Received: 16 February 2023 | Accepted: 21 April 2023

DOI: 10.1002/plr2.20318

Journal of Plant Registrations

DESCRIPTION OF PLANT GENETIC MATERIALS

‘Newell’ bermudagrass: A public release from the USDA *Cynodon* collection

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Assigned to Associate Editor Michael Casler.

Funding information
National Institute of Food and Agriculture, Grant/Award Numbers: 2022-67013-36252, Hatch project 1018058

Abstract

Warm-season perennial grasses are the backbone of the pasture-based livestock industry in the lower southeastern United States, and bermudagrass (*Cynodon* spp.) is the most widely planted forage species, covering ~15 million ha. The genus *Cynodon* is native to southern Africa, and germplasm collections possess high genetic and phenotypic variability. The USDA National Plant Germplasm System maintains a collection of bermudagrass plant introductions (PIs) in Griffin, GA, and USDA-ARS, Tifton, GA, maintains additional germplasm. Multi-location trials were established in four states (Florida, Georgia, North Carolina, and Oklahoma) to screen *Cynodon* germplasm for herbage accumulation (HA), nutritive value (NV), and bermudagrass stem maggot (BSM) (*Atherigona reversura* Villeneuve). Due to the large genotype × environment interaction for HA, we focused on selecting accessions adapted to South Georgia and Florida, and further studies were performed in Florida. Several PIs showed improved HA and NV compared with ‘Tifton 85’. PI 316510, originally introduced from Ingelheim, Germany, produced high HA in Citra, FL, and Tifton, GA, with improved NV traits. In addition, PI 316510 had faster establishment and similar BSM tolerance to Tifton 85. We confirmed PI 316510 as tetraploid ($2n = 4x = 36$) through chromosome counts and flow cytometry, and it is genetically distinct from other commercial cultivars. PI 316510 has been publicly released under the name ‘Newell’, and it is vegetatively propagated. Planting material can be requested from the UF-IFAS Forage Breeding program.

Abbreviations: BSM, bermudagrass stem maggot; CP, crude protein; DAP, days after planting; HA, herbage accumulation; NPGS, National Plant Germplasm System; NV, nutritive value.

1 | INTRODUCTION

Bermudagrass (*Cynodon* spp.) is used in various agricultural systems in the southeastern United States (Taliaferro et al., 2004). The species is used for grazing, hay, and silage and is also of importance as turfgrass (Sollenberger et al., 2020). The genus *Cynodon* comprises 15 species and two cultivars (de Wet & Harlan, 1970), and *C. dactylon* (L.) Pers. is one of the most genetically variable and economically important species. The species *C. aethiopicus*, *C. plectostachyus*, and *C. nlemfuensis* are commonly known as stargrass; the other species are commonly known as bermudagrass (Taliaferro et al., 1997). The name "bermudagrass" is used herein to refer to all genotypes used in this study. All *Cynodon* species have a basic chromosome number of nine ($x = 9$), and ploidy levels range from $2n = 2x = 18$ to $2n = 6x = 54$ (Anderson et al., 2009; Grossman et al., 2021; Taliaferro et al., 1997).

The USDA-ARS National Plant Germplasm System (NPGS) maintains a *Cynodon* germplasm collection, and accessions are accessible through GRIN-GLOBAL (https://www.grin-global.org). Most accessions were collected around the world and include various species; however, passport information, ploidy level, and taxonomy are incomplete or unknown for many accessions. PI 316510 was collected by Dr. Glenn Burton in Ingelheim, Germany, in 1966. It was growing in a sod border around crop fields in a calcareous, sandy soil in a valley. This location was characterized by a mean temperature of 7.1°C and frost temperatures for about 4 weeks (https://www.grin-global.org). A bermudagrass forage core collection was assembled using phenotype evaluations for herbage accumulation (HA), cold tolerance, and other traits (Anderson, 2005; Anderson et al., 2009). The accessions included in the forage core collection are composed of plant introductions (PIs) and interspecific hybrids from Dr. Glenn Burton's former breeding program, and broad genetic diversity has been documented in this germplasm (Anderson, 2005; Anderson et al., 2009). The forage core collection, in addition to several PIs obtained from GRIN and several commercial cultivars, showed significant phenotypic variation for crude protein (CP), P, in vitro organic matter digestibility, and neutral detergent fiber concentrations (de Souza et al., 2020) and for HA and bermudagrass stem maggot (BSM; *Atherigona reversura* Villeneuve) tolerance in Florida (Grossman et al., 2021). The latter two studies were limited to data collected in Florida; however, there is increasing interest in the use of bermudagrass in the transition zone as a forage and nutrient receiver crop (Baxter et al., 2022; Spearman et al., 2021). More recently, de Souza et al. (2023) reported several PIs exhibiting greater HA and nutritive value (NV) in a multi-year study conducted in Ardmore, OK, and these PIs were well adapted to northern latitudes. Anderson et al. (2021) and Baxter et al. (2022) reported accessions and breeding lines with improved cold tolerance in trials performed in the field in north Georgia under controlled conditions.

Core Ideas

- PI 316510 produced high herbage accumulation in Citra, FL, and Tifton, GA.
- PI 316510 showed improved nutritive value.
- PI 316510 had faster establishment and similar bermudagrass stem maggot tolerance to Tifton 85.
- PI 316510 has been publicly released under the name 'Newell'.

