Nutritive quality of Coastal bermudagrass treated with plant growth-promoting rhizobacteria

by

Connie Lea Fike

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Approved by

Elizabeth Wagner, Chair, Associate Professor of Animal Sciences
David W. Held, Associate Professor of Entomology
Russell Muntifering, Professor of Animal Sciences
Courteney Holland, Extension Specialist

Abstract

Plant growth-promoting rhizobacteria (PGPR) are naturally occurring, non-pathogenic soil bacteria that aggressively colonize plant roots. These beneficial bacteria increase nutrient uptake, pest resistance, drought tolerance, and promote root and top growth. Biofertilization with PGPR may enable reductions in nitrogen applications in hay production. The objective of this preliminary study was to determine the nutritive quality and biomass yield of Coastal bermudagrass (*Cynodon dactylon*) treated with PGPR in a hay production scenario. Bermudagrass sod was harvested during winter dormancy from a field maintained by a commercial hay grower. Sod was rinsed free of native soil and transplanted into 52 pots (0.0929 m^2 each) containing locally sourced field soil. Bermudagrass was treated with N and irrigated 3 times per week for 15 min during establishment (68 d). Each pot was an experimental unit. Pots were arranged into four blocks and assigned treatments using a randomized complete block design. Each block contained 13 pots including one untreated control, and each block represented a replicate. Treatments were arranged in a factorial design with PGPR (Blend 20 from Auburn University), 56 kg/ha of N (full rate), and 28 kg/ha of N (half rate) each applied at different time intervals. Initial plant heights were measured using a grazing ruler, and pots were given an initial treatment at d 0. Pots were irrigated as needed. On d 28, 56, 91 and 119, plant height was measured between 0700-0800 h, then forage was harvested to a 5.08 cm stubble height, and biomass (per 0.0929 m^2) was calculated. Forage was sealed in plastic bags and immediately transported to the laboratory for processing. Pots were re-treated following the assigned treatment schedule post-harvest. Dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) concentrations were determined for each

harvest. Statistical analysis was performed using JMP 12.0.1 (SAS, Inc.) with significance set at $P < 0.05$. Data were analyzed using the MANOVA procedure. Individual date × treatment interactions were analyzed using LSMeans Contrast procedure. The control was similar to PGPR for biomass production, DM, NDF, ADF, and ADL. Full rate of N and PGPR differed in biomass production, but PGPR was similar to half rate of N at some harvest dates. PGPR was similar to the half rate of N when evaluating DM, and was similar to full rate of N at some harvest dates. For ADF, NDF and ADL, PGPR was similar to the full rate and half rate of N at some harvest dates. This study is one of the first reports on the effect of PGPR on nutritive quality of forage-type bermudagrasses. Further research is needed to explore the efficacy of biofertilization of forage bermudagrass on a larger scale.

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INTRODUCTION

Bermudagrass (*Cynodon dactylon*) is a warm-season perennial forage that is widely used in grazing and hay systems in the Southeast. 'Coastal' is an F_1 hybrid of 'Tift common bermuda' crossed with a South African bermudagrass variety. The variety was released in 1943 by the USDA and the University of Georgia Coastal Plain Experiment Station in Tifton, GA. 'Coastal' is best adapted to the Coastal Plain and lower Piedmont areas. It is estimated that there are approximately 6 million hectares of this variety in the Southern United States (Lee et al., 2013). Due to the inability to produce an adequate amount of seeds 'Coastal', like other hybrids, must be sprigged for establishment. 'Coastal' is not as cold-tolerant as 'Tifton 44' but is more drought tolerant and higher yielding than 'common' bermudagrass. 'Coastal' grows taller than common bermudagrass and is coarse-stemmed, growing both rhizomes and stolons. It is also highly responsive to N fertilization (Ball and Pinkerton, 2002; Ball et al., 2015).

