ($P \leq 0.05$).

The lower percent green foliage observed in late February and March 2013 compared with the previous year is due to a freeze that occurred two days between March 2 and March 15, 2013 and one day between March 16 and April 1, 2013 (Table 2.6), which affected ability of bermudagrass to regrow. For both years, even though biotypes differed in initial response to cool weather in the fall, all biotypes had initiated significant regrowth by February 1, 2012 and by

February 15, 2013 (Tables 2.8 and 2.10). Assuming that regrowth of bermudagrass from stolons and rhizomes can occur at a soil temperature of 20 C (Satorre et al. 1996) and based on the maximum soil temperature data collected in 2012 and 2013 (Table 2.6) in the present study, regrowth would be expected in mid-January to early February.

Table 2.10. Percent green foliage assessments every 15 days from December 1, 2012 through March 1, 2013 for bermudagrass biotypes separated into three groups.^a

Group ^b	Bermudagrass green foliage (%)						
	Dec 1, 2012	Dec 15, 2012	Jan 1, 2013	Jan 15, 2013	Feb 1, 2013	Feb 15, 2013	Mar 1, 2013
1	86 a ^c	51 cd	18 g	3 jk	6 hij	39 def	65 bc
2	73 b	28 ef	6 hi	1 k	6 ij	31 ef	56 cd
3	46 d	11 ghijk	3 ijk	0 k	3 ijk	25 fgh	43 cde

^a Bermudagrass biotypes planted May 23, 2011. Grouping of the biotypes was based on similarity in response for the December 1, 2012 rating (see Table 2.7). Green foliage based on 0 - 100%, where 0= no green foliage and 100 = total area of plot with green foliage.

^b Group 1 represented by biotypes A, E, K, L, and S; Group 2 included biotypes B, C, D, G, H, I, J, M, N, Q, and R; Group 3 included biotypes F, P, and T (see Table 2.1 for information on bermudagrass biotypes).

^c Means in columns and rows followed by the same letter are not significantly different ($P \leq 0.05$).

Averaged across the December, 2012 through March, 2013 time period, percent green foliage for the biotypes in Group 1 ranged from 29% for biotype L to 51% for biotype E with an average of 38% for the five biotypes (Table 2.9). Percent green foliage for the Group 2 biotypes ranged from 18% for biotype C to 35% for biotypes G, H, and Q. The average green foliage percentage for the Group 2 biotypes was 29% and less than for the Group 1 biotypes. For the Group 3 biotypes, average green foliage percentage ranged from 9 to 24% with an average of 18%, which was less than for the Group 2 biotypes. For both 2011/2012 and 2012/2013, biotypes A, K, and S were included in Group 1 (Table 2.9). However, biotype T included in Group 1 in 2011/2012 was in Group 3 in 2012/2013.

The UPGMA tree generated by Jaccard's similarity coefficient grouped the 19 biotypes into two major clusters, A and B (Figure 2.1). Biotypes A, B, C, J, K, L, N, Q, and T formed cluster A. Cluster B is comprised of the biotypes D, F, G, H, I, M, P, and R. Biotypes E and S

did not fall in either A or B clusters but have a similarity coefficient (percentage of common alleles) of 0.42 and 0.40, respectively. For cluster A, biotypes K and L are most similar with a similarity coefficient of 0.70. For the ground cover data (Table 2.4) biotypes K and L are included in Group 2 and for plant height (Table 2.4) are included in Group 3. Biotypes K, L and N have a similarity coefficient of 0.59. Unlike biotypes K and L, biotype N appears in Group 3 for ground cover but for plant height all biotypes are included in Group 3. Although biotypes J and Q in cluster A have a similarity coefficient of 0.62, biotype J appears in Group 3 for ground cover whereas biotype Q appears in Group 1. For plant height, biotype J appears in Group 4 and Q appears in Group 1. Biotypes A and B in cluster A have a similarity coefficient of 0.57 although A is in Group 1 for ground cover and B is in Group 2; both biotypes are in Group 2 for plant height.

For cluster B, biotypes G and I have similarity coefficients of 0.66 and G, I, and M have a coefficient of 0.63 (Figure 2.1). Biotypes G and I are in Group 2 for ground cover and G, I, and M are in either Group 3 or 4 for plant height (Table 2.4). Biotypes D and F with a similarity coefficient of 0.62 are both found in Group 2 for groundcover and Group 3 for plant height. Biotypes G, H, I, and M have a similarity coefficient of 0.61 and all are in Group 2 for ground cover; biotype H is in Group 4 for plant height. Of interest is that biotype Q in cluster A and biotype R in cluster B with similarity coefficient of only 0.44 are two of the most aggressive in respect to groundcover development and plant height.

In the sugarcane growing area of Louisiana, observations suggest that bermudagrass can vary in its ability to establish as well as growth characteristics and aggressiveness in competing with sugarcane. Biotypes that were most aggressive include A collected in St. Martinville, Q collected in Port Allen, and R collected at the LSU AgCenter Sugar Research Station in St. Gabriel. These biotypes established very rapidly and were tall-growing, with long internodes and

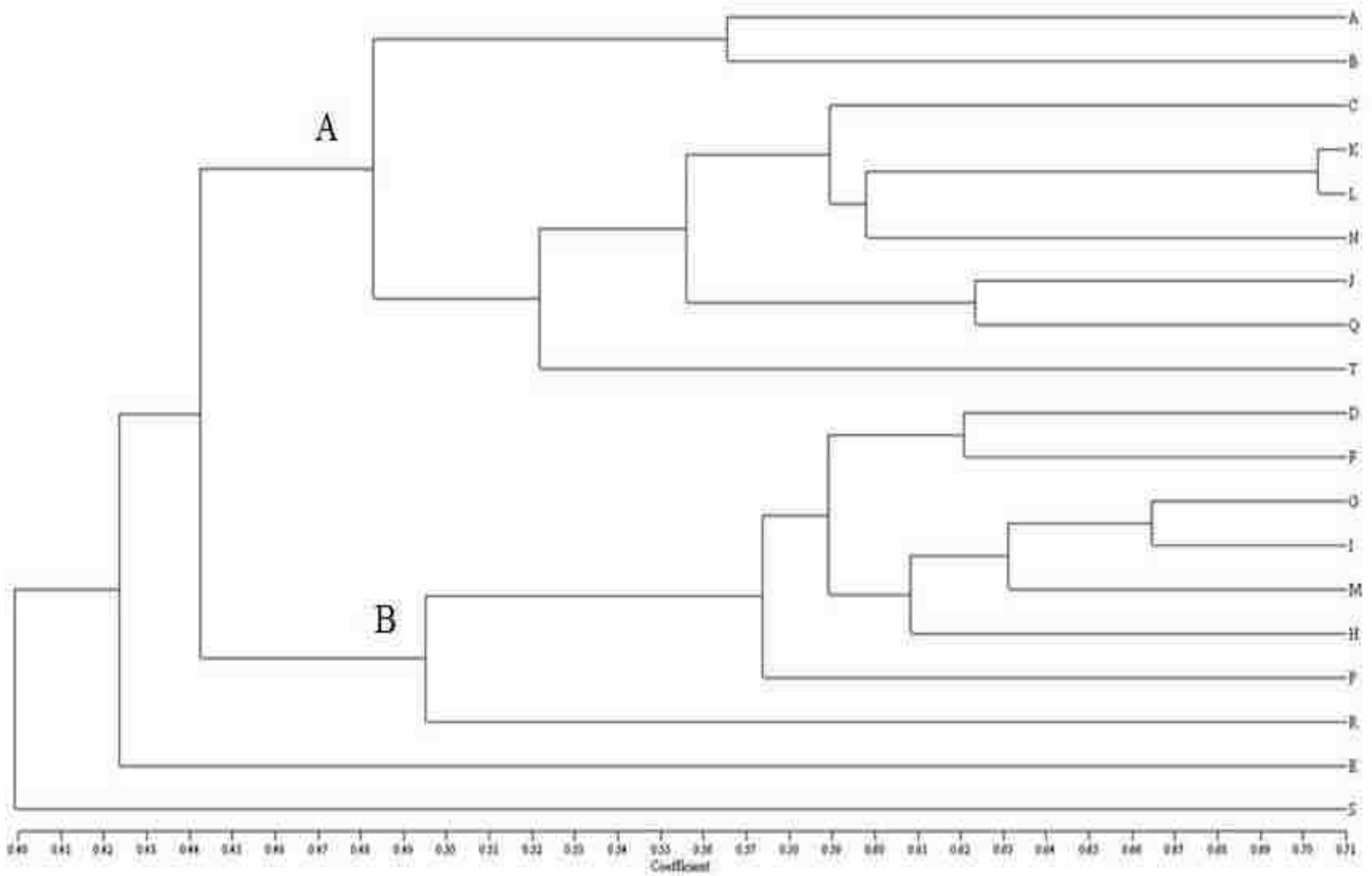


Figure 2.1. Bermudagrass Biotype Genetic Similarity Dendrogram

wide leaves. Biotypes A and Q were also able to retain green foliage later into the winter and to initiate growth in the spring earlier than some of the other biotypes.

There were also biotypes of bermudagrass that were very slow to establish and were short growing. These biotypes, considered less aggressive, were J collected in Samuels, N collected in New Iberia, and T collected at the LSU AgCenter, Northeast Research Station in St. Joseph. Based on groundcover, these three biotypes were an average of 5.3 times slower to establish compared with biotypes A, Q, and R. For biotypes J, N, and T, plant height was an average of 61% less than for biotypes A, Q, and R. In the first year of the study, bermudagrass dry weight was an average of 7.8 times greater for biotypes A, Q, and R compared with biotypes J, N, and T. Although differences were observed between biotypes A and Q and among biotypes J, N, and T in their ability to establish, morphology, and cold tolerance, all of the biotypes were clustered in the same group based on Jaccard's similarity coefficient. Variation in growth of bermudagrass throughout the sugarcane growing area may be the result of genetic mutations and the transfer and propagation of strains which were developed for specific uses such as pastures. Results from this research help to illustrate differences observed in bermudagrass present in sugarcane in Louisiana and suggest that growers consider adjusting control programs in fields where bermudagrass has been more aggressive and competitive with sugarcane.

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CHAPTER 3: BERMUDAGRASS BIOTYPE RESPONSE TO GLYPHOSATE

INTRODUCTION

Sugarcane (*Saccharum* spp. hybrids) is grown in Louisiana, Florida, and Texas in the continental U.S. Approximately 40% of U.S. sugar obtained from sugarcane is produced in Louisiana, accounting for 17.4% of total U.S. sugar production (Salassi et al. 2011). In 2011, 489 producers grew sugarcane on 165,000 hectares in 23 parishes (Anonymous 2011, Salassi et al. 2011). Average sugarcane yield from total acres amounted to 70.1 Mg ha⁻¹ with approximately 8,100 kg of sugar produced per harvested hectare. Sugarcane leads Louisiana's agricultural row crops in total crop market value. In 2011, the sugarcane industry supplied Louisiana's economy with 2.5 to 3.0 billion dollars.

Bermudagrass (*Cynodon dactylon* L. Pers.) is a major weed problem in Louisiana sugarcane fields. The perennial nature of sugarcane combined with wide row spacing provides a favorable environment for bermudagrass (Holm et al. 1977). Sugarcane in Louisiana is planted during August and September. Producers generally harvest three to four crops before stubble is destroyed and fields are fallowed in preparation for planting (Etheredge et al. 2009). Bermudagrass cannot be completely controlled in sugarcane with either preemergence or postemergence herbicides (Anonymous 2013). Bermudagrass infestation, however, can be reduced when metribuzin or terbacil are applied prior to weed emergence in the spring (Richard 1993).

The fallow period is the ideal time to reduce bermudagrass infestation (Etheredge et al. 2009; Miller et al. 1999). Richard (1997) conducted studies to compare the effectiveness of several combinations of cultural and herbicide programs to determine the best methods for bermudagrass control in fallowed sugarcane fields. Results showed that sugar yield in the plant-cane crop and first-ratoon crop were increased 33 and 71%, respectively when bermudagrass was

controlled in fallow with only herbicide programs. In contrast, sugar yield was increased 11 to 20% when only a tillage program was used in fallow. Results suggest that the combination of both tillage and herbicide would be most effective. Glyphosate at 2.1 to 3.4 kg ae/ha is recommended for control of bermudagrass in fallowed sugarcane fields (Anonymous 2013; Etheredge et al. 2009). Glyphosate is a foliar applied, non-selective herbicide that inhibits the shikimate acid pathway by disrupting 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (Vencill 2002). The interruption of this pathway prevents formation of the amino acids tryptophan, tyrosine and phenylalanine thereby suppressing syntheses of essential proteins in the target plant.

Bermudagrass thrives in well-drained, fertile soil (Heath et al. 1985). This vigorous grass may be spread by seed, stolons, and rhizomes (Rocheouste 1962). Propagation of bermudagrass most commonly occurs through the transport of stolons and rhizomes. *Cynodon dactylon*, a tetraploid, is more fertile than the diploid species *Cynodon transvaalensis* (Duble 2010). The genus *Cynodon* has the ability to cross-pollinate and is highly self-incompatible, therefore, out-crossing is common (Burton 1947). Sexual reproduction of a species leads to the transfer of genes and the adaptation of a species to a particular environment.