The current hybrid bermudagrass cultivars were developed decades ago by Drs. Glenn Burton and Wayne Hanna in Tifton, GA. These cultivars provided vital ecosystem services and revolutionized the forage and livestock industry in the region (Bade, 2000; Corriher & Redmon, 2009). 'Coastal' and Tifton 85 are the two most planted forage bermudagrass cultivars. Coastal is a hybrid between 'Tift' bermudagrass and an accession from South Africa, and it was released in 1943. Tifton 85 is an interspecific hybrid between 'Tifton 68', a *C. nlemfuensis* hexaploid ($2n = 6x = 54$), and plant introduction (PI) 290884, a tetraploid *C. dactylon* (Burton et al., 1993). In Florida, stargrass cultivars have been utilized, including 'Ona', 'Florona', and 'Florico'; most recently, the bermudagrass cultivar 'Mislevy' was released in 2019 (Vendramini et al., 2021). Climate change, emerging pests, and the need for greater productivity require more investments in bermudagrass breeding, considering that most cultivars were released before the BSM became a serious problem in 2010 (Corriher & Redmon, 2009). Bermudagrass stem maggot was first discovered in the United States in 2009 in Los Angeles, CA, and it has since spread throughout the southeastern United States and worldwide (Baxter et al., 2019; Patitucci et al., 2016; Ribeiro et al., 2016). The BSM damages all bermudagrass cultivars, leading to yield reductions of up to 50%, but fine-textured cultivars exhibit greater susceptibility (Baxter et al., 2019). Furthermore, improving NV in bermudagrass is crucial for the livestock industry because NV and forage intake are the greatest factors affecting animal performance (Sollenberger & Vanzant, 2011). Breeding and selection in bermudagrass have resulted in improved cultivars exhibiting greater NV (Burton & Monson, 1972; Burton et al., 1967, 1984, 1993).

The USDA-NPGS and the core collection preserve unique sources of diversity for *Cynodon* spp. germplasm that can be exploited for breeding. The main goal of this study was to evaluate a vegetatively propagated *Cynodon* spp. germplasm collection for HA, NV, and BSM, and to select from these

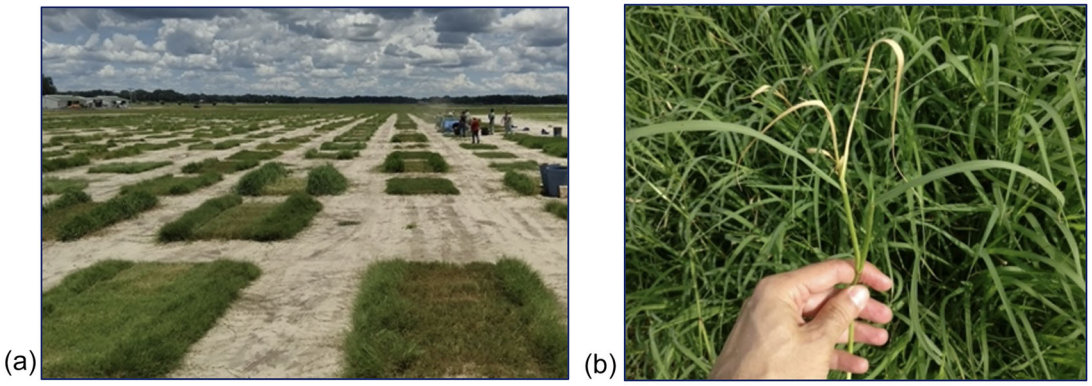

FIGURE 1 (a) Replicated germplasm screening for herbage accumulation (HA) using 286 accessions of *Cynodon* spp. across the southeastern United States. (b) Stem tip chlorosis and necrosis cause by bermudagrass stem maggot (*Atherigona reversura* Villeneuve), a new threat to bermudagrass pastures in the southeastern United States.

evaluations accessions that could be explored for public cultivar releases. Several studies were performed over multiple locations, years, and management practices that resulted in the selection and release of the new bermudagrass cultivar Newell.

2 | METHODS

2.1 | Study 1: Multi-year and multi-state germplasm screening for HA, NV, and BSM

A collection of 286 accessions of *Cynodon* spp. were vegetatively planted using plugs in replicated trials in Citra (29.4 N, −82.2 W), Ona (27.4 N, −81.9 W), and Marianna (30.9 N, −85.1 W), FL; Tifton, GA (31.5 N, −83.5 W); Jackson Springs, NC (35.2 N, −79.7 W); and Ardmore, OK (34.2 N, −97.1 W) (Figure 1a). Trials were established as a row-column design with two replicates in all locations except Ardmore, OK. In Ardmore, OK, a split-plot treatment arrangement was implemented with two nitrogen (N) rates (whole-plots) and genotypes (sub-plots). The N rates consisted of nonfertilized and N-fertilized treatments, and the N-fertilized plots received N after each harvest with split application of 150 kg N ha^{-1} in June and August 2016 and received 150 kg N ha^{-1} in April, June, and July 2017 (de Souza et al., 2023). Plots were harvested to determine HA. The number of harvests ranged from three (Jackson Springs, NC) to seven (Citra, FL) per year, and data from 2 years were used in this study. Additionally, four NV traits were measured in Citra, FL, using wet chemistry analysis for 11 harvests for a selected group of 15 genotypes following the methods described by de Souza et al. (2020). Visual ratings for BSM damage were collected multiple times during the summer in Ona and Citra, FL, and Tifton, GA, using a scale of 0–5 (where 0 = no visible damage and 5 = >90% damage) in Tifton, GA, and a scale of 0–9 (where 0 = no visible damage and 9 = >90% damage) in Citra and Ona, FL (Figure 1b). Variance components were estimated using linear mixed models in ASReml-R (Butler et al., 2017) in R (R Development Core Team, 2020) using a repeated measures model to account for multiple harvests within location, and genetic parameters were reported for HA. Significant effects of variance components were tested using a likelihood ratio test (LRT) with a χ^2 test with 1 degree of freedom (Satorra & Saris, 1985). Principal component analyses were performed with the *prcomp* function in R using a correlation matrix of the genotypic values obtained with the multi-harvest model. The genotype × location interaction was estimated using Pearson correlations using predictive values for HA.