Plant growth-promoting rhizobacteria (PGPR) are non-pathogenic, soil inhabiting, beneficial bacteria that are able to colonize the seeds and roots of plants (rhizosphere) (Kloepper and Schroth, 1978). For PGPR to be beneficial, the bacteria must efficiently colonize the root surface of the host plant. These bacteria benefit their host plants through increasing drought tolerance, pest resistance, nutrient uptake, and promoting root and top growth. Beneficial effects can be either through direct or indirect mechanisms. Direct mechanisms include: N_2 fixation (biofertilization), plant stress control, increasing availability of soil nutrients, and stimulation of root growth (Lugtenberg and Kamilova, 2009; Vessey, 2003; Nelson, 2004). In addition, PGPR are able to suppress severity of pathogens through induced systemic resistance (ISR), reducing

the level of disease, antibiosis, and through competition for nutrients as a means of indirectly stimulating plant growth (Lugtenberg and Kamilova, 2009; Nelson, 2004).

Despite extensive research in agronomic crops using bacterial inoculants, there are relatively few studies describing the effect of PGPR on grasses, especially forage-type grasses. Baltensperger et al. (1978) evaluated the effect of nitrogen-fixing bacterial inoculants on eight genotypes of turf-type bermudagrasses at varying N fertilization rates in a greenhouse. There were no differences among genotypes. However, increased biomass and foliar nitrogen were seen due to bacterial inoculation (Baltensperger et al., 1978). A similar study was performed by Coy et al. (2014) to evaluate 16 bacterial blends for growth promotion in Tifway (*Cynodon dactylon (L). Pers. x Cynodon transvaalensis Burtt-Davy*), a hybrid turf-type bermudagrass, under growth chamber and greenhouse conditions in two experiments. The bacterial strains were isolated by Auburn University's Department of Entomology and Plant Pathology. Coy et al. (2014) reported root and/or top growth with certain blends as a result of inoculation, and concluded that Blends 19, 20, MC 2, and MC 3 should be evaluated further for use in grass systems (Coy et al., 2014). Blends developed by Auburn University have shown improvements in growth in various crop systems but there is little known about the physiological effects on the plant in terms of changes in nutritive quality. For this reason, a preliminary study was conducted to determine the effects of Blend 20 (Auburn University's Department of Entomology and Plant Pathology) on the nutritive quality of forage-type 'Coastal' bermudagrass under a hay production scenario.

LITERATURE REVIEW

COASTAL BERMUDAGRASS

History and characteristics

Bermudagrass is a sod-forming grass that is grown throughout the Southeast United States for hay, pasture, and turf. There are many varieties of bermudagrass utilized by cattle producers and horse owners in the Southeast, but one of the most commonly used varieties is 'Coastal' bermudagrass. 'Coastal' was the first hybrid developed for use in southern forage programs, and is a cross between 'Tift common bermuda' and a bermudagrass variety introduced from South Africa (Burton, 1948). It was released by the USDA-ARS and the University of Georgia Coastal Plain Experiment Station in 1943 (Burton, 1948; Lee et al., 2013). The cultivar is light green, coarse-stemmed, tall growing, and spreads using rhizomes and stolons. Hybrid bermudagrass varieties are considered sterile due to their inability to produce enough viable seeds for propagation. This characteristic requires 'Coastal' and other hybrids to be established from vegetative planting material, such as rhizomes or stolons, commonly called sprigs.

When compared to 'common' bermudagrass, 'Coastal' has improved vigor and higher yields. It can yield up to twice as much as common bermudagrasses and is better suited for hay production or grazing fields (Lee, 2013). Burton (1948) stated that 'Coastal' is relatively drought tolerant, is generally higher producing in late summer and early fall, and goes into dormancy later compared to 'common'. 'Common' sod has a greater incidence of weeds and is less resistant to leaf spot (*Helminthosporium*) than 'Coastal' that is managed properly (Burton, 1948). Another advantage to 'Coastal' is its greater resistance to root knot nematode compared to

‘common’ and when grown in association with root knot susceptible legumes performance of the legume increases (Burton, 1948; Lee et al., 2013).

‘Coastal’ is more cold or frost tolerant than ‘common’ (Burton, 1948) but less winter hardy than ‘Tifton 44’ (Burton and Monson, 1978). Bermudagrass as well as other warm-season plants store carbohydrates as starch in the leaves of plants (Longland and Byrd, 2006). Bermudagrass, during winter dormancy, relies on stored carbohydrate reserves to survive the winter and uses those reserves to begin growing in the early spring until leaves are better developed to sustain growth (Ball et al., 2015; Lee et al., 2013; Ball and Pinkerton, 2002).