In Louisiana sugarcane fields, bermudagrass plant height, growth rate, leaf width, and internode length can vary considerably. Plant populations that vary phenotypically are considered to be biotypes. Biotypes are defined by the Weed Science Society of America (WSSA) as “a population within a species with distinct genetic variation” (Vencill 2002). It is possible that bermudagrass biotypes exist that are more competitive with sugarcane and that are less susceptible to herbicides. Growers have reported variability in bermudagrass control with glyphosate. Bryson and Wills (1985) reported variable response to glyphosate for 17 biotypes of

bermudagrass collected cotton fields and tree nurseries in Mississippi, Arkansas, Louisiana, and Tennessee.

The objective of this study was to evaluate control and regrowth of 19 bermudagrass biotypes collected in Louisiana treated with glyphosate during the initial establishment period and when bermudagrass was well established.

MATERIALS AND METHODS

In August and September 2010, 20 bermudagrass biotypes were collected at 12 outfield locations (5 heavy and 7 light soils) used by the Louisiana sugarcane breeding programs (LSU AgCenter Sugar Research Station, USDA Agricultural Research Service, and the American Sugarcane League); five from sugarcane farms in Louisiana, and three from LSU AgCenter Research Stations (Sugar Research Station, Dean Lee Research and Extension Center, and Northeast Research Station) (Table 3.1). Whole plants were collected and planted in 11.4 L pots and were used as “mother plants” for later propagation. Because of the inability to establish and overall lack of vigor, biotype O collected in Jeanerette, LA was omitted.

Greenhouse Study. On March 28, 2011 and April 13, 2012, two to three inch sections of bermudagrass stolons from “mother plants” were planted into 10 cm pots containing a 2:1 river silt and Jiffy Mix Plus¹ mixture in the greenhouse and were watered and fertilized weekly with a Miracle-Gro² water-soluble 24-8-16 fertilizer solution. The greenhouse was maintained at 29 C +/- 15 C. The experiment was set up as a randomized complete block design with 4 replications.

¹ A sterile soil mix with an optimal blend of sphagnum and vermiculite with MagAmp slow release fertilizer (7-40-6). Jiffy Products of America, Inc., 600 Industrial Parkway, Norwalk, OH 44857.

² An all purpose water soluble fertilizer. Scotts Miracle-Gro Products, Inc. 14111 Scottslawn Road, Marysville, OH 43041.

Table 3.1. Bermudagrass biotypes collected in Louisiana and evaluated for control with glyphosate in greenhouse and field studies.^a

Biotype	Grower	Farm	Location	Parish
Outfield sites ^b				
A	Lawrence Levert	St. John	St. Martinville	St. Martin
B	Ronald Hebert	Ronald Hebert	Jeanerette	Iberia
C	Brett Allain	Allain	Baldwin	St. Mary
D	Wilson Judice	Frank Martin	Centerville/Calumet	St. Mary
E	Pete Lanaux	Lanaux	Lucy	St. John the Baptist
F	Brian Graugnard	Bon Secour	Vacherie	St. James
G	Joel Landry	Glenwood	Napoleonville	Assumption
H	Howard Robichaux	Mary	Raceland	Lafourche
I	Danny Naquin	Magnolia	Schriever	Terrebonne
J	Joe Beard III	Brunswick	Samuels	Point Coupee
K	Todd Andre	Alma	Allon	Point Coupee
L	Al Landry	Landry Farm	Plaquemine	Iberville
Off-Station nursery site ^b				
M	Blake Newton	Bunkie	Bunkie	Avoyelles
Other sites ^b				
N	Ronnie Gonsoulin	Airport Road	New Iberia	Iberia
O	Ronald Hebert	Bayside	Jeanerette	Iberia
P	Mike Cremaldi	Cremaldi Farms	Patterson	St. Mary
Q	Kerny Gros	Barrowza Plantation	Port Allen	West Baton Rouge
R	LSU AgCenter	Sugar Research Station	St. Gabriel	Iberville
S	LSU AgCenter	Dean Lee Research Stn	Alexandria	Rapides
T	LSU Agcenter	Northeast Res. Station	St. Joseph	Tensas

^a Biotype O was not evaluated because of the inability to establish.

^b Outfield sites are locations where sugarcane cultivar trials are conducted by the LSU AgCenter. The off-station nursery is used for sugarcane cultivar seed increases. Other sites included sugarcane farms where bermudagrass control concerns have been expressed. Collections were also made at three LSU AgCenter Research Stations where crops other than sugarcane are grown.

On May 23, 2011 and June 12, 2012, after bermudagrass had established, bermudagrass biotypes were treated with glyphosate³ at 0.84 and 1.68 kg/ha using a CO₂ back pack sprayer delivering 140 liters of water per hectare with spray pressure of 193 kPa. A nontreated was included for comparison. At application, bermudagrass longest stolon length ranged from 13 cm for biotype H, K, and P to 40 cm for biotype A in 2011 and from 9 cm for biotype N to 35 cm for biotype S in 2012 (Table 3.2). Bermudagrass percent control was evaluated at 7, 14, and 28 days

³ Roundup WeatherMAX, a potassium salt of glyphosate plus surfactant. Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, MO 63167.

after treatment (DAT) based on a scale of 0 to 100% with 0 = no control and 100 = plant death.

At 28 DAT, bermudagrass above ground biomass was harvested at soil level and dried at 60 C for 3 days. Twenty-eight days after collecting biomass, bermudagrass regrowth was rated using a scale of 0 to 5 with 0 = no regrowth and 5 = regrowth equal to the nontreated.

Field Study. On March 28, 2011, two to three inch sections of bermudagrass stolons from the original “mother plants” were planted into 5 cm pots containing a 2:1 river silt and Jiffy Mix Plus mixture in the greenhouse and were watered and fertilized weekly with a Miracle-Gro water-soluble 24-8-16 fertilizer solution. The greenhouse was maintained at 29 C +/- 15 C. On May 23, 2011, plants were transplanted into the field on a Cancienne Silt Loam soil at the Central Research Station, Ben Hur Research Farm, Baton Rouge, LA. The experimental design was a randomized complete block with three replications. Plot size was 1.5 x 1.5 meters. Two plants were planted in the center of each plot 0.6 meters from one another. Plots were irrigated as needed to promote bermudagrass establishment. Nitrogen fertilizer was applied on July 1, 2011 using ammonium nitrate⁴ (34-0-0) at a rate of 46 kg N/ha (Twidwell 2009). Alleys between plots were 1.5 meters wide and were sprayed with glyphosate using a hooded sprayer as needed to prevent bermudagrass encroachment from adjoining plots. On August 11, 2011 (79 days after planting in the field), bermudagrass biotypes were treated with 2.24 kg/ha glyphosate using a CO₂ back pack sprayer at 140 liters of water per hectare with spray pressure of 193 kPa. Bermudagrass height ranged from 7 cm for biotype T to 30 cm for biotype A (Table 3.2). Bermudagrass biotype percent control was determined at 7, 14, and 28 DAT based on the scale previously described. After the final rating, bermudagrass top growth was removed and regrowth 28 days later was not observed for any of the biotypes. The following year, on April 27, 2012, bermudagrass top growth from plots that had not been treated the previous year was removed.

⁴ Red Fox Fertilizer. 356 E. Inez Road. Dothan, AL 36301.

Fertilizer was not applied in 2012. Alleys between plots were treated as needed as described for the previous year. On June 26, 2012, bermudagrass biotypes ranging in height from 12 cm for biotype I to 35 cm for biotype S (Table 3.2) and well established were treated with 2.24 kg/ha of glyphosate as described previously. Bermudagrass control was determined 7, 14, and 28 DAT as described previously. Bermudagrass top growth was removed and regrowth of bermudagrass was determined 28 days later based on ground cover of each plot where 0 = no regrowth and 100% = total ground cover. Because of the greater sensitivity of bermudagrass in the first year (establishment year) compared with the following year, data were analyzed separately for the two years.

Table 3.2. Bermudagrass biotype stolon length at time of glyphosate application in the greenhouse study and bermudagrass average height at time of glyphosate application in the field study.

Biotypes ^c	Greenhouse study ^a		Field study ^b	
	Stolon length (cm)		Plant height (cm)	
	May 23, 2011	June 12, 2012	August 11, 2011	June 26, 2012
A	40/3 ^d	23/1 ^d	30	22
B	21/2	16/2	14	23
C	17/3	21/2	15	14
D	24/6	13/1	11	16
E	17/5	13/2	19	28
F	21/5	19/2	12	13
G	14/4	16/1	10	19
H	13/4	15/2	17	16
I	17/4	14/1	10	12
J	19/2	23/1	17	17
K	13/2	15/1	14	20
L	25/3	27/1	16	14
M	16/1	18/1	9	17
N	14/4	9/1	9	18
P	13/1	25/1	16	12
Q	20/5	11/1	25	25
R	24/4	14/1	26	19
S	39/5	35/2	26	35
T	17/5	24/1	7	15

^a Glyphosate applied 56 days after planting (DAP) of bermudagrass in 2011 and 60 DAP in 2012.

^b Glyphosate applied 80 DAP of bermudagrass in 2011 and 400 DAP in 2012.

^c See Table 3.1 for information on bermudagrass biotypes.

^d Because multiple stolons were present in each pot, length of longest/shortest stolon is provided.

For each of the parameters measured for both the greenhouse and field studies, means for the 19 biotypes were plotted and separated into groups based on similarity in response to glyphosate treatments. Therefore, data are presented both for individual biotypes and for groups containing several biotypes based on similarity in response. Data were subjected to the Proc Mixed Procedure in SAS (SAS Institute 2012) with experiments (where appropriate) and replications considered random effects. Least square means were calculated and mean separation was performed at $P \leq 0.05$. Letter groupings were converted using the PDMIX800 macro in SAS (Saxton 1998).

RESULTS AND DISCUSSION

Greenhouse Study. For bermudagrass control 7, 14, and 28 DAT, bermudagrass biotypes were separated into three groups based on similarity in response to glyphosate. Averaged across glyphosate rates of 0.84 and 1.68 kg/ha, bermudagrass control 28 DAT was 73% and greatest for the biotypes in Group 3 (D, F, I, M, and P) (Table 3.3). Control 28 DAT for biotypes in Group 2 (B, G, H, J, and T) and for biotypes in Group 1 (A, C, E, K, L, N, Q, R, and S) averaged 61% and 57%, respectively, and control was equivalent to that for biotypes in Group 3 14 DAT (60%). Bermudagrass control averaged across glyphosate rates 7 DAT was 32% and lowest for the Group 1 biotypes. Bermudagrass was controlled 7 DAT 38% for biotypes in Group 2 and 46% for biotypes in Group 3; for biotypes in Group 1 14 DAT bermudagrass was controlled 45%. Averaged across bermudagrass biotype groups and glyphosate rates, control was 39% 7 DAT and increased to 64% 28 DAT.

For bermudagrass dry weight 28 days after glyphosate application, biomass regardless of glyphosate rate (0, 0.84, and 1.68 kg/ha) was greatest for the Group 1 biotypes (A, C, L, and S) and lowest for the Group 3 biotypes (D, G, H, M, N, and P) (Table 3.4). Averaged across biotype groups, dry weight was 2.4 g/plant when glyphosate was not applied and was decreased 21% for

glyphosate at 0.84 kg/ha and 42% at 1.68 kg/ha. Regardless of glyphosate rate, bermudagrass regrowth, as also noted for dry weight, was greatest for the Group 1 biotypes (A, C, E, N, and Q) and lowest for the Group 3 biotypes (D, G, L, P, and R) (Table 3.4). For glyphosate at 1.68 kg/ha, very little regrowth was observed for the Group 2 biotypes (B, F, H, I, J, K, M, S, and T) (rating of 1.0) and for the Group 3 biotypes (rating of 0.2). Regrowth averaged across biotype groups was 4.5 where glyphosate was not applied and regrowth was reduced to an average of 3.6 for glyphosate at 0.84 kg/ha and to 1.0 for 1.68 kg/ha.

Table 3.3. Control of bermudagrass biotypes 7, 14, and 28 days after treatment (DAT) with glyphosate at 0.84 and 1.68 kg/ha in the greenhouse study.^a

Biotype group ^b	7 DAT			14 DAT			28 DAT		
	0.84 kg/ha	1.68 kg/ha	Group avg.	0.84 kg/ha	1.68 kg/ha	Group avg.	0.84 kg/ha	1.68 kg/ha	Group avg.
1	27	37	32 f ^c	35	55	45 d	48	65	57 bc
2	32	45	38 e	47	61	54 c	54	68	61 b
3	34	59	46 d	48	72	60 bc	60	87	73 a
DAT avg.	— 39 c ^d —			— 53 b —			— 64 a —		

^a Two to three inch stolon sections of each biotype were planted into 10 cm pots on March 28, 2011 and April 13, 2012. Glyphosate was applied when bermudagrass stolon length ranged from 13 to 40 cm in 2011 and 9 to 35 cm in 2012 (see Table 3.2). Bermudagrass control based on a scale of 0 to 100% with 0 = no control and 100% = plant death.

^b Grouping of biotypes was based on similarity in response to glyphosate. For dry weight Group 1 was represented by biotypes A, C, E, K, L, N, Q, R, and S; Group 2 included biotypes B, G, H, J, and T; Group 3 included biotypes D, F, I, M, and P (see Table 3.1 for information on bermudagrass biotypes and Table 3.5 for individual biotype response to glyphosate averaged across glyphosate rates and DAT).

^c Biotype group means averaged across glyphosate rates for the rating dates followed by the same letter are not significantly different ($P \leq 0.05$).

^d DAT means averaged across biotype groups and glyphosate rates for 7, 14, and 28 DAT followed by the same letter are not significantly different ($P \leq 0.05$).