2.2 | Study 2: Additional experiments in Florida

2.2.1 | Multi-location trials in Florida for HA

Three trials were planted in the spring 2017 in Ona, Marianna, and Hague, FL, and were allowed to establish during the first year. Plugs were vegetatively propagated for each genotype vegetatively and used for establishing the experiments under a randomized complete block design with 10 selected genotypes and four replicates (1.8 m by 4.6 m plot size). These 10 entries included Tifton 85, Jiggs, Florida 44 as controls and seven PIs selected for HA in the trial with 286 accessions. Herbage accumulation was measured five times in each location in 2018 and 2019, and plots were harvested approximately every 32 days after the staging harvest in April each year. Herbage accumulation was collected to a 10-cm stubble height from each plot, and fresh biomass was weighed before subsamples (~500 g) were taken, weighed, dried in a forced-air oven at 60°C for 72 h, and weighed again to estimate HA in kg ha^{-1} on a dry matter basis. Linear mixed models were implemented in ASReml-R in R following a repeated measures model to account for multiple harvests and multiple locations, and the 10 genotypes were considered as

fixed effects. Tukey's HSD test ($p \leq 0.05$) was performed to compare genotypes within locations.

2.2.2 | Establishment trials

A trial was planted in Gainesville, FL, on May 28, 2020, using Tifton 85, Jiggs, Mislevy, PI 316510, and entry 286. Plots (1.5 m by 12 m) were established using ~1600 kg ha^{-1} of fresh cut tops (>6 weeks growth) following a randomized complete block design with four replicates and a split-plot treatment arrangement. Herbicide treatments were dicamba (279 g active ingredient [a.i.] ha^{-1}) + 2,4-D ($C_8H_6Cl_2O_3$, 799 g a.i. ha^{-1}; Weedmaster, 7 days after planting [DAP]), aminopyralid (92.7 g a.i. ha^{-1}) + florpyrauxifen-benzyl (9.3 g a.i. ha^{-1}; Duracor, 7 DAP), sulfosulfuron (52.5 g a.i. ha^{-1}, Outrider, 14 DAP), and a control. Plots were fertilized with 37, 4, and 23 kg ha^{-1} of N, P, and K 30 DAP and with 60, 7, and 39 kg ha^{-1} of N, P, and K 60 DAP. Plots were visually assessed 30, 60, and 90 DAP for bermudagrass cover using a scale of 0–5, where 5 is equivalent to 100% cover.

An on-farm trial was established on April 10, 2018, at the North Florida Holsteins dairy farm in Bell, FL (29.7 N, −82.8 W). The five genotypes included in this study were Tifton 85, PI 316510, and three other selected accessions (276, 282, and 242). The goal for this on-farm trial was to serve as a breeding nursery to provide growers with planting material to start their nurseries. The experiment located in Citra, FL, was used as the source of vegetative plant material, mowed with a disk mower to a 5-cm stubble height at 7:00 a.m. The vegetative plant material placed in individual bags by genotype and transported to the farm. The soil at the experimental area was prepared by tilling with a shallow disc-harrowing, and the vegetative plant material was uniformly distributed on the soil surface. Each plot was 400 m^2 (40 m by 10 m), and 100 kg of fresh tops were planted per plot to account for the recommended rate of 2700 kg ha^{-1} (Hancock, 2016). A cultipacker and roller were used to increase soil–plant contact. The experimental area was irrigated to promote establishment and plots were fertilized with 50 and 27 kg ha^{-1} of N and K, respectively, 30 DAP. Herbage accumulation was assessed by clipping an area of 0.25 m^2 at a 5-cm stubble in five locations within each plot on June 17, 2019. Samples were weighed, and a subsample (~500 g per plot) was weighed and dried in a forced-air oven at 60°C for 72 h to estimate HA in kg dry matter ha^{-1}.

2.3 | Study 3: Ploidy determination

The protocol for chromosome counts and for ploidy determination are described in detail by Grossman et al. (2021). In summary, root tips were collected from the accessions potted in the greenhouse and pretreated with 0.0025% cycloheximide (Sigma-Aldrich) for 2 h at room temperature. Root tips were washed in distilled water and fixed in ethanol/acetic acid (3:1) solution overnight. Cell wall digestion was performed with an enzyme mix consisting of 10 µL of pecti-nase/cellulase solution (100:200 units) (Fisher Scientific), 5 µL of 5% pectolyase (MP Biomedicals), and 5 µL of 5% cytohelicase (Sigma-Aldrich) for 2 h and 15 min at 37°C. Slides were prepared by the cell dissociation and air-drying technique.