Establishment and management

Approximately 6 million hectares of ‘Coastal’ has been established for hay and grazing in the southern United States (Lee et al., 2013). It is best adapted to the Coastal Plain and lower Piedmont regions where the typical seasonal production is May through September or October, depending on how far north the area expands. Bermudagrass is deep-rooted, requires well-drained soils, and can be adapted to sandy soils because it uses water efficiently (Burton, 1948). ‘Coastal’ can be sprigged, using a commercial sprigging machine, from January until late July, but ideally sprigs should be in winter dormancy when established (Ball et al., 2015). Planting while the grass is still in winter dormancy is highly successful for a few reasons. Proper soil moisture is key to successful establishment and, typically, soil moisture conditions are more favorable in the late winter or early spring (Lee et al., 2013). Sprigs that have been dug during winter dormancy have higher stored energy reserves to initiate growth once temperatures rise compared to sprigs dug in early spring. Though sprigs are the best source of vegetative material, ‘Coastal’ can also be established using mature stems of plants referred to as topgrowth. Topgrowth used for establishment should have six or more nodes and be six to seven weeks old

(Lee et al., 2013). Weed control is a major factor for successful establishment of bermudagrass varieties, and stands should be treated using a pre- and post-emergent herbicide to control broadleaf weeds (Hansen et al., 2000). To reduce weed competition, nitrogen fertilizer should not be applied at planting. The first nitrogen application can be applied at rates of 33.6-56 kg/ha when stolons reach 7-15 cm in length, and the second application can be applied 30 days later (Jennings et al., 2013). New stands of 'Coastal' should either be lightly grazed or mowed for hay the first year of establishment (USDA-ARS, 2016), and grass should not be grazed until the grass is 15-20 cm tall (Hansen et al., 2000).

The most important factor effecting forage quality is stage of maturity. There is a decline in digestibility as plants grow from a leafy, vegetative stage to the reproductive stage when the plant begins producing seeds (Blaser, 1962). For example, Norman and Richardson (1937) reported an increase in structural carbohydrates (cellulose and lignin) as ryegrass (*Lolium perenne*) reached the reproductive stage. Increases in cellulose and lignin over time can also be seen in perennial grasses such as bermudagrass. Forages cut for hay during boot stage will have less fiber and lignin compared to hay cut during full bloom (Blaser, 1962). Shorter harvest intervals result in less mature, higher quality forages. Less mature forages tend to have a lower dry matter content and a higher crude protein (CP) content than forages subject to more time between harvests (Overman and Scholtz, 2003) There is a decline in CP of 'Coastal' bermudagrass when harvests were delayed from 2 to 8 weeks regardless of varying rates of applied nitrogen (Prine and Burton, 1956). Net CP losses are seen during mature stages of growth due to large decreases in leaf/stem ratios and increase in concentration of structural carbohydrates (Blaser, 1962).

Reductions in digestible energy can be attributed to increases in structural carbohydrates, lignification, and a reduction of soluble carbohydrates and digestible protein when used for energy (Blaser, 1962). Ethredge et al. (1973) reported that harvest interval and harvest clipping height both had influences on yield and quality. The highest average energy production was obtained from the 3-wk clipping frequency and 0-cm clipping height, while the lowest average energy production was from the 7-wk interval and the 14-cm height. It was noted that plots clipped on 7-wk intervals and at a 14-cm height accumulated dry, dead stubble later in the season compared to plots with a shorter harvest interval and clipping height (Ethredge et al., 1973). Harvesting grasses at 4-wk intervals compared to 8-wk intervals increases crude protein, ether extract, and ash content of the forage but lower crude fiber, cellulose, acid detergent fiber (ADF), acid detergent lignin (ADL), and cell wall content values are seen in younger forages (Utley et al., 1971). The current standard harvest interval is 28 days, or 4 weeks, for 'Coastal' bermudagrass. When the variety was first released, Burton (1948) determined that the ideal cutting interval for 'Coastal' was 4-5 weeks based on the crude protein content of four hay cuttings that were chemically analyzed (Burton, 1948).

Nitrogen Fertilization

Nitrogen fertilizer is a necessary input for productive, high-quality forages, and bermudagrass is highly responsive to nitrogen applications (Wilkinson and Langdale, 1974). Burton and Jackson (1962) evaluated the effect of rate and frequency of application of six nitrogen sources on 'Coastal' and determined that for most forms of nitrogen applied, forage yield increased. It was