In Table 3.5 bermudagrass control, dry weight, and regrowth rating data are presented for the 19 biotypes arranged by group. Control values are averaged across rating dates (7, 14, and 28 DAT) and glyphosate rates of 0.84 and 1.68 kg/ha. For the Group 1 biotypes, bermudagrass control was lowest and ranged from 41% for biotype L to 47% for biotype S. In Group 2, control ranged from 49% for biotype J to 53% for biotypes B, G, and T. Bermudagrass control in Group

3 ranged from 57% for biotypes F and I to 64% for biotype P. Averaged across biotypes within each group bermudagrass was controlled 45% for Group 1 and 60% for Group 3.

Table 3.4. Dry biomass and regrowth of bermudagrass biotypes following glyphosate applied at 0, 0.84, and 1.68 kg/ha in the greenhouse study.^a

Biotype group ^b	Dry weight (g/plant)			Regrowth rating		
	None	0.84 kg/ha	1.68 kg/ha	None	0.84 kg/ha	1.68 kg/ha
1	3.4	2.5	1.9	4.9	4.4	1.9
2	2.1	1.9	1.3	4.8	3.8	1.0
3	1.6	1.2	1.0	3.8	2.7	0.2
Rate avg.	2.4 a ^c	1.9 b	1.4 c	4.5 a ^c	3.6 b	1.0 c

^a Two to three inch stolon sections of each biotype were planted into 10 cm pots on March 28, 2011 and April 13, 2012. Glyphosate was applied when bermudagrass stolon length ranged from 13 to 40 cm in 2011 and 9 to 35 cm in 2012 (see Table 3.2). Dry weight for bermudagrass measured 28 days after glyphosate application. Regrowth ratings made 28 days after dry weight harvest were based on a scale of 0 to 5 with 0 = no regrowth and 5 = regrowth equal to the nontreated.

^b Grouping of biotypes was based on similarity in response to glyphosate. For dry weight Group 1 was represented by biotypes A, C, L, and S; Group 2 included biotypes B, E, F, I, J, K, Q, R, and T; Group 3 included biotypes D, G, H, M, N, and P. For regrowth ratings Group 1 was represented by biotypes A, C, E, N, and Q; Group 2 included biotypes B, F, H, I, J, K, M, S, and T; Group 3 included biotypes D, G, L, P, and R (see Table 3.1 for information on bermudagrass biotypes and Table 3.5 for individual biotype response to glyphosate averaged across glyphosate rates).

^c Glyphosate rate means averaged across biotype groups for each parameter followed by the same letter are not significantly different ($P \leq 0.05$).

Bermudagrass dry weight per plant averaged across glyphosate rates of 0, 0.84, and 1.68 kg/ha was greatest for Group 1 biotypes and ranged from 2.4 g for biotype L to 2.9 g for biotype C (Table 3.5). For the Group 2 biotypes, dry weight per plant ranged from 1.6 g for biotype B, I, and T to 2.1 g for biotype Q. Dry weight in Group 3 ranged from 1.1 g for biotype G to 1.4 g for biotype D. Averaged across biotypes in Group 1, dry weight was 2.6 g and was 1.4 times that of the average for biotypes in Group 2 and twice that for biotypes in Group 3. Dry weight averaged 38% greater for the Group 2 biotypes compared with the Group 3 biotypes. Regrowth ratings (0 to 5 scale) averaged across glyphosate rates of 0, 0.84, and 1.68 kg/ha ranged from 3.4 for biotype N to 4.3 for biotype C in Group 1, from 2.9 for biotype H to 3.3 for biotypes I and S in Group 2, and from 1.7 for biotype D to 2.6 for biotypes G and R in Group 3 (Table 3.5).

Bermudagrass regrowth averaged across biotypes in Group 1 was 3.7 and decreased to an average of 3.2 for Group 2 and 2.2 for Group 3.

Table 3.5. Control of bermudagrass and dry biomass and regrowth for 19 biotypes presented individually and by groups in the greenhouse study.^a

Bermudagrass biotype-group	Control (%) ^b	Bermudagrass biotype/group	Dry weight (g/plant) ^c	Bermudagrass biotype/group	Regrowth rating ^c
A - Group 1	45 defg ^d	A - Group 1	2.6 ab	A - Group 1	3.8 ab
C - Group 1	44 fg	C - Group 1	2.9 a	C - Group 1	4.3 a
E - Group 1	45 defg	L - Group 1	2.4 abc	E - Group 1	3.5 ab
K - Group 1	45 efg	S - Group 1	2.5 ab	N - Group 1	3.4 ab
L - Group 1	41 g	Group 1 avg.	2.6 A	Q - Group 1	3.6 ab
N - Group 1	44 fg			Group 1 avg.	3.7 A
Q - Group 1	46 defg	B - Group 2	1.6 bcd		
R - Group 1	46 defg	E - Group 2	2.0 abcd	B - Group 2	3.1 abc
S - Group 1	47 c-g	F - Group 2	1.8 bcd	F - Group 2	3.1 abcd
Group 1 avg.	45 C ^e	I - Group 2	1.6 bcd	H - Group 2	2.9 bcd
		J - Group 2	1.7 bcd	I - Group 2	3.3 ab
B - Group 2	53 a-g	K - Group 2	1.9 abcd	J - Group 2	3.1 abcd
G - Group 2	53 a-f	Q - Group 2	2.1 abcd	K - Group 2	3.2 ab
H - Group 2	51 b-g	R - Group 2	1.9 abcd	M - Group 2	3.1 abc
J - Group 2	49 b-g	T - Group 2	1.6 bcd	S - Group 2	3.3 ab
T - Group 2	53 a-g	Group 2 avg.	1.8 B	T - Group 2	3.2 ab
Group 2 avg.	51 B			Group 2 avg.	3.2 B
		D - Group 3	1.4 cd		
D - Group 3	59 abc	G - Group 3	1.1 d	D - Group 3	1.7 d
F - Group 3	57 abcd	H - Group 3	1.2 d	G - Group 3	2.6 bcd
I - Group 3	57 a-e	M - Group 3	1.3 d	L - Group 3	2.5 bcd
M - Group 3	61 ab	N - Group 3	1.2 d	P - Group 3	1.8 cd
P - Group 3	64 a	P - Group 3	1.3 d	R - Group 3	2.6 bcd
Group 3 avg.	60 A	Group 3 avg.	1.3 C	Group 3 avg.	2.2 C

^a See Table 3.1 for information on bermudagrass biotypes. Two to three inch stolon sections of each biotype were planted into 10 cm pots on March 28, 2011 and April 13, 2012. Glyphosate was applied when bermudagrass stolon length ranged from 13 to 40 cm in 2011 and 9 to 35 cm in 2012 (see Table 3.2). Grouping of biotypes was based on similarity in response to glyphosate for each growth parameter.

^b Control based on a scale of 0 to 100% with 0 = no control and 100% = plant death. Control data averaged across ratings made 7, 14, and 28 days after treatment and glyphosate rates of 0.84 and 1.68 kg/ha.

^c Dry weight for bermudagrass measured 28 days after glyphosate application and averaged across glyphosate rates of 0, 0.84, and 1.68 kg/ha. Regrowth ratings made 28 days after dry weight harvest and averaged across glyphosate rates of 0, 0.84, and 1.68 kg/ha were based on a scale of 0 to 5 with 0 = no regrowth and 5 = regrowth equal to the nontreated.

^d Biotype means within each column followed by the same lower case letter are not significantly different ($P \leq 0.05$).

^e Group means (averaged across biotypes) within each column followed by the same upper case letter are not significantly different ($P \leq 0.05$).

For the greenhouse study based on visual ratings and dry weight biomass 28 DAT, and on regrowth rating (Tables 3.3 and 3.4), none of the bermudagrass biotypes were completely controlled with glyphosate at the highest rate of 1.68 kg/ha. However, variability in response to glyphosate was observed among bermudagrass biotypes. Bermudagrass biotypes in Group 1 least sensitive to glyphosate based on lowest average control for 7, 14, and 28 DAT, greatest dry weight, and greatest regrowth would include A and C (Table 3.5). The Group 1, biotypes E, N, and Q would also be considered less sensitive to glyphosate for control and regrowth. Biotypes K and S were included in Group 1 for control, but were in Group 2 for regrowth. Because regrowth was statistically equivalent to that of the Group 1 biotypes, biotypes K and S would also be considered less sensitive to glyphosate. Biotypes L and R were also included in Group 1 for control, but were included in Group 3 for regrowth. Therefore, based on bermudagrass control and regrowth, biotypes A (St. Martinville), C (Baldwin), E (Lucy), K (Allon), N (New Iberia), Q (Port Allen), and S (Alexandria) were least sensitive to glyphosate (average control of 44 to 47% and regrowth of 3.2 to 4.3 with 0= no regrowth and 5= regrowth equal to nontreated).

Bermudagrass biotypes most sensitive to glyphosate (Group 3 biotypes) would include D and P based on greatest average control and lowest dry weight and regrowth; M based on control and dry weight; and G based on dry weight and regrowth. Biotypes F and I were also included in Group 3 for control but were assigned to Group 2 for dry weight and regrowth. Biotype H was included in Group 3 for dry weight but was assigned to Group 2 for control and regrowth. Biotype L assigned to Group 3 for regrowth was included in Group 1 for control and dry weight. Therefore, based on bermudagrass control and regrowth, biotypes D (Centerville) and P (Patterson) were most sensitive to glyphosate (average control of 59 and 64% and regrowth of 1.7 and 1.8 with 0= no regrowth and 5= regrowth equal to nontreated).

Field Study. Grouping of the biotypes was based on similarity in response observed for control in 2011 and the same groupings were also used in 2012. In 2011, control with glyphosate at 2.24 kg/ha for bermudagrass planted 79 days earlier was lowest for the Group 1 biotypes (A, E, N, R, and S) compared with the Group 3 biotypes (B, F, G, H, I, L, P, and T); 49 versus 89%, respectively, at 7 DAT and 86 versus 98%, respectively, at 14 DAT (Table 3.6). At 14 DAT control of Group 2 biotypes (C, D, J, K, M, and Q) was 95% and equivalent to that for the Group 3 biotypes. At 28 DAT, however, bermudagrass control was equivalent for the three biotype groups (99 to 100%). In 2012 when bermudagrass was well established, control with glyphosate at 2.24 kg/ha was much less than observed the previous year. At 7 DAT bermudagrass was controlled no more than 18% for the biotype groups (Table 3.6). Bermudagrass control 14 DAT was 19% for the Group 1 biotypes and increased to 30% for the Group 3 biotypes. Control of the biotype groups was equivalent 28 DAT and ranged from 41 to 52%.

Table 3.6. Control of bermudagrass biotypes 7, 14, and 28 days after treatment (DAT) with glyphosate at 2.24 kg/ha in the field study in 2011 and 2012.^a

Biotype group ^b	2011 Bermudagrass control (%) ^c			2012 Bermudagrass control (%) ^c		
	7 DAT	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT
1	49 f ^d	86 d	99 a	11 f	19 d	41 ab
2	65 e	95 bc	100 a	14 e	24 cd	44 a
3	89 cd	98 ab	100 a	18 d	30 bc	52 a

^a Two established bermudagrass plants for each biotype were planted in the center of each 1.5 x 1.5 meter plot 0.6 meters from one another on May 23, 2011. Glyphosate was applied on August 11, 2011 when height ranged from 7 to 30 cm (see Table 3.2). In 2012 glyphosate was applied on June 26 to bermudagrass that had not been treated the previous year and height ranged from 12 to 35 cm (see Table 3.2).

^b Grouping of biotypes was based on similarity in response to glyphosate. Group 1 was represented by biotypes A, E, N, R, and S; Group 2 included biotypes C, D, J, K, M, and Q; Group 3 included biotypes B, F, G, H, I, L, P, and T (see Table 3.1 for information on bermudagrass biotypes and Table 3.7 for individual biotype response to glyphosate averaged across 7, 14, and 28 DAT).

^c Control based on a scale of 0 to 100% with 0 = no control and 100% = plant death.

^d Biotype group means within each year for the 7, 14, and 28 DAT ratings followed by the same letter are not significantly different ($P \leq 0.05$).

Table 3.7. Control of bermudagrass and regrowth based on ground cover for 19 biotypes presented individually and by groups in the field study.^a

Bermudagrass biotype - group	2011 Control (%) ^b	2012 Control (%)	Bermudagrass biotype - group	2012 Regrowth (%) ^b
A - Group 1	79 def ^c	17 e	A - Group 1	66 ab ^b
E - Group 1	82 cdef	28 abcde	C - Group 1	86 a
N - Group 1	82 cdef	22 de	J - Group 1	78 a
R - Group 1	71 f	24 de	Q - Group 1	79 a
S - Group 1	77 ef	34 abc	S - Group 1	79 a
Group 1 avg	78 C ^d	24 C	T - Group 1	85 a
			Group 1 avg	79 A
C - Group 2	84 bcdf	17 e		
D - Group 2	88 abcde	38 a	B - Group 2	52 abc
J - Group 2	85 abcde	23 de	E - Group 2	30 abc
K - Group 2	88 abcde	28 abcde	G - Group 2	43 abc
M - Group 2	89 abcde	37 ab	H - Group 2	48 abc
Q - Group 2	85 abcdef	25 cde	I - Group 2	37 abc
Group 2 avg	86 B	27 B	K - Group 2	38 abc
			N - Group 2	46 abc
B - Group 3	93 abc	36 abc	Group 2 avg	42 B
F - Group 3	99 a	38 a		
G - Group 3	98 a	26 bcde	D - Group 3	2 e
H - Group 3	95 abc	31 abcd	F - Group 3	4 de
I - Group 3	96 abc	31 abcd	L - Group 3	19 bcd
L - Group 3	93 abcd	25 de	M - Group 3	18 bcd
P - Group 3	97 ab	38 a	P - Group 3	5 de
T - Group 3	95 abc	19 e	R - Group 3	15 cd
Group 3 avg.	96 A	34 A	Group 3 avg.	11 C

^a See Table 3.1 for information on bermudagrass biotypes. Two established bermudagrass plants for each biotype were planted in the center of each 1.5 x 1.5 meter plot 0.6 meters from one another on May 23, 2011. Glyphosate was applied on August 11, 2011 when height ranged from 7 to 30 cm (see Table 3.2). In 2012 glyphosate was applied on June 26 to bermudagrass that had not been treated the previous year and height ranged from 12 to 35 cm (see Table 3.2). Grouping of biotypes was based on similarity in response to glyphosate.