For flow cytometry, nucleic extraction buffer and CyStain Propidium Iodide Absolute P staining solution were prepared according to the CyStain PI Absolute P Kit instructions (Sysmex America), kept at 4°C, and protected from light. Internal standards included sorghum (*Sorghum bicolor* L.) with a 1C DNA content of 1.74 pg, rice (*Oryza sativa* L. ssp. japonica 'Nipponbare') with a 1C DNA content of 0.897 pg, and the maize (*Zea mays* L.) inbred line B73 with a genome size of 5.64 pg/2C. Additional bermudagrass standards included diploid (*Cynodon transvaalensis* Burtt Davy) breeding line AB33 (Pang et al., 2010), tetraploid [*Cynodon dactylon* (L.) Pers.] 'Celebration', and the PIs with counted chromosomes. For each accession, ~0.5 cm^2 of healthy, young bermudagrass leaf tissue from a minimum of two separate leaves was collected from the potted plants in the greenhouse and placed in petri dishes. A similar amount of the internal standard was co-chopped with the sample. To extract the nucleic DNA, 300 µL of nucleic extraction buffer was pipetted into the petri dishes, the leaves were chopped until completely macerated, and an additional 200 µL of extraction buffer was added. The petri dishes were placed on a slant to move the extraction buffer and tissue to the bottom of the plates to sit for an additional 30–60 s. The solution was filtered through a Celltrics 50-µm filter into 5-mL test tubes. Once the filter was removed, 2 mL of staining solution containing propidium iodide, RNAase, and staining buffer was added to the tubes. The final solution was incubated in the dark at 4°C for 30–60 minutes in a closed container with ice. The BD Accur C6 Flow Cytometer (BD Biosciences) was used to obtain 2C DNA content in picograms. The 2C DNA content, measured in picograms, was calculated by multiplying the mean value G1 sample peak by the internal standard DNA content and dividing the product by the mean value G1 internal standard.

2.4 | Study 4: Molecular fingerprinting

Plant material of Alicia, Russell, Jiggs, T85, Tifton 292, Newell, and T68 were provided by Lisa Baxter, Bill Anderson, and Esteban Rios. Leaf samples (~0.2 g) were placed into 2-mL tubes with four Zn-plated BBs (Daisy Outdoor Products) and placed into liquid N_2. Samples were repeatedly removed from the liquid N_2 and ground on a vortexer for <10 s and then placed back into the liquid N_2 until the plant material was a fine powder. DNA was extracted from shoot tissue using a

TABLE 1 List of primers used for genotyping six bermudagrass cultivars and PI 316510.

Primer name	Forward	Reverse	Repeat Motif[a]	Expected size (bp)
Comp62333_c0_seq3	GCCGAATCTAGCCCCGAC	CAAACTCCTCCGCGAGCA	(GA)7	180
Comp61529_c0_seq1	CGCCCTTCCTGAACTGCA	TCTCCTCCGCAGTCCTCC	(TGC)5	131
Comp60891_c0_seq12	CCAATCTGACGCCGGGTT	CGACGTCAGTTGAGGCGT	(CGC)7	168
Comp62384_c0_seq6	GTGGTGACCTGGCTGTCC	TCCGTCCTTTTCCGTGCG	(GAA)6	131
Comp61826_c0_seq6	CGTTGCTAGGCGAGGAGG	CGCTGCTGTCTTCTTGGC	(GCA)6	119

[a]Repeat motif length is from the bermudagrass cultivar Tifgreen.

TABLE 2 Broad-sense heritability (H^2), standard error for the H^2, and genotype × harvest interaction within location for herbage accumulation collected in 286 bermudagrass accessions evaluated across multiple years.

Location	H^2	SE	r_B
Jackson Springs, NC	0.33**	0.04	0.91
Ardmore, OK	0.44**	0.03	0.87
Tifton, GA	0.43**	0.02	0.73
Citra, FL	0.54**	0.02	0.86
Marianna, FL	0.08^{NS}	0.03	0.85

Note. Likelihood ratio test for the genotype random component: ns, nonsignificant.
**Significant at the 0.01 probability level.

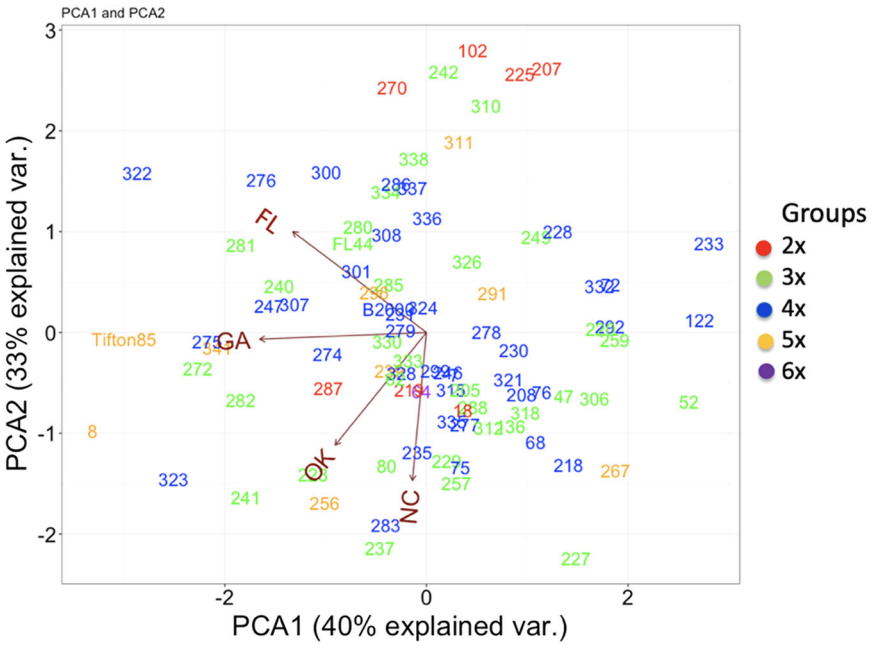

FIGURE 2 Principal component analysis (PCA) showing genotype by environment interaction for herbage accumulation across four locations (Citra, FL; Tifton, GA; Ardmore, OK; Jackson Springs, NC) in 286 bermudagrass accessions.

GeneJet Plant Genomic DNA Purification Kit (Thermo Fisher Scientific).