^b Control based on a scale of 0 to 100% with 0 = no control and 100% = plant death. Control data averaged across rating made 7, 14, and 28 days after treatment for glyphosate at 2.24 kg/ha. Grouping of biotypes was based on similarity in response observed for control in 2011 and the same grouping was also used for 2012. At the 28 day rating bermudagrass biomass was removed and 28 days later regrowth ratings were made based on groundcover of plots where 0 = no regrowth and 100% = total ground cover. Regrowth was not observed for any of the biotypes in 2011 but differences were observed in 2012.

^c Biotype means within each column followed by the same lower case letter are not significantly different ($P \leq 0.05$).

Bermudagrass control with glyphosate averaged across ratings made at 7, 14, and 28 DAT for the biotypes ranged from 71% for biotype R to 99% for biotype F in 2011 and from 17% for biotypes A and C to 38% for biotypes D, F, and P in 2012 (Table 3.7). For the Group 1

biotypes in 2011, average control ranged from 71% for biotype R to 82% for biotypes E and N with a Group average of 78%. In 2012, control of the Group 1 biotypes averaged 24% and ranged from 17% for biotype A to 34% for biotype S. For the Group 2 biotypes, bermudagrass control was lowest for biotype C in 2011 (84%) and in 2012 (17%) and was greatest for biotype M in 2011 (89%) and for biotype D in 2012 (38%). Averaged across biotypes, control in 2011 was 86% and in 2012 was 27%. In both years, average control of the Group 2 biotypes was greater than for the Group 1 biotypes. For the Group 3 biotypes, bermudagrass control was lowest for biotypes B and L (93%) in 2011 and for biotype T (19%) in 2012. For both years, bermudagrass control for the Group 3 biotypes was greatest for biotype F; 99% control in 2011 and for biotypes F and P; 38% control in 2012. Averaged across biotypes for Group 3, bermudagrass control was 96% in 2011 and 34% in 2012 and for both years control was greater than for Groups 1 and 2.

An estimate of bermudagrass regrowth was made in the field study based on percent ground cover within each plot 28 days following removal of bermudagrass foliage after the final control rating. In 2011, regrowth was not observed for any of the biotypes indicating that the glyphosate rate of 2.24 kg/ha was sufficient to provide complete control that year (data not shown). However in 2012 when bermudagrass was well established and control was no more than 38%, differences among the biotypes in susceptibility to glyphosate were assessed based on regrowth (Table 3.7). For the biotypes in Group 1, regrowth ranged from 66% ground cover for biotype A to 86% for biotype C. Regrowth ranged from 30% groundcover for biotype E to 52% for biotype B in Group 2 and from 2% for biotype D to 19% for biotype L in Group 3. Averaged across biotypes, regrowth in Group 1 was 79% and was greater than for Group 2 (42%) and Group 3 (11%).

For the field study in 2011, average control of bermudagrass 28 DAT for the Group 1, 2, and 3 biotypes was 99 to 100% (Table 3.6) and regrowth was not observed. However in 2012 when bermudagrass was more established, average control of the biotypes 28 DAT in Groups 1, 2, and 3 was 41 to 52% (Table 3.6) and differences in regrowth among biotypes and groups were observed (Table 3.7). Even though differences in response were observed between years, some consistencies occurred. In Table 3.7 control data are presented for both years as an average across rating dates (7, 14, and 28 DAT) and regrowth data based on ground cover 28 days after foliage removal 28 DAT. For the field study, bermudagrass biotypes least sensitive to glyphosate based on lowest control for both years and greatest regrowth would include A, C, J, and Q (Table 3.7). Biotype S was considered less sensitive to glyphosate for control the first year and regrowth; Biotype E for control both years but not for regrowth; and Biotype T for regrowth but not for control. Therefore, based on bermudagrass control both years and regrowth, biotypes A (St. Martinville), C (Baldwin), J (Samuels), and Q (Port Allen) were least sensitive to glyphosate (average control of 79 to 85% in 2011 and 17 to 25% in 2012 and regrowth of 66 to 86% groundcover) (Table 3.7). Biotypes most sensitive to glyphosate based on greatest control both years and least regrowth would include D, F, M, and P. Biotype L was most sensitive to glyphosate for control the first year and regrowth; biotypes B, H, and I for control both years but not for regrowth; and biotype R for regrowth but not for control. Therefore, based on bermudagrass control both years and regrowth, biotypes D (Centerville), F (Vacherie), M (Bunkie), and P (Paterson) were most sensitive to glyphosate (average control of 88 to 99% in 2011 and 37 to 38% in 2012 and regrowth of 2 to 18% groundcover) (Table 3.7). Bryson and Wills (1985) reported a range in control of 17 biotypes of bermudagrass with glyphosate at 1.12 kg/ha of 38 to 87% seven weeks after treatment.

For both the greenhouse and field studies some overall conclusions can be made regarding response of bermudagrass biotypes collected in Louisiana to glyphosate. Bermudagrass biotypes least sensitive to glyphosate based on consistency in control and regrowth response following glyphosate application in both greenhouse and field studies would include biotypes A (St. Martinville), C (Baldwin), and Q (Port Allen). Bermudagrass biotypes most sensitive to glyphosate based on consistency in response would include D (Centerville) and P (Patterson). The bermudagrass biotypes evaluated in this study do not represent all of the possible genetic variability in bermudagrass in Louisiana, but are representative of bermudagrass present at sites where sugarcane has been grown for more than 80 years and at two sites where crops other than sugarcane have been grown.

Differences in sensitivity to glyphosate observed in this study may help to explain the variation in bermudagrass control with glyphosate observed in fallowed fields in the sugarcane producing area of Louisiana. Results showed that glyphosate was most effective when applied to bermudagrass during early establishment. In sugarcane fields after several years of production bermudagrass can become well established. It would be critical during the fallowed period that intensive tillage programs that fragment bermudagrass stolons and rhizomes be implemented prior to glyphosate application. Glyphosate should be applied when stolon growth is first initiated and before stolons begin to root and spread. A tillage operation 7 to 10 days following application along with one or more follow-up applications of glyphosate, as needed, should help to improve long-term bermudagrass control.

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CHAPTER 4: BERMUDAGRASS (*CYNODON DACTYLON*) INTERFERENCE WITH SUGARCANE

INTRODUCTION

Sugarcane (*Saccharum* spp. hybrids) is a subtropical perennial crop grown in Louisiana, Florida, and Texas in the continental U.S. Approximately 40% of U.S. sugar is obtained from sugarcane produced in Louisiana, accounting for 17.4% of total U.S. sugar production (Salassi et al. 2011). In Louisiana, sugarcane is planted in August and September and three to four crops are harvested before stubble is destroyed and fields are fallowed in preparation for planting (Baucum and Rice 2011; Etheredge et al. 2009). During the summer fallow period weeds are controlled by cultivation and use of glyphosate herbicide. Once the crop is planted, cultivation is limited to the row sides and middles, which can favor re-establishment of perennial weeds (Richard and Dalley 2007). Perennial weeds can become increasingly problematic in the ratoon crops (Miller et al. 1999; Etheredge et al. 2010).

Weeds most troublesome in Louisiana sugarcane include bermudagrass (*Cynodon dactylon* L. Pers.), johnsongrass (*Sorghum halepense* L. Pers.), morningglory (*Ipomoea* spp.), and itchgrass (*Rottboellia cochinchinensis* Lour. W. Clayton) (Hackett et al. 2011). Gibson and Liebman (2003) define competitiveness as “the relative ability of a plant to obtain a specific resource when in competition with another plant”. Inadequate supply of water, nutrients, and sunlight contribute to competition between weeds and the desired crop (Aldrich and Kremer 1997). The ability of sugarcane to compete with weeds can be dependent on stalk population, canopy development, and ratooning ability (Richard and Dalley 2007). Research suggests that the growth stage of sugarcane most susceptible to weed competition is early in the season when the crop is tillering (Blanco et al. 1984; Fadayomi and Abayomi 1988; Lencse and Griffin 1991; Millhollon 1992; Turner 1985).

Because sugarcane is grown on wide rows and the row top is undisturbed throughout the crop cycle, bermudagrass can establish rapidly (Holm et al. 1977). A dense cover of bermudagrass on top of sugarcane rows can physically suppress sugarcane growth (Richard 1996). Richard (1995) reported that bermudagrass biomass increased 340% between plant cane and first stubble and 490% between first stubble and second stubble. Weed control in sugarcane is dependent on both the competitive ability of the crop and the effectiveness of herbicide treatments. Richard (1993) evaluated several herbicides for their effect on growth of bermudagrass and sugarcane yield. In the plant-cane crop, bermudagrass biomass was 10.7 times greater in plots when fenac was applied compared with metribuzin. Sugarcane yield loss when fenac was applied was 10% in plant cane, 6% in first-ratoon, and 14% in second ratoon crops. Fenac was more injurious to sugarcane than metribuzin and terbacil. When bermudagrass was treated with dalapon, sugarcane stalk population was increased 12% (Richard 1996).

Millhollon (1995) determined that when johnsongrass was allowed to compete with sugarcane all season, yield was reduced an average of 43%. However, if johnsongrass was removed early in the season, sugarcane yield reduction was less severe. Lencse and Griffin (1991) reported that when itchgrass was allowed to compete season long, sugarcane stalk population, yield, and sugar yield were reduced 34, 42, and 43%, respectively. Season long competition of morningglory reduced sugarcane yield 36% (Bhullar et al. 2012). Jones and Griffin (2009) and Millhollon (1988) reported reduction in sugarcane yield of 24 to 39% with season long red morningglory (*Ipomoea coccinia* L.) competition.

Based on the current weed control recommendations in sugarcane in Louisiana, none of the soil-applied preemergence herbicides provide complete control of bermudagrass and postemergence herbicide options are not available (Anonymous 2013). It is imperative, therefore, that bermudagrass be effectively controlled during the fallow period so that infestation at

planting is minimal. It is then anticipated that herbicide applied at planting along with competition from sugarcane will be sufficient to allow sugarcane to establish and for yield to be maximized. Other factors affecting competition would include the rate of germination of sugarcane seed pieces along with the ability of sugarcane to produce root and shoot biomass.

The objectives of this research were to evaluate the competition between sugarcane and bermudagrass when bermudagrass was planted at several densities and to compare the level of competition among several sugarcane cultivars and bermudagrass based on the differences in cultivar growth rate and production of shoot and root biomass.

MATERIALS AND METHODS

Research was conducted in August and September 2011 at LSU AgCenter Sugar Research Station, St. Gabriel, Louisiana, coinciding with normal sugarcane planting time. Two studies were conducted with the first evaluating the competition between a single sugarcane cultivar and bermudagrass at four planting densities (density study) and a second study evaluating competition between six sugarcane cultivars and bermudagrass at two planting densities (cultivar study). Prior to initiation of each experiment, sugarcane and bermudagrass were pre-germinated for ten days by planting single sugarcane eyepieces and single node bermudagrass stem cuttings in trays filled with Jiffy Mix Plus¹ potting soil. This procedure was followed to represent what would happen in the field with planted sugarcane and fragmented stem pieces of bermudagrass present due to tillage of seedbed. This planting procedure helped to assure that sugarcane and bermudagrass were initiating growth at the same time and that each had an equal chance to compete with one another. After germination and emergence, sugarcane was transplanted into the center of a 26.5 L pot (surface area of 0.093 m²) filled with one part

¹ A sterile soil mix with an optimal blend of sphagnum and vermiculite with MagAmp slow release fertilizer (7-40-6). Jiffy Products of America, Inc., 600 Industrial Parkway, Norwalk, OH 44857.

sterilized Commerce silt loam soil (fine-silty, mixed, super-active, nonacid, thermic Fluvaquentic Endoaquepts), one part sterilized sand, and one part Jiffy Mix Plus.

For the density study, 0, 1, 2, or 4 bermudagrass plants were transplanted into each pot and evenly spaced around the transplanted sugarcane seed piece of the cultivar ‘HoCP 96-540’ (Tew et al. 2005). In the cultivar study, 0 or 2 bermudagrass plants were transplanted and evenly spaced in pots containing the cultivars HoCP 96-540, ‘L 97-128’ (Gravois et al. 2008), ‘L 99-226’ (Bischoff et al. 2009), ‘HoCP 00-950’ (Tew et al. 2009), ‘L 01-283’ (Gravois et al. 2010), and ‘L 03-371’ (Gravois et al. 2012). For both studies, experiments were conducted outside with each pot under a drip irrigation watering system that delivered 1.02 cm of water per day. The experimental design for each experiment was a Randomized Complete Block with four replications and experiments were repeated. Because root production was limited by soil volume in the pots, experiments were terminated 56 days after transplanting (DAP) of sugarcane and bermudagrass.