Bermudagrass DNA fragments were amplified from each sample using five polymorphic bermudagrass SSR markers (Table 1). The markers were created by Dr. Keenan Amundsen (University of Nebraska) from RNA sequencing data from the bermudagrass turfgrass cultivars Tifgreen, MiniVerde, and TifEagle. Each PCR reaction contained 2 µL of 5x Colorless GoTaq Flexi buffer (Promega), 1 µL of 25 mM $MgCl_2$, 0.8 µL of 2.5 mM dNTP mix, 1.8 µL of 1 mM M13 primer (M13-TGTAAAACGACGGCCAGT) 5′ labeled with either FAM or HEX, 0.5 µL of 1 mM of forward primer with a 5′M13 tag, 2 µL of 1 mM of reverse primer, 0.04 µL of GoTaq Flexi DNA polymerase, 0.86 µL of water, and 1 µL of sample DNA diluted to 2.5 ng μL^{-1}. The thermocycler conditions were an initial denaturation at 94°C for 3 min, 39 cycles of 94°C for 30 s, 50°C for 1 min, 72°C for 1 min and 10s, and a final elongation step at 72°C for 40 min. Polymerase chain reaction ampli-

TABLE 3 Predicted herbage accumulation (HA), crude protein (CP), phosphorous (P), in vitro organic matter digestibility (IVOMD), and neutral detergent fiber (NDF) concentrations for PI 316510, Florida 44, and Tifton 85 in Citra, FL.

Genotype	HA-2015 ($n = 5$)	HA-2016 ($n = 6$)	CP ($n = 11$)	P ($n = 11$)	IVOMD ($n = 11$)	NDF ($n = 11$)
			g kg^{-1}			
PI 316510	21,450	21,150	139a	3.2a	557a	662a
Florida 44	17,020	17,250	127a	2.9ab	461b	667a
Tifton 85	18,100	19,480	133a	2.8b	541a	693b

Note: HA represents predicted values across 286 accessions; post hoc comparisons were not conducted. Nutritive value traits are means for 11 harvests, and Tukey HSD test was performed among 15 selected accessions. Means with the same letter do not differ statistically ($P \leq 0.05$). For more details, see de Souza et al. (2020).

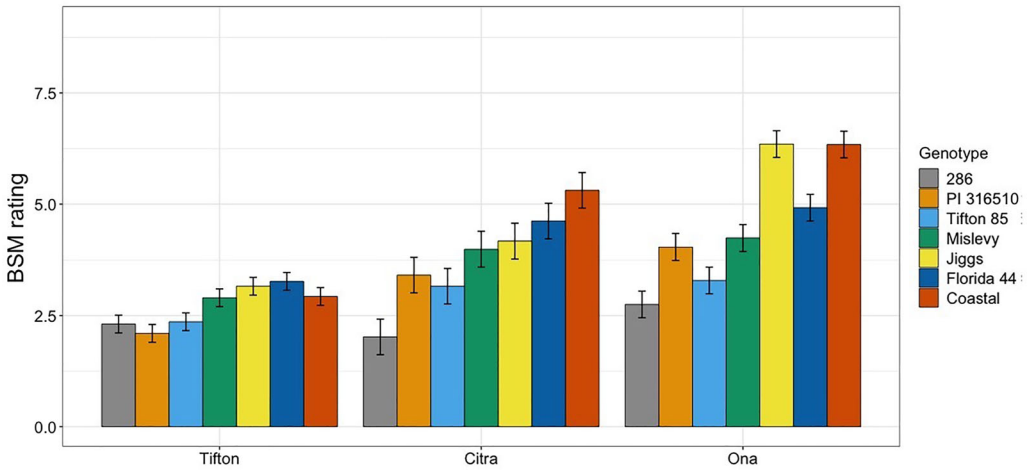

FIGURE 3 Bermudagrass stem maggot (BSM) ratings across three locations. Rating on a scale of 1–9 (where 1 = no visible damage and 9 = >90% damage) in Citra and Ona, FL, and on a scale of 0–5 (where 0 = no visible damage and 5 = >90% damage) in Tifton, GA. Values represent predicted values and their corresponding standard error for 286 accessions. Post hoc comparisons were not conducted.

cons were then diluted with 20 μL of molecular biology grade water. To load the PCR fragments onto the SeqStudio Genetic Analyzer (Thermo Fisher Scientific), each sample contained 8.5 μL of Hi-Di formamide (Thermo Fisher Scientific), 0.5 μL of GeneScan 500 ROX dye size standard, and 1 μL of diluted PCR sample. Samples were denatured on a thermocycler at 94°C for 5 minutes, then loaded onto the SeqStudio, and run using the default program "fragment analysis." The fragment size data were analyzed using GeneMapper software version 6 (Thermo Fisher Scientific). For each sized fragment, samples were coded as 1 if they had the allele of a particular size or 0 if they did not have the allele of a particular size. There were no missing data. The Excel ADD-IN, GenALEx v6.5 (Peakall & Smouse, 2006) was used to generate a principal coordinate analysis based on genetic distance.

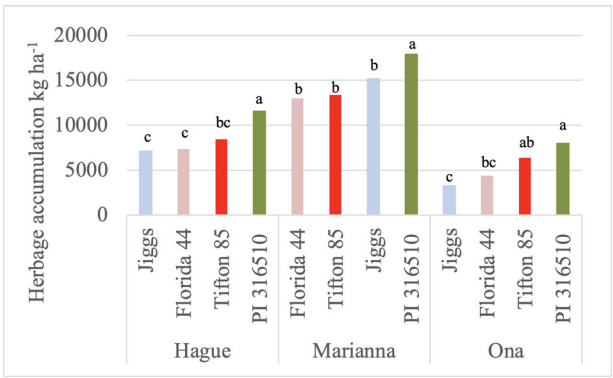

FIGURE 4 Mean herbage accumulation (HA; kg ha^{-1}) per year in Citra, Ona, and Marianna, FL, across 10 harvests performed between April 2018 and October 2019.

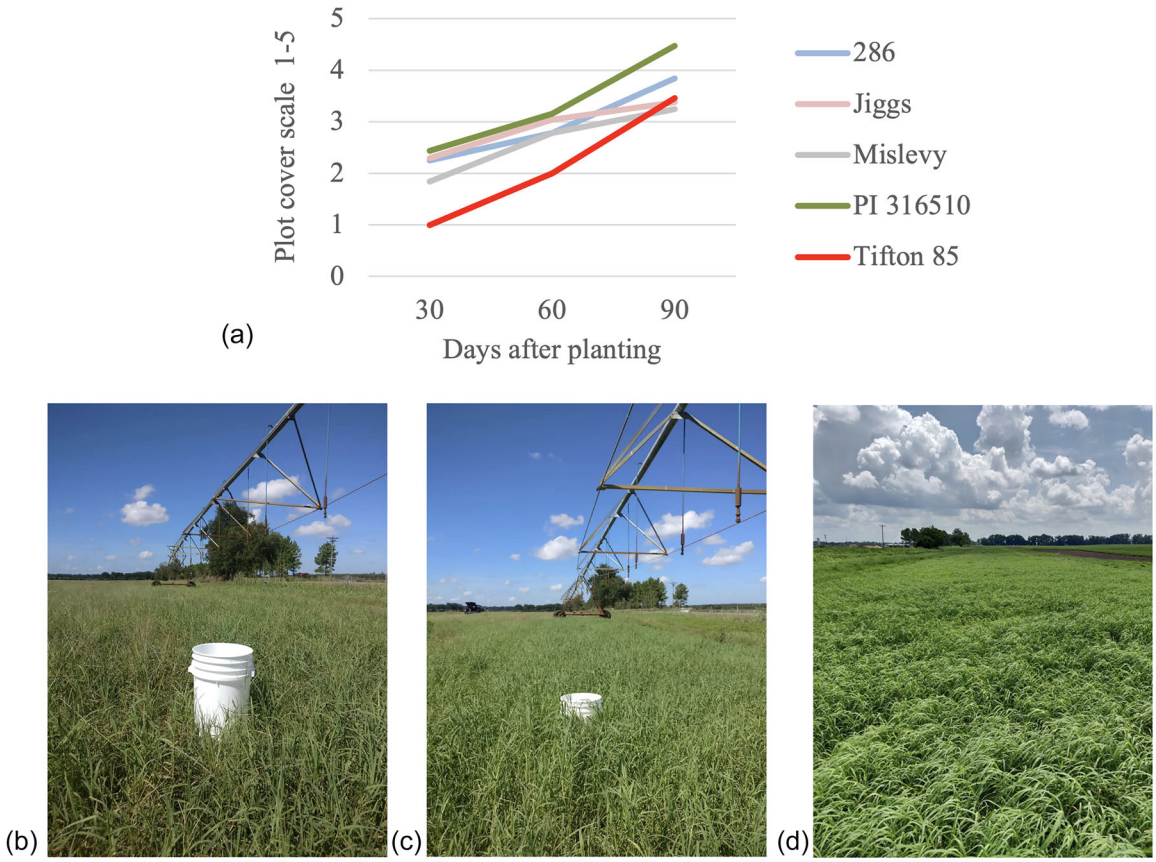

FIGURE 5 (a) Bermudagrass plot cover ratings 30, 60, and 90 days after planting in Gainesville, FL, for three cultivars (Tifton 85, Jiggs, and Mislevy) and two selected accessions (PI 316510 and 286). On-farm trial 60 days after planting at the North Florida Holsteins dairy farm in Bell, FL. (b) Tifton 85. (c) PI 316510. (d) PI 316510 plot prior to the harvest performed on June 17, 2019.

3 | RESULTS

3.1 | Study 1: Multi-year and multi-state germplasm screening for HA, NV, and BSM

The genetic variance for HA was significant in all locations for the single-location analyses (LRT; $p < 0.01$), except for Marianna, FL (Table 2); thus, Marianna was excluded from the multi-location analysis. The broad-sense heritability (H^2) estimate for HA for the multi-location model was 0.12 ± 0.02, and the genotype × location interaction was significant, given the low correlation (0.32) among predicted values across locations. These two parameters indicated the presence of genetic variation in the whole collection and showed a strong genotype by environment interaction for HA. A principal component analysis performed with genotypic values estimated for HA across four locations (Citra, FL; Tifton, GA; Jackson Springs, NC; and Ardmore, OK) demonstrated a clear genotype × environment effect for HA (Figure 2). Some genotypes produced greater HA in Florida (entry 322: PI 316510), compared with genotypes more adapted to higher latitudes (entries 283, 237, 256). Tifton 85 was the commercial cultivar that produced the greatest HA across locations (Figure 2).

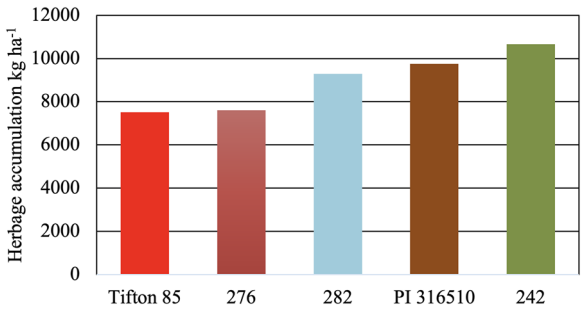

FIGURE 6 Herbage accumulation (kg ha^{-1}) in a single harvest performed in an on-farm trial conducted at the North Florida Holsteins dairy farm in Bell, FL. Mean values represent five subsamples per plot. Statistical analyses were not performed due to the lack of replications.