For the density and the cultivar studies, bermudagrass percent coverage of the surface area of pots was determined visually 14, 28, 42, and 56 DAP. In both studies, sugarcane shoot population was determined 56 DAP by counting the number of sugarcane shoots per pot. Sugarcane leaf length measured from the soil surface to tip of the longest leaf and leaf collar height measured from the soil surface to the uppermost visible leaf collar were determined 42 and 56 DAP. Shoot (above ground) biomass of both bermudagrass and sugarcane was harvested 56 DAP. Roots were washed free of soil and separated into bermudagrass and sugarcane components. Biomass samples were dried at 60 C for 48 hours and re-weighed to determine dry weight.

Data for both the density and cultivar studies were subjected to Mixed Procedure in SAS (SAS Institute 2012) with experiments and replications considered random effects. Least square

means were calculated and mean separation was performed at $P \leq 0.05$. Letter groupings were converted using the PDMIX800 macro in SAS (Saxton 1998).

RESULTS AND DISCUSSION

Bermudagrass Density Study. For bermudagrass groundcover, there was no significant effect due to planting density or an interaction between planting density and DAP. This shows that based on groundcover, 1 bermudagrass plant per pot was able to establish as rapidly as 4 plants per pot. Significant increase in growth of bermudagrass, based on percent coverage of the pot surface area, was observed from 14 to 42 DAP (average of 17 to 69%) (Table 4.1). By 56 DAP, bermudagrass coverage averaged across bermudagrass density was 75%.

Table 4.1. Bermudagrass ground cover 14, 28, 42, and 56 days after planting (DAP) at a density of 0, 1, 2, or 4 plants per pot with ‘HoCP 96-540’ sugarcane.^a

Bermudagrass density plants/pot	Bermudagrass ground cover (%)				Density average
	14 DAP	28 DAP	42 DAP	56 DAP	
0	--	--	--	--	--
1	12	48	67	75	50
2	18	51	60	66	49
4	21	65	80	83	62
DAP average ^b	17 c	55 b	69 a	75 a	--

^aBermudagrass and sugarcane were pre-germinated from single node stem cuttings and planted in 26.5 L pots with a surface area of 0.093m³. Ground cover represents the surface area of the pots.

^bDAP means averaged across bermudagrass density followed by the same letter are not significantly different ($P \leq 0.05$).

At 56 DAP, a planting density of 4 bermudagrass plants per pot resulted in bermudagrass shoot and root weight 1.7 and 2.1 times greater, respectively, compared to a planting density of only one bermudagrass plant (Table 4.2). Averaged across bermudagrass planting densities, bermudagrass shoot weight averaged 3.6 times more than root weight. In a similar study conducted to evaluate purple nutsedge competition with sugarcane, Etheredge et al. (2010) reported that purple nutsedge root weight averaged 3.4 times that of shoot weight. The results of this study and the present study illustrate the difference in resource allocation between 2 different

weeds. Etheredge et al. (2010) reported a production of 37.3 tubers per pot 64 days after 1 tuber of nutsedge was planted with ‘LCP 85-384’ sugarcane. The ability of bermudagrass to rapidly produce shoot biomass even at a low plant density in the present study suggests that bermudagrass would be highly competitive with sugarcane at planting.

Table 4.2. Bermudagrass shoot and root weight 56 days after planting at a density of 0, 1, 2, or 4 plants per pot with ‘HoCP 96-540’ sugarcane.^a

Bermudagrass density plants/pot	Bermudagrass shoot weight (g/pot)	Bermudagrass root weight (g/pot)
0	--	--
1	19.1 b ^b	4.4 b
2	22.8 ab	7.0 ab
4	32.9 a	9.4 a

^aBermudagrass and sugarcane pre-germinated from single node stem cuttings and planted in 26.5 L pots with a surface area of 0.093m³.

^bMeans in each column followed by the same letter are not significantly different ($P \leq 0.05$).

Sugarcane longest leaf length for HoCP 96-540, averaged across 42 and 56 DAP, was equivalent when grown alone or with 1 or 2 bermudagrass plants per pot (Table 4.3). Sugarcane leaf length, however, was reduced 12% when in competition with 4 bermudagrass plants per pot. Sugarcane uppermost collar height was not negatively affected by bermudagrass competition. As expected, both sugarcane leaf length and collar height increased from 42 to 56 DAP.

Table 4.3. ‘HoCP 96-540’ sugarcane leaf length and uppermost collar height 42 and 56 days after planting (DAP) with bermudagrass at a density of 0, 1, 2, or 4 plants per pot.^a

Bermudagrass density plants/pot	Sugarcane leaf length (cm)			Sugarcane collar height (cm)		
	42 DAP	56 DAP	Density average ^b	42 DAP	56 DAP	Density average ^b
0	115	122	118 a	26.9	30.3	28.6 a
1	109	113	111 ab	25.0	27.4	26.2 a
2	107	113	110 ab	23.4	26.4	24.9 a
4	102	105	104 b	22.8	24.3	23.6 a
DAP average ^c	108 b	113 a	--	24.5 b	27.1 a	--

^aBermudagrass and sugarcane were pre-germinated from single node stem cuttings and planted in 26.5 L pots with a surface area of 0.093m³. Leaf length represents measurement from soil surface to tip of longest leaf.

^bDensity means averaged across 42 and 56 DAP followed by the same letter are not significantly different ($P \leq 0.05$).

^cDAP means averaged across bermudagrass density followed by the same letter are not significantly different ($P \leq 0.05$).

Sugarcane shoot number, 56 DAP of sugarcane with bermudagrass at 1, 2, and 4 plants per pot was reduced an average of 50% compared with sugarcane grown alone, and differences in shoot number were not observed among the bermudagrass planting densities (Table 4.4). For sugarcane shoot weight, a single bermudagrass plant reduced sugarcane growth 51% and reduction in shoot growth was equivalent to that observed for 2 or 4 bermudagrass plants (Table 4.4). Root weight of sugarcane was not negatively affected when sugarcane was grown with 1 bermudagrass plant, but root weight was reduced an average of 39% when grown with 2 or 4 bermudagrass plants. Etheredge et al. (2010) reported that LCP 85-384 shoot dry weight was not negatively affected by an initial planting density of 2 purple nutsedge tubers per pot, but sugarcane root weight was reduced an average of 50% when 1 or 2 tubers were planted per pot.

Table 4.4. ‘HoCP 96-540’ sugarcane shoot population and shoot and root dry weight 56 days after planting with bermudagrass at a density of 0, 1, 2, or 4 plants per pot.^a

Bermudagrass density plants/pot	Sugarcane shoot number per pot (no./pot)	Sugarcane shoot weight (g/pot)	Sugarcane root weight (g/pot)
0	8.9 a ^b	38.9 a	25.6 a
1	4.4 b	19.1 b	19.1 ab
2	4.6 b	17.4 b	16.3 b
4	4.3 b	13.0 b	15.2 b

^aBermudagrass and sugarcane pre-germinated from a single node stem cutting were planted in 26.5 L pots with a surface area of 0.093m³.

^bMeans within each column followed by the same letter are not significantly different ($P \leq 0.05$).

Sugarcane Cultivar Study. To further evaluate the competitiveness of bermudagrass with sugarcane, 6 sugarcane cultivars were grown with bermudagrass at densities of 0 and 2 plants per pot. For bermudagrass groundcover, there was no significant effect due to sugarcane cultivar or an interaction between cultivar and DAP. This shows that bermudagrass at a plant density of 2 plants per pot was able to establish and compete with sugarcane regardless of the cultivar. Averaged across sugarcane cultivars, bermudagrass coverage of the pot surface area increased from 10% 14 DAP to 52% 56 DAP (Table 4.5). For sugarcane longest leaf length and uppermost collar height 42 and 56 DAP and shoot population 56 DAP, a significant effect due to

bermudagrass competition and for the interaction between bermudagrass competition and cultivar was not observed. Averaged across sugarcane cultivars, longest leaf length regardless of bermudagrass competition level increased from 42 to 56 DAP (Table 4.6). Averaged across bermudagrass density and DAP, sugarcane leaf length was greatest for L 97-128 and L 99-226 (146 and 149 cm, respectively) and least for HoCP 96-540 and L 01-283 (117 and 103 cm, respectively). Sugarcane uppermost collar height averaged over bermudagrass density and DAP was greatest for L 97-128, L 99-226, and HoCP 00-950 (36.3 to 39.3 cm) and least for L 01-283 (22.2 cm) (Table 4.7).

Sugarcane shoot population 56 DAP averaged across bermudagrass densities was greatest for L 97-128, L 99-226, and L 03-371 (5.5 to 5.8 shoots per pot) and averaged 1.7 times that for HoCP 00-950 and L 01-283 (3.1 and 3.4 shoots per pot) (Table 4.8).

Table 4.5. Bermudagrass ground cover 14, 28, 42, and 56 days after planting (DAP) of 0 or 2 bermudagrass plants per pot with six sugarcane cultivars.^a

Cultivar	Bermudagrass ground cover (%)				Cultivar average
	14 DAP	28 DAP	42 DAP	56 DAP	
HoCP 96-540	9	40	55	60	41
L 97-128	10	31	44	50	34
L 99-226	11	24	32	33	25
HoCP 00-950	10	42	46	58	39
L 01-283	11	35	48	52	36
L 03-371	8	34	47	60	37
DAP average ^b	10 d	34 c	45 b	52 a	--

^aBermudagrass and sugarcane pre-germinated from single node stem cuttings and planted in 26.5 L pots with a surface area of 0.093m³.

^bDAP means averaged across sugarcane cultivars and bermudagrass density followed by the same letter are not significantly different ($P \leq 0.05$).

Table 4.6. Sugarcane leaf length of six cultivars 42 and 56 days after planting (DAP) with bermudagrass (BG) at a density of 0 (-BG) or 2 (+BG) plants per pot.^a

Cultivar	Sugarcane leaf length (cm)				Cultivar average ^b
	42 DAP		56 DAP		
	- BG	+ BG	- BG	+ BG	
HoCP 96-540	119	110	123	117	117 d
L 97-128	142	139	151	154	146 ab
L 99-226	148	143	155	152	149 a
HoCP 00-950	138	129	142	136	136 bc
L 01-283	102	100	109	101	103 e
L 03-371	125	121	139	129	128 c
Density average ^c	129 bc	123 c	137 a	131 ab	--

^aBermudagrass and sugarcane were pre-germinated from single node stem cuttings and planted in 26.5 L pots with a surface area of 0.093m³. Leaf length represents measurement from soil surface to tip of longest leaf.

^bCultivar means averaged across bermudagrass density and DAP followed by the same letter are not significantly different ($P \leq 0.05$).

^cBermudagrass density means averaged across sugarcane cultivars for 42 and 56 DAP followed by the same letter are not significantly different ($P < 0.05$).

Table 4.7. Sugarcane uppermost collar height of six cultivars 42 and 56 days after planting (DAP) with bermudagrass (BG) at a density of 0 (-BG) or 2 (+BG) plants per pot.^a

Cultivar	Sugarcane collar height (cm)				Cultivar average ^b
	42 DAP		56 DAP		
	- BG	+ BG	- BG	+ BG	
HoCP 96-540	25.0	25.6	29.3	30.2	27.5 a
L 97-128	35.8	35.1	44.4	41.8	39.3 a
L 99-226	35.2	32.7	40.8	37.3	36.5 a
HoCP 00-950	34.8	31.8	41.2	37.3	36.3 a
L 01-283	20.6	19.6	25.0	23.7	22.2 c
L 03-371	26.5	26.2	30.4	29.3	28.1 b
Density average	29.6	28.5	35.2	33.3	--

^aBermudagrass and sugarcane were pre-germinated from single node stem cuttings and planted in 26.5 L pots with a surface area of 0.093m³

^bCultivar means averaged across bermudagrass density and DAP followed by the same letter are not significantly different ($P \leq 0.05$).

Table 4.8. Sugarcane shoot population of six cultivars 56 days after planting with bermudagrass (BG) at a density of 0 (-BG) or 2 (+BG) plants per pot.^a

Cultivar	Sugarcane shoot population (no./pot)		
	- BG	+ BG	Cultivar average ^b
HoCP 96-540	5.3	4.3	4.8 ab
L 97-128	5.4	5.6	5.5 a
L 99-226	6.1	5.4	5.8 a
HoCP 00-950	3.3	2.9	3.1 b
L 01-283	3.9	2.9	3.4 b
L 03-371	5.3	5.8	5.5 a
Density average	4.9	4.5	--

^aBermudagrass and sugarcane pre-germinated from a single node stem cutting were planted in 26.5 L pots with a surface area of 0.093m³.

^bCultivar means averaged across bermudagrass density followed by the same letter are not significantly different ($P \leq 0.05$).

Competition between sugarcane and 2 bermudagrass plants per pot for 56 days reduced sugarcane shoot weight an average of 17% (Table 4.9). For the cultivars, the reduction in shoot weight due to bermudagrass competition ranged from 2 and 4% for HoCP 96-540 and L 97-128 to 21 to 27% for L 99-226, HoCP 00-950, L 01-283 and L 03-371. Sugarcane root weight was reduced an average of 14% (Table 4.9). For the cultivars, the reduction in root weight due to bermudagrass competition was 0 to 15% for HoCP 96-540, L 99-226, and HoCP 00-950 and 21 to 31% for the other cultivars. Averaged across bermudagrass density, sugarcane shoot weight was greatest for L 97-128 and L 99-226 (71.6 and 67.8 g/pot, respectively) and averaged 2.8 times that for HoCP 96-540 and L 01-283. Sugarcane root weight for L 97-128 and L 99-226 was equivalent (35.7 and 36.6 g/pot, respectively) and averaged 1.9 times greater than for HoCP 96-540, HoCP 00-950, L 01-283, and L 03-371. Etheredge et al. (2010) reported that based on shoot and root dry weight after 64 days of competition with purple nutsedge that L 97-128 was at least twice as competitive as LCP 85-384, Ho 95-988, and HoCP 96-540.

Table 4.9. Sugarcane shoot and root weight of six cultivars 56 days after planting (DAP) with bermudagrass (BG) at a density of 0 (-BG) or 2 (+BG) plants per pot.^a

Cultivar	Sugarcane shoot weight (g/pot)			Sugarcane root weight (g/pot)		
	- BG	+ BG	Cultivar average ^b	- BG	+ BG	Cultivar average ^b
HoCP 96-540	26.7	26.2	26.4 d	17.9	16.6	17.3 b
L 97-128	72.9	70.3	71.6 a	40.3	31.0	35.7 a
L 99-226	76.0	59.7	67.8 ab	35.3	37.9	36.6 a
HoCP 00-950	57.3	44.3	50.8 bc	20.3	17.3	18.8 b
L 01-283	26.9	20.3	23.6 d	22.4	17.8	20.1 b
L 03-371	47.8	34.8	41.3 cd	23.0	15.8	19.4 b
Density average ^c	51.3 a	42.6 b	--	26.5 a	22.7 b	--

^aBermudagrass and sugarcane were pre-germinated from single node stem cuttings and planted in 26.5 L pots with a surface area of 0.093m³.

^bCultivar means averaged across bermudagrass density followed by the same letter are not significantly different ($P \leq 0.05$).

^cBermudagrass density means averaged across sugarcane cultivars followed by the same letter are not significantly different ($P \leq 0.05$).

Bermudagrass shoot and root weights were also determined to assess the ability to compete with sugarcane. Although not significant, bermudagrass shoot weight was numerically greatest when grown with HoCP 96-540, L 01-283, and L 03-371 and least for L 99-226 (Table 4.10). Bermudagrass root weight was greatest when bermudagrass was grown with L 01-283 and lowest when bermudagrass was grown with L 97-128, L 99-226, and HoCP 00-950.

Results from both the bermudagrass density and the sugarcane cultivar studies show that bermudagrass can be highly competitive with sugarcane. When bermudagrass and HoCP 96-540 sugarcane were planted together with each having an equal chance to establish, bermudagrass at a density of 2 plants per pot decreased sugarcane shoot population 48%, shoot weight 55%, and root weight 36% (Table 4.4). In the sugarcane cultivar study, two bermudagrass plants per pot did not reduce population of HoCP 96-540, L 97-128, L 99-226, HoCP 00-950, L 01-283, and L 03-371 (Table 4.8), but reduced shoot and root weight an average of 17 and 14%, respectively (Table 4.9). Bittencourt et al. (2010) reported that sugarcane cultivars vary in regard to how rapidly they emerge and produce tillers after planting in August and September and in the spring

following the winter dormant period. These characteristics can directly affect the ability of sugarcane to compete with weeds early in the growing season.

Table 4.10. Bermudagrass shoot and root weight 56 days after planting at a density 2 plants per pot with six sugarcane cultivars. ^a

Cultivar	Bermudagrass shoot weight		Bermudagrass root weight	
	Shoot weight g/pot	Percent of total shoot weight/pot (%)	Root weight (g/pot)	Percent of total root weight/pot (%)
HoCP 96-540	16.4 a ^b	39 ^c	5.8 ab ^b	26 ^c
L 97-128	15.0 a	18	3.3 b	10
L 99-226	8.5 a	13	3.2 b	8
HoCP 00-950	14.3 a	24	3.9 b	18
L 01-283	17.2 a	46	8.9 a	33
L 03-371	19.3 a	36	6.5 ab	29

^aBermudagrass and sugarcane were pre-germinated from single node stem cuttings and planted in 26.5 L pots with a surface area of 0.093m³.

^bMeans within each column followed by the same letter are not significantly different (P ≤ 0.05).

^cValues represent percent of total weight (bermudagrass + sugarcane) of shoot and root biomass per pot represented by bermudagrass. Example: sugarcane shoot weight for HoCP 96-540 grown with bermudagrass of 26.2 g/pot (See Table 4.9) plus bermudagrass shoot weight when grown with HoCP 96-540 of 16.4 g/pot (See Table 4.10) equals a total shoot weight of 42.6 g/pot. Percent of total shoot weight represented by bermudagrass equals 39% (16.4 / 42.6 x 100)

In the density study, of the total shoot biomass produced per pot, (HoCP 96-540 sugarcane plus bermudagrass) bermudagrass shoot weight 56 days after planting of 2 plants per pot represented 57% and sugarcane shoot biomass represented 43% of the total biomass; bermudagrass root weight represented 30% of the total root biomass per pot and sugarcane root biomass represented 70% of the total biomass (Table 4.2 and 4.4). In the cultivar study, of the total shoot biomass produced per pot, bermudagrass shoot weight 56 days after planting of 2 bermudagrass plants per pot (the same density of bermudagrass evaluated in the density study) represented 36 to 46% when grown with the sugarcane cultivars HoCP 96-540, L 01-283, and L 03-371, but only 13 to 24% for L 97-128, L 99-226, and HoCP 00-950 (Table 4.10). Expressing total shoot biomass (bermudagrass plus sugarcane) as the percentage represented by sugarcane, shows shoot biomass to be lowest for HoCP 96-540, L 01-283, L 03-371 (54 to 64% of total

biomass) and greatest for L 97-128, L 99-226, and HoCP 00-950 (76 to 87% of total biomass) (Table 4.9).

Of the total root biomass produced per pot, bermudagrass root weight represented 26 to 33% for HoCP 96-540, L 01-283, and L 03-371, but only 8 to 18% for L 97-128, L 99-226, and HoCP 00-950. Of the total root biomass produced, sugarcane root weight would represent 67 to 74% for the cultivars HoCP 96-540, L 01-283, and L 03-371 and 82 to 92% of the total biomass for the cultivars L 97-128, L 99-226, and HoCP 00-950. Of interest is that in both studies where 2 bermudagrass plants per pot were grown with HoCP 96-540, there was very close agreement in percent of total root biomass represented by bermudagrass and sugarcane (26 and 30% bermudagrass and 70 and 74% sugarcane).

Results show the ability of the cultivars L 97-128, L 99-226, and HoCP 00-950 to rapidly produce shoot and root biomass which would enhance their competitiveness with bermudagrass. The cultivars HoCP 96-540, L 01-283, and L 03-371, which were slower to establish would be less competitive with bermudagrass. Furthermore, based on shoot and root growth of sugarcane relative to bermudagrass, L 01-283 may be more negatively affected by bermudagrass competition than HoCP 96-540. Tew et al. (2005) reported that HoCP 96-540 is slow to establish at planting and Bittencourt et al. (2010) reported that in the spring following the winter dormant period, HoCP 96-540 emerged later and produced fewer shoots per row compared with L 97-128 and L 99-226.

Herbicides currently used at sugarcane planting for control of bermudagrass include clomazone, metribuzine, and tebuthiuron (Anonymous 2013). These herbicides provide only suppression of bermudagrass (60 to 80% control) and because of their short residual activity it is recommended that metribuzin be applied 60 days following initial herbicide application to help prevent bermudagrass re-establishment. This research highlights the ability of bermudagrass to

rapidly establish from stem nodes and to aggressively compete with sugarcane at planting. Furthermore, results show that competitiveness of bermudagrass with sugarcane can be affected by sugarcane cultivar. Successful management of bermudagrass is dependent upon both the competitive ability of the sugarcane cultivar and the effectiveness of the herbicide in suppressing bermudagrass re-establishment.

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CHAPTER 5: ALLELOPATHIC EFFECT OF BERMUDAGRASS (*CYNODON DACTYLON*) LEACHATE ON SUGARCANE

INTRODUCTION

Sugarcane (*Saccharum* spp. hybrids) is grown in Louisiana, Florida, and Texas in the continental U.S. Approximately 40% of U.S. sugar is obtained from sugarcane produced in Louisiana, accounting for 17.4% of total U.S. sugar production (Salassi et al. 2011). In Louisiana, sugarcane is planted in August and September and grown for 3 to 5 years before the crop is destroyed and replanted. Bermudagrass (*Cynodon dactylon* L. Pers.) is a serious weed problem in sugarcane both in Louisiana and throughout the world. The perennial nature of sugarcane and slow early season growth, combined with wide row spacing, provide a favorable environment for bermudagrass growth (Holm et al. 1977). Not only can bermudagrass compete with crops for water, nutrients, light, and space, but bermudagrass rhizomes and foliage can also produce chemicals that inhibit growth of corn (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.) (Vasilakoglou et al. 2005). Total fresh weight and root length of cotton were reduced when exposed to bermudagrass exudates. This process which can affect the level of competition between a weed and the crop is referred to as allelopathy.

Asaduzzaman et al. (2010) defines allelopathy as “both beneficial and deleterious biochemical interaction between plants and weeds, and/or plants and microorganisms through the production of chemical compounds that escape into the environment and subsequently influence the growth and development of neighboring plants”. The beneficial consequence is to help assure reproduction and survival of weeds over time. The deleterious consequence would be the negative effect on a desirable crop. Weed interference encompasses the competition among plants for light, water, space, and nutrients, but also includes the possibility that allelopathy may have a contributing effect (Rice 1984; Putnam 1985).

Allelopathy was studied in 300 BC by Theophrastus who concluded that “odors” from cabbage negatively affected grapevines (*Vitis* spp.) (Willis 1985). Romeo (2000) stated that almost 40% of allelopathic papers submitted to the Journal of Chemical Ecology are rejected because of the current state of knowledge and the lack of tests to determine allelopathic compounds. In a review of allelopathic evidence in Poaceae, Sanchez-Moreiras et al. (2004) stated that in early studies, the mode of action of allelochemicals from Poaceae was studied by measuring rate of seed germination, seedling emergence, and root and shoot growth, but in recent years techniques have evolved that allow for biochemical and physiological investigation to determine specific compounds and sites of action.

Putnam and Tang (1986) after examining several allelopathic studies came to the conclusion that single phytotoxins did not create an allelopathic effect, but rather it was several compounds acting together that caused the effect. Allelochemicals have been divided into a range of major chemical groups including but not limited to simple water-soluble organic acids, simple unsaturated lactones, long chain fatty acids, simple phenols, benzoic acid and its derivatives, cinnamic acid and its derivatives, flavonoids, tannins, amino acids, and polypeptides (Putnam and Tang 1986; Rice 1984; Whittaker and Feeney 1971). Pontif and McGawley (2008) found that leachate from morningglory (*Ipomoea* spp.) and johnsongrass (*Sorghum halepense* L.) roots reduced reproduction of *Rotylenchulus reniformis* in soybean (*Glycine max* L.), indicating that allelopathic compounds can affect more than just plant species.

Bermudagrass, tall fescue (*Festuca arundinacea* Schreb.) and cutleaf evening primrose (*Oenothera laciniata* Hill) leachates reduced pecan (*Carya illinoensis* (Wangenh.) K. Koch) root weight by 17%, trunk weight by 22%, and total pecan plant dry weight by 19% (Smith et al. 2001). Total fresh weight of young peach (*Prunus persica* L.) trees grown with bermudagrass was reduced up to 86% in the year of planting and up to 87% in the second year (Weller et al.

1985). Researchers suspected that some of the fresh weight reduction was due to allelopathic effects of bermudagrass on the young peach tree roots. Extracts of purple nutsedge (*Cyperus notundus* L.) and bermudagrass applied to soybean plants grown in containers decreased soybean vigor and the effect was more severe for bermudagrass (Velu and Rajagopal 1996). Soybean yield when plants were treated with bermudagrass extracts was reduced 2% compared with the nontreated control.

In another study soil collected from two sites, one with and one without bermudagrass was potted and used to grow nine vegetable crops (Meissner et al. 1989). Shoot dry mass for all crops was reduced when grown in soil previously infested with bermudagrass. For barley grown in soil containing bermudagrass, johnsongrass and purple nutsedge roots, Horowitz and Friedman (1971) showed a proportional decrease in barley (*Hordeum vulgare* L.) seed radical length growth as concentration of plant root residue in soil was increased.

In Louisiana, it is not uncommon for bermudagrass to be present in planted sugarcane or for bermudagrass residue to be present in the soil as a consequence of field preparation for sugarcane planting. In both cases it is possible that allelochemicals from bermudagrass could be released into the soil and could affect the growth and development of sugarcane. Research was conducted to evaluate the effects of leachates from roots of bermudagrass on the growth of sugarcane.

MATERIALS AND METHODS

Research was conducted in August 2011 at the LSU AgCenter Sugar Research Station, St. Gabriel, La. Six sugarcane cultivars; 'HoCP 96-540' (Tew et al. 2005), 'L 97-128' (Gravois et al. 2008), 'L 99-226' (Bischoff et al. 2009), 'HoCP 00-950' (Tew et al. 2009), 'L 01-283' (Gravois et al. 2010), and 'L 03-371' (Gravois et al. 2012). were included. Ten days prior to

experiment initiation, single node segments of each sugarcane cultivar were planted in trays with 10 cm cells containing Jiffy Mix Plus¹ potting soil.

Pots (3.7 L) containing a soil mixture of one part sterilized commerce silt loam (fine-silty, mixed, super-active, nonacid, thermic Fluvaquentic Endoaquepts), one part sterilized sand, and one part Jiffy Mix Plus with a surface area of approximately 0.021 m² were planted with a pre-germinated eyepiece of each sugarcane cultivar. Bermudagrass stolons collected from the Sugar Research Station at St. Gabriel were planted in coco fiber hanging baskets (30 cm diameter and 12 cm depth) containing sterilized perlite (treatment baskets) and baskets were watered with greenhouse tap water until bermudagrass foliage covered 50% of the surface area. A second set of hanging baskets contained only sterilized perlite (control baskets). Each hanging basket (treatment and control) was suspended 50 cm above the greenhouse bench with a 30 cm plastic funnel affixed to the bottom. Leachate was collected in an 18.9 L container through the clear plastic tubing connected to the bottom of the funnel under each hanging basket. Sterile 2 L plastic bottles were used to transfer water from the holding container to the sugarcane pots. The aluminum foil wrapped collection bottles were washed with water after each use. Foil was wrapped around the collection bottles to prevent photodegradation of potential allelopathic chemicals. Bermudagrass leachate extraction methods were modified from those described by Pontif and McGawley (2008).

Six blocks (replications) were set up in the greenhouse and within blocks each sugarcane cultivar was planted into two pots. One pot received leachate from four hanging baskets containing bermudagrass and the other pot received leachate from the non-bermudagrass control. The experiment was initiated 72 hr after sugarcane was transplanted to the 3.7 L pots. Each

¹ A sterile soil mix with an optimal blend of sphagnum and vermiculite with MagAmp slow release fertilizer (7-40-6). Jiffy Products of America, Inc., 600 Industrial Parkway, Norwalk, OH 44857.

morning, 1.5 L of water was added to each hanging basket. Approximately 6 L of leachate was collected from both the bermudagrass and control baskets. Leachate (200 ml) collected from either bermudagrass or the control pots was added immediately to designated pots. Sugarcane was watered only once per day because 150 ml of leachate was deemed sufficient to keep soil in the pots near field capacity (Viator et al. 2006).

Throughout the experiment, air temperature in the greenhouse ranged from 21 to 31 C and relative humidity ranged from 15 to 96%. Weekly pH measurements of tap water, bermudagrass and control leachates averaged 8.3, 8.4, and 8.4, respectively. Leaf length (longest leaf), leaf number, and collar height (measured from soil to uppermost collar) of sugarcane were recorded 14, 28, and 42 days after planting (DAP). At 42 DAP, tiller height (measured from soil to tip of tiller), and tiller number were determined. Following removal of above ground sugarcane biomass at 42 DAP, roots were washed free of soil. Shoot and root biomass samples were dried at 60 C for 48 hr and weighed to determine dry weight. The experimental design was a 2 (bermudagrass leachate or control leachate) x 6 (sugarcane cultivars) factorial in a Randomized Complete Block with six replications. The experiment was repeated twice.

At 28 days after experiments were initiated, three 10 ml samples of bermudagrass leachate from coco fiber baskets containing bermudagrass and perlite, three samples of control leachate from fiber baskets containing perlite, three samples of greenhouse tap water, and three samples of leachate from only coco fiber baskets were collected for chemical analysis. Leachate samples were transported on ice to the LSU Department of Environmental Sciences Response & Chemical Assessment Team Laboratory. Samples were filtered through Whatman #2 filter paper and washed with ethyl acetate. Aqueous portions of the samples were discarded. A 1 ml injection of the leachate sample was processed in a GC/MS with a hold temperature of 270 C. Helium was used as the carrier gas at a rate of 1 ml/min. The GC oven was held at 80 C for 1 minute and

increased to 125 C at 5 C increments followed by an increase in temperature to 325 C at 10 C increments. The oven was held at 325 C for 4 minutes. Each sample had a run time of 34 minutes. A capillary column that is 30-m by 0.25 μm diameter and a 0.5 μm DB-5 film capillary column were used in the GC. The MS was operated in scan mode, employed by electron ionization. Methods for extraction and operation of instrumentation were modified from Viator et al (2006). Processing leachate samples on the scan mode aided in determining the identity of chemicals present in the bermudagrass leachates. Chemicals were identified by their retention times and molecular weights using the Wiley library chemical database. Compounds identified by the GC/MS with quality values less than 70% should not be used to actively identify the tentative compound (Fontenot 2009). The chemicals found in most abundance were recorded.

Data were subjected to Mixed Procedure in SAS (SAS Institute 2012) with experiments and replications considered random effects. Least square means were calculated and mean separation was performed at $P \leq 0.05$. Letter groupings were converted using the PDMIX800 macro in SAS (Saxton 1998).

RESULTS AND DISCUSSION

For sugarcane longest leaf length, leaf number per pot, and collar height, 14, 28, and 42 DAP, analysis of variance did not show a significant cultivar x leachate source (bermudagrass vs no bermudagrass control) interaction or a significant effect due to leachate source. For all cultivars, as expected, sugarcane leaf length, leaf number, and collar height, regardless of leachate source increased numerically from 14 to 42 DAP (Tables 5.1, 5.2, and 5.3). Averaged across leachate source and DAP, sugarcane leaf length for HoCP 96-540, L 97-128, and HoCP 00-950 ranged from 107 to 109 cm and for L 99-226, L 01-283, and L 03-371 ranged from 99 to 105 cm (Table 5.1).

Sugarcane leaf number averaged across leachate sources and DAP was greatest for HoCP 00-950 (Table 5.2). Sugarcane collar height was greatest for HoCP 96-540 (26 cm) and least for L 01-283 (20 cm) (Table 5.3).

At 42 DAP, when the experiments were terminated, the cultivar x leachate source interaction as well as leachate source effect were not significant for sugarcane tiller height, tiller number, shoot weight, or root weight (Table 5.4). A significant cultivar effect, however, was observed for sugarcane tiller height, tiller number, and sugarcane shoot weight.

Averaged across leachate sources, tiller height was greatest for L 99-226 and L 03-371 (64 and 58 cm, respectively); tiller height was shortest for HoCP 96-540 and HoCP 00-950 (1 and 6 cm, respectively) but were not significantly different from L 98-128 and L 01-283 (16 and 20 cm, respectively) (Table 5.4). Total number of tillers for L 99-226 and L 03-371 averaged 2 and was greater than for the other cultivars (Table 5.4). Although HoCP 00-950 did not produce any tillers, shoot weight was greater than all other cultivars.

Chemical compounds present in tap water and leachate collected from coco baskets, perlite, and bermudagrass are shown in Table 5.5. For all compounds identified, the QF was 72 to 91%. Because leachate samples were filtered through paper and washed with ethyl acetate, compounds present in ethyl acetate alone were determined. In ethyl acetate and ethyl acetate plus ammonium sulfate, dodecamethyl cyclohexasiloxane, decamethyl cyclopentasiloxane, and guanidine were identified. In the tap water, decamethyl cyclopentasiloxane and dodecamethyl cyclohexasiloxane were also identified along with 2-methyl-3-indazolone-N-D1, tetracosamethylcyclododecasiloxane, and heptamethyl-3-3-bis. In the leachate collected dodecamethyl cyclohexasiloxane, isobutyl nonyl ester, and 2-ethylhexyl hexyl ester phthalic acid, bis (2-methylpropyl) ester, mono (2-ethylhexyl) ester, diisooctyl ester benzenedicarboxylic acid, and methyl ester heptadecanoic acid were identified. For the coco basket, perlite, and

Table 5.1. The effect of leachate collected from actively growing bermudagrass and from a no bermudagrass control on sugarcane longest leaf length 14, 28, and 42 days after planting (DAP).^a

Sugarcane leaf length (cm)									
Bermudagrass leachate					Control leachate				
Cultivar	14 DAP	28 DAP	42 DAP	Cultivar average	14 DAP	28 DAP	42 DAP	Cultivar average	Cultivar average ^b
HoCP 96-540	69	125	134	109	67	122	131	107	108 a ^c
L 97-128	75	123	130	109	78	119	126	108	109 a
L 99-226	65	119	125	103	69	114	120	101	102 bc
HoCP 00-950	69	123	133	108	70	120	128	106	107 ab
L 01-283	66	115	121	101	68	110	115	98	99 c
L 03-371	78	115	117	103	76	120	125	107	105 ab

^aSugarcane planted in 3.7 L pots and grown in the greenhouse. Leachate collected from coco baskets containing bermudagrass grown in perlite or containing only perlite (no bermudagrass control) was used as the only water source.

^bData averaged across leachate source and DAP.

^cMeans followed by the same letter are not significantly different ($P \leq 0.05$).

Table 5.2. The effect of leachate collected from actively growing bermudagrass and from a no bermudagrass control on sugarcane leaf number 14, 28, and 42 days after planting (DAP).^a

Sugarcane leaf number (no./pot)									
Bermudagrass leachate					Control leachate				
Cultivar	14 DAP	28 DAP	42 DAP	Cultivar average	14 DAP	28 DAP	42 DAP	Cultivar average	Cultivar average ^b
HoCP 96-540	7	9	9	8	7	9	9	8	8 b ^c
L 97-128	7	8	10	8	7	8	9	8	8 b
L 99-226	6	8	9	8	6	8	10	8	8 b
HoCP 00-950	7	9	10	9	7	9	10	9	9 a
L 01-283	7	8	9	8	6	9	9	8	8 b
L 03-371	7	9	10	9	7	9	9	8	8 b

^aSugarcane planted in 3.7 L pots and grown in the greenhouse. Leachate collected from coco baskets containing bermudagrass grown in perlite or containing only perlite (no bermudagrass control) was used as the only water source.

^bData averaged across leachate source and DAP.

^cMeans followed by the same letter are not significantly different ($P \leq 0.05$).

bermudagrass leachate, several compounds as expected were identical that were also present in the coco basket and perlite leachate. However, one compound not previously identified was found in the bermudagrass leachate, hexamethyl tetracosahexane. This compound is also known as squalene (QF factor of 90%; CAS number 111-02-4). The chemical structure of squalene is $C_{30}H_{50}$ with a molecular weight of 410.7 g mol^{-1} .

An EPA review reported that squalene is produced in both plants and animals (FDA 2009). In plants, squalene is a precursor to triterpenoids and steroids. There is a wide variety of triterpenoids that can be found in large quantities in plant latex's and resins. The function of triterpenoids is thought to be a chemical defense against pathogens and herbivores (Abe et al. 1993). Kalinova et al. (2007) found that buckwheat (*Fagopyrum esculentum* M.) released squalene from the roots when germinated in agar; however, in field experiments squalene was not detected. Squalene extracted from the agar and present during germination of lettuce (*Lactuca sativa* L.), mustard (*Sinapis alba* L.), and Dutch clover (*Trifolium repens* L.) seeds was shown to stimulate and not inhibit radical growth. Squalene extracted from plants has not been proven to have allelopathic functions.

The negative effects of bermudagrass leachate on sugarcane tiller height, tiller number, or shoot and root biomass were not observed in this study. These findings are in contrast to those reported by Velu and Rajagopal (1995), Smith et al. (2001), and Vasilakoglou et al. (2005). In the case of Velu and Rajagopal (1995) where negative effects on soybean growth were observed, bermudagrass plant material was either soaked in water for 24 hr with the water used to irrigate soybean or 50 g of live bermudagrass cuttings were added to the potting media. The soaking of plant material in water may have increased concentration of allelochemicals as compared to the procedure used in the present study where leachate was collected from actively growing bermudagrass. Vasilakoglou et al. (2005) dried and ground bermudagrass to create liquid

Table 5.3. The effect of leachate collected from actively growing bermudagrass and from a no bermudagrass control on sugarcane uppermost collar height 14, 28, and 42 days after planting (DAP).^a

Cultivar	Sugarcane collar height (cm)									
	Bermudagrass leachate				Control leachate					
	14 DAP	28 DAP	42 DAP	Cultivar average	14 DAP	28 DAP	42 DAP	Cultivar average	Cultivar average ^b	
HoCP 96-540	15	27	36	26	15	27	34	25	26 a ^c	
L 97-128	14	24	30	22	14	24	29	22	22 bc	
L 99-226	13	24	26	21	13	23	26	21	21 cd	
HoCP 00-950	13	25	33	24	14	24	30	23	23 b	
L 01-283	12	22	25	20	13	21	26	20	20 d	
L 03-371	15	24	26	22	14	25	27	22	22 bc	

^aSugarcane planted in 3.7 L pots and grown in the greenhouse. Leachate collected from coco baskets containing bermudagrass grown in perlite or containing only perlite (no bermudagrass control) was used as the only water source.

^bData averaged across leachate source and DAP.

^cMeans followed by the same letter are not significantly different ($P \leq 0.05$).

Table 5.4. The effect of leachate collected from actively growing bermudagrass (BG) and from a no bermudagrass control on sugarcane tiller height, tiller number, and root and shoot weight 42 days after sugarcane planting (DAP).^a

Cultivar	Tiller height (cm)			Tiller number (no./pot)			Shoot weight (g/pot)			Root weight (g/pot)		
	BG	Control	Cultivar	BG	Control	Cultivar	BG	Control	Cultivar	BG	Control	Cultivar
	leachate	leachate	average	leachate	leachate	average	leachate	leachate	average	leachate	leachate	average
HoCP 96-540	6	7	6 b ^b	1	1	1 bc ^b	10	10	10 b ^b	6	7	6 a ^b
L 97-128	18	14	16 b	1	1	1 b	10	10	10 b	7	7	7 a
L 99-226	60	67	64 a	2	2	2 a	10	10	10 b	8	7	8 a
HoCP 00-950	2	1	1 b	0	0	0 c	12	11	12 a	7	7	7 a
L 01-283	20	21	20 b	1	1	1 b	9	9	9 b	8	8	8 a
L 03-371	49	48	58 a	2	2	2 a	9	9	9 b	8	7	8 a

^aSugarcane planted in 3.7 L pots and grown in the greenhouse. Leachate collected from coco baskets containing bermudagrass grown in perlite or containing only perlite (no bermudagrass control) was used as the only water source.

^bData averaged across leachate source. Means followed by the same letter are not significantly different ($P \leq 0.05$).

Table 5.5. Chemical compounds isolated from tap water and leachate collected from coco baskets, perlite, and bermudagrass in the sugarcane and bermudagrass allelopathy greenhouse study.^a

Water source	RT	Wiley Library ID	CAS#	QF ^b
Ethyl Acetate	16.301	Cyclohexasiloxane, dodecamethyl	000540-97-6	90
	10.958	Cyclopentasiloxane, decamethyl	000541-02-6	83
	11.858	Guanidine	000113-00-8	83
	11.042	Cyclopentasiloxane, decamethyl	000541-02-6	91
Ethyl Acetate + Na ₂ SO ₄	11.048	Cyclopentasiloxane, decamethyl	000541-02-6	91
	16.301	Cyclohexasiloxane, dodecamethyl	000540-97-6	91
Tap water	21.384	2-Methyl-3-Indazolone-N-D1	054120-67-1	80
	11.043	Cyclopentasiloxane, decamethyl	000541-02-6	90
	16.301	Cyclohexasiloxane, dodecamethyl	000540-97-6	90
	26.717	Tetracosamethylcyclododecasiloxane	018919-94-3	74
	28.009	Heptamethyl-3-3-bis	038147-00-1	72
Coco fiber basket	24.254	Phthalic acid, isobutyl nonyl ester	1000309-04-4	72
	11.053	Cyclopentasiloxane, decamethyl	000541-02-6	90
	16.301	Cyclohexasiloxane, dodecamethyl	000540-97-6	87
Coco fiber basket + perlite	24.286	Benzenedicarboxylic acid, bis (2-methylpropyl) ester	000084-69-5	72
	30.248	Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	004376-20-9	91
	11.048	Cyclopentasiloxane, decamethyl	000541-02-6	91
	16.301	Cyclohexasiloxane, dodecamethyl	000540-97-6	90
	30.317	Phthalic acid, 2-ethylhexyl hexyl ester	1000309-02-5	72
	25.832	Heptadecanoic acid, methyl ester	001731-92-6	90
	30.317	Benzenedicarboxylic acid, diisooctyl ester	027554-26-3	90
Coco fiber basket + perlite + bermudagrass	30.254	Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	004376-20-9	90
	30.248	Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	004376-20-9	91
	11.042	Cyclopentasiloxane, decamethyl	000541-02-6	91
	16.301	Cyclohexasiloxane, dodecamethyl	000540-97-6	91
	32.330	Tetracosahexaene, hexamethyl ^c	000111-02-4	90
	25.843	Heptadecanoic acid, methyl ester	001731-92-6	90

^aChemicals from ethyl acetate and ethyl acetate plus sodium sulfate wash, tap water, and from leachate samples collected from coco fiber baskets, coco fiber baskets containing perlite, and coco baskets containing perlite and actively growing bermudagrass were processed using GC/MS. Specific chemicals were identified by their retention times (RT) and molecular weights using the Wiley Library chemical database.

^bA quality factor (QF) >70 considered to be true identification.

^cTetracosahexaene, hexamethyl qualene (squalene) identified in the water source containing actively growing bermudagrass.

extracts used to water cotton and corn. Again, the exposure to plant material extract may have amplified the allelopathic effects of bermudagrass. Additionally, a portion of this study was conducted in the lab in hydroponic culture which would eliminate absorption of allelochemicals to soil which could lessen the negative effect on the crops. In research conducted by Smith et al. (2001), the methodology to evaluate allelopathy using bermudagrass leachate as a water solution was the same as used in the present study with the exception that rhizomes rather than stolons were used as planting material. It is possible that use of rhizomes for planting may have promoted more rapid bermudagrass establishment and greater underground biomass (rhizome + roots) which may have increased production of allelochemicals.

Squalene, a possible allelopathic compound, was identified in leachate collected from watering of bermudagrass grown in coco baskets containing perlite in the present study. Putnam and Tang (1986) concluded that allelopathic effects were not caused by single phytotoxins but rather by several compounds acting together. Although squalene has been reported to be allelopathic (Kalinova et al. 2007) it is possible that other compounds such as these identified in Table 5.5 may act with squalene to produce allelopathic effects. In Louisiana, when sugarcane is planted in August and September, bermudagrass can emerge with the crop, and with rainfall, allelochemicals could be leached into the soil and taken up by the sugarcane plant. Another method of sugarcane exposure to allelochemicals at planting would be through degradation of bermudagrass that was actively growing prior to planting and killed during land preparation. The decomposition of bermudagrass as a source of leachate was not investigated in the present study.

Results from this study show that squalene is produced by actively growing bermudagrass and can be moved from a living plant to the soil solution. The concentration of squalene in bermudagrass leachate was not measured in this study, but would be expected to vary depending on both the amount of bermudagrass root and shoot biomass present when soil is

prepared for sugarcane planting and the level of bermudagrass competition during early crop establishment. Bermudagrass interference with sugarcane implies that the crop and weed would be competing for water, light, nutrients, and space, but also that allelochemicals produced by bermudagrass could also be a contributing factor. Regardless of whether or not allelochemicals produced by bermudagrass are detrimental to sugarcane growth, it is imperative from the standpoint of maximizing sugarcane stand establishment and yield potential during the crop cycle that effective bermudagrass control programs be implemented. A fallow program that combines tillage and timely glyphosate applications followed by an effective preemergence herbicide at planting would be essential to the management of bermudagrass in sugarcane.

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CHAPTER 6: SUMMARY

In Louisiana, sugarcane is typically harvested three to four times from a single planting. Replanting is required when disease and weed pressure begins to affect sugarcane stalk population and yield. Fields that will be replanted are fallowed following the last crop harvest until replanting in August or September. During the fallow period fields are cultivated and treated with herbicide to destroy sugarcane and control annual and perennial weeds. Bermudagrass is especially troublesome in sugarcane and can become well established over the crop cycle. Growers have reported variability in growth characteristics and competitiveness of bermudagrass in the sugarcane crop and also in susceptibility of bermudagrass to glyphosate applied during the fallow period. Research was conducted to compare growth, biomass production, genetics, and glyphosate response for bermudagrass biotypes collected in Louisiana and to evaluate bermudagrass competition with sugarcane at planting.

The first objective was to compare growth characteristics of 20 bermudagrass biotypes collected from Louisiana sugarcane fields and at locations where sugarcane is not grown. Biotypes that were most aggressive included A collected in St. Martinville, Q collected in Port Allen, and R collected at the LSU AgCenter Sugar Research Station in St. Gabriel. These biotypes established rapidly and were tall-growing, with long internodes and wide leaves. Biotypes A and Q were also able to retain green foliage later into the winter and to initiate growth in the spring earlier than some of the other biotypes. There were also biotypes of bermudagrass that were slow to establish and were short growing. These biotypes, considered less aggressive, were J collected in Samuels, N collected in New Iberia, and T collected at the LSU AgCenter, Northeast Research Station in St. Joseph. Based on groundcover, biotypes J, N, and T were an average of 5.3 times slower to establish compared with biotypes A, Q, and R and plant height averaged 61% less. In the first year of the study, bermudagrass dry weight was 7.8

times greater for biotypes A, Q, and R compared with biotypes J, N, and T. Although differences were observed between these biotypes in their ability to establish and in morphology, and cold tolerance, all were clustered in the same group based on Jaccard's similarity coefficient, indicating the presence of common alleles.

A second objective was to evaluate initial control and regrowth potential of the bermudagrass biotypes when treated with glyphosate during the initial establishment period and after establishment. Bermudagrass biotypes least sensitive to glyphosate included A collected in St. Martinville, C collected in Baldwin, and Q collected in Port Allen. Bermudagrass considered most sensitive to glyphosate were collected in Vacherie (biotype F) and Patterson (biotype P). Differences in susceptibility to glyphosate observed in this study may help to explain the variation in bermudagrass control with glyphosate observed in fallowed fields in the sugarcane producing area of Louisiana. Of significance and to be expected was that glyphosate was most effective when applied to bermudagrass during early establishment. Therefore, it would be critical that intensive tillage programs that fragment bermudagrass stolons and rhizomes be implemented prior to glyphosate application. In fields where bermudagrass control with glyphosate has been difficult in the past, glyphosate should be applied when stolon growth is first initiated and before stolons begin to root and spread. A tillage operation 7 to 10 days following application along with one or more follow-up applications of glyphosate, as needed, will help to improve long term bermudagrass control.

The third objective was to evaluate competition between sugarcane and bermudagrass at planting. When bermudagrass and 'HoCP 96-540' sugarcane were planted together with each having an equal chance to establish, bermudagrass at a density of two plants per pot decreased sugarcane shoot population 48%, shoot weight 55%, and root weight 36%. Of the total shoot biomass produced per pot, (sugarcane plus bermudagrass) bermudagrass shoot weight 56 days

after planting of two plants per pot represented 57% and sugarcane shoot biomass represented 43% of the total biomass; bermudagrass root weight represented 30% of the total root biomass per pot and sugarcane root biomass represented 70% of the total biomass. In another study, two bermudagrass plants per pot did not reduce shoot population of HoCP 96-540, 'L 97-128', 'L 99-226', 'HoCP 00-950', 'L 01-283', and 'L 03-371', but shoot and root weight were reduced an average of 17 and 14%, respectively. Of the total shoot biomass produced per pot, bermudagrass shoot weight 56 days after planting of two bermudagrass plants per pot represented 36 to 46% when grown with the sugarcane cultivars HoCP 96-540, L 01-283, and L 03-371, but only 13 to 24% for L 97-128, L 99-226, and HoCP 00-950. Expressing total shoot biomass (bermudagrass plus sugarcane) as the percentage represented by sugarcane, shoot biomass was lowest for HoCP 96-540, L 01-283, L 03-371 (54 to 64% of total biomass) and greatest for L 97-128, L 99-226, and HoCP 00-950 (76 to 87% of total biomass). Of the total root biomass produced per pot, bermudagrass root weight represented 26 to 33% for HoCP 96-540, L 01-283, and L 03-371, but only 8 to 18% for L 97-128, L 99-226, and HoCP 00-950. Of the total root biomass produced, sugarcane root weight represented 67 to 74% for the cultivars HoCP 96-540, L 01-283, and L 03-371 and 82 to 92% of the total biomass for the cultivars L 97-128, L 99-226, and HoCP 00-950. In both of the competition studies where two bermudagrass plants per pot were grown with HoCP 96-540; there was very close agreement in percent of total root biomass represented by bermudagrass and sugarcane.

Results from both the bermudagrass density and the sugarcane cultivar studies show that bermudagrass can be highly competitive with sugarcane. The ability of the cultivars L 97-128, L 99-226, and HoCP 00-950 to rapidly produce shoot and root biomass would enhance their competitiveness with bermudagrass. The cultivars HoCP 96-540, L 01-283, and L 03-371, which were slower to establish would be less competitive with bermudagrass. Furthermore, based on

shoot and root growth of sugarcane relative to bermudagrass, L 01-283 may be more negatively affected by bermudagrass competition than HoCP 96-540. Because herbicides currently used in sugarcane provide only suppression of bermudagrass with limited residual activity, successful management of bermudagrass would be dependent on the competitive ability of the sugarcane cultivar.

The last objective was to evaluate the allelopathic potential of bermudagrass on sugarcane growth. This was accomplished by collection of soil leachate from actively growing bermudagrass plants and use of leachate as the sole water source for bermudagrass planted from stem cuttings. Bermudagrass leachate did not affect sugarcane tiller height, tiller number, or shoot and root biomass. Analysis of leachate showed presence of squalene (hexamethyl tetracosahexane), a precursor to triterpenoids and steroids.

APPENDIX

Biotype photos are included for a visual representation of growth habit differences.

Biotype A



Biotype D



Biotype B



Biotype E



Biotype C



Biotype F



Biotype G



Biotype J



Biotype H



Biotype K



Biotype I



Biotype L



Biotype M



Biotype P



Biotype N



Biotype Q



Biotype O



Biotype R



Biotype S



Biotype T



VITA

Dexter Fontenot was born in Eunice, Louisiana, and grew up on the family farm where rice, soybeans, and crawfish were produced. Upon completing high school in Mamou in 1999, he attended Louisiana State University where he earned a Bachelor of Science degree in horticulture in 2003. After working on the family farm for two years and getting married, he decided to return to LSU where as a graduate assistant under Dr. Charles Johnson with Dr. Edward Bush he completed a Master of Science degree in 2007. Dexter then accepted a Research Associate position at the LSU AgCenter Sugar Research Station in St. Gabriel, where he coordinated photoperiod and crossing of sugarcane. In 2008 he enrolled in the graduate program under the direction of Dr. Jim Griffin and is currently a candidate for the degree of Doctor of Philosophy in agronomy. He is also currently employed by Kleentek, a division of Certis USA, LLC.