The predicted values for HA in Citra, FL, for three genotypes are presented in Table 3. PI 316510 had the greatest HA in 2015 and 2016 and greater HA than all controls in both years. At Tifton, GA, the HA for PI 316510 were less than Tifton 85 in 2015, but there was no difference in 2016 (data not presented). PI 316510 had greater P and lower neutral detergent fiber concentration than Tifton 85, and

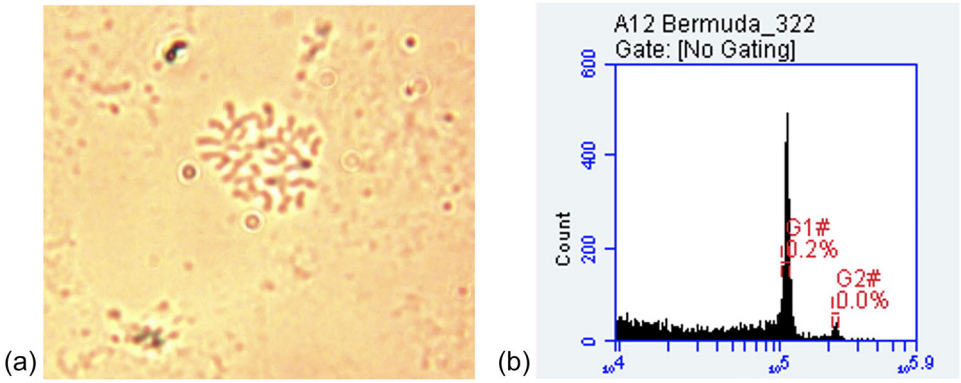

FIGURE 7 (a) Chromosomes of PI 316510 root tips under 400× magnification ($2n = 4x = 36$). (b) histogram of PI 316510 depicting the propidium iodide florescence area signals (FL2A) of the sample nucleic DNA.

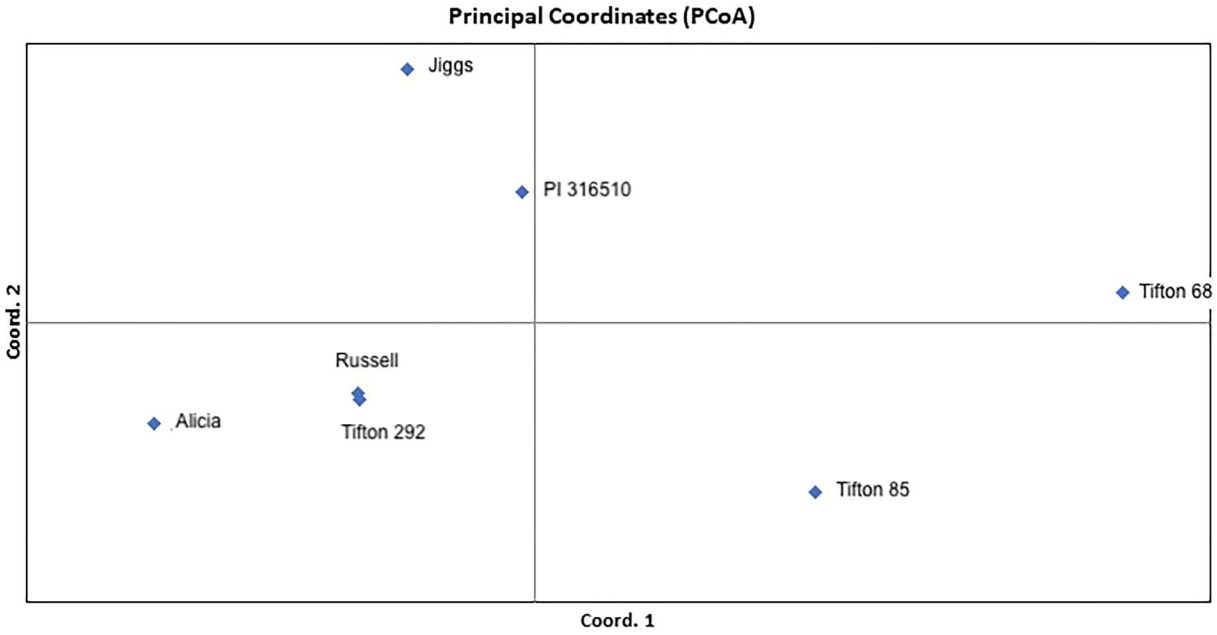

FIGURE 8 Principal coordinates analysis for six commercial bermudagrass cultivars and PI 316510 performed with five polymorphic bermudagrass simple sequence repeat markers.

similar CP and in vitro organic matter digestibility in Citra, FL (Table 3).

All entries were visually rated for BSM damage and none of the entries exhibited complete BSM resistance. Ratings in Tifton, GA (scale of 0–5, where 0 = no visible damage and 5 = >90% damage) were lower than Citra and Ona, FL (scale of 0–9, where 0 = no visible damage and 9 = >90% damage) due to different scale used in trials. Tifton85 had the least BSM damage for commercial cultivars, whereas Coastal, Florida 44, and Jiggs ranked among the most susceptible, especially in Citra and Ona, FL (Figure 3). PI 316510 had similar BSM damage to Tifton 85 in Tifton, GA, and Citra, FL, and similar BSM damage as Mislevy in Ona, FL. Entry 286 had the lowest BSM ratings across locations.

3.2 | Study 2: Additional experiments in Florida

3.2.1 | Multi-location trials in Florida for herbage accumulation

The averaged HA over 10 harvests, five harvests performed in 2018 and five in 2019, is presented in Figure 4. Despite

the considerable location variability for HA, the genotype × location interaction was not significant and PI 316510 had greater HA than Jiggs and Florida 44 across all locations, and greater than Tifton 85 in Hague and Marianna (Figure 4). Tifton 85 and Jiggs are currently among the most popular cultivars being cultivated in Florida and South Georgia, and these results show the advantage of PI 316510 for improved HA.

3.2.2 | Establishment trial

Tifton 85, Jiggs, and Mislevy had a slower establishment than the two selected accessions and 90 DAP reached an average of 3.4, which corresponds to approximately 80–90% of the plot covered with bermudagrass (Figure 5a). PI 316510 had faster establishment than Tifton85 and reached 4.5 by 90 DAP, representing 90–100% plot coverage (Figure 5a). Also, PI 316510 reached a rating of 3.2 by Day 60, which was similar to the rating recorded for Tifton 85 90 DAP. The improved rate for establishment possesses a relative advantage to cover the area faster, which reduces the potential for weed competition and may also affect HA production in the year of establishment. These two hypotheses will be further studied in future trials with PI 316510 and other cultivars.

The lack of replication in the on-farm trial prevented us from performing statistical analyses, but visual differences were observed in this trial (Figure 5b and c). Similar to the results in Figure 5a, Tifton 85 had a slower establishment (Figure 5b) and PI 316510 covered the area and had more biomass 60 DAP (Figure 5c). One year after the establishment, plots were sampled for HA and three accessions (242, PI 316510, and 282) produced greater HA than Tifton 85 (Figure 6). Despite the limited data available, the greater yield recorded for PI 316510 compared with Tifton 85 in replicated trials over multiple years and locations (Figures 2 and 4; Table 1) seem to maintain in on-farm trials; however, further studies will be conducted in Florida and Georgia to develop management recommendations for establishment practices and to conduct long-term HA performance in on-farm trials.

3.3 | Study 3: Ploidy determination

Chromosome counts and flow cytometry confirmed that the ploidy of PI 316510 is $2n = 4x = 36$ (Grossman et al., 2021) (Figure 7). The tetraploid nature of PI 316510 represents an important finding because it can be used as a parent in crosses to continue the development of improved bermudagrass cultivars in the future.

3.4 | Study 4: Molecular fingerprinting

Genotyping of PI 316510 and the bermudagrass cultivars Alicia, Russell, Tifton 292, Tifton 85, Tifton 68, and Jiggs revealed that PI 316510 is not closely related to any of the bermudagrass cultivars commonly used for forage (Figure 8). The release of unrelated cultivars is important because plants with greater diversity may be able to better withstand abiotic and biotic stresses than genetically homogenous lines (Ramanatha Rao & Hodgkin, 2002). In the past, bermudagrass cultivars have been accidentally released that were already existing cultivars or released with incorrect parentage information (Harris-Shultz et al., 2010; Wang et al., 2010), and thus the use of genotyping new cultivar releases prevents this from occurring.

4 | CONCLUSION

PI 316510 was obtained from the USDA-NPGS and was tested in multi-location and multi-year trials, showing greater HA and NV, faster establishment, and similar BSM tolerance compared with Tifton 85. PI 316510 is tetraploid and could be used as a parent in crosses to continue the development of improved bermudagrass cultivars. The cultivar name for PI 316510 is 'Newell', in honor of Dr. Wilmon Newell's legacy in Florida and the University of Florida. The on-site trials at the Plant Science Research and Education Unit in Citra, FL, and the on-farm trial located at the North Florida Holsteins dairy farm serve as breeding stocks to distribute the planting material to stakeholders.

AUTHOR CONTRIBUTIONS

E. F. Rios: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; visualization; writing—original draft. **Y. Lopez**: Data curation; investigation; writing—review and editing. **P. Munoz**: Conceptualization; funding acquisition; investigation; methodology; resources; writing—review and editing. **J. C. B. Dubeux Jr**: Investigation; methodology; resources; writing—review and editing. **J. M. B. Vendramini**: Data curation; investigation; methodology; resources; writing—review and editing. **M. Wallau**: Data curation; formal analysis; investigation; methodology; resources; writing—review and editing. **A. J. Grossman**: Data curation; investigation; methodology; validation; writing—review and editing. **W. Anderson**: Conceptualization; data curation; formal analysis; investigation; methodology; resources; supervision; writing—review and editing. **L. Baxter**: Investigation; methodology; resources;

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ACKNOWLEDGMENTS

This research was partially funded by the USDA National Institute of Food and Agriculture project 2022-67013-36252, and National Institute of Food and Agriculture, Grant/Award Number Hatch project 1018058. The authors thank the Forage Breeding and Genetics Lab members and staff at the Plant Science Research and Education Unit, Citra, FL, for providing help during data collection. The authors greatly thank the Florida Milk-Checkoff program and the Florida Cattlemen Enhancement Board funding for their support.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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How to cite this article: Rios, E. F., Lopez, Y., Munoz, P., Dubeux, J. C. B., Vendramini, J. M. B., Wallau, M., Grossman, A. J., Anderson, W., Baxter, L., Harris-Shultz, K., Castillo, M. S., Saha, M. C., Quesenberry, K., Blount, A., Reith, P., & Kenworthy, K. (2023). 'Newell' bermudagrass: A public release from the USDA *Cynodon* collection. *Journal of Plant Registrations*, 1–11. https://doi.org/10.1002/plr2.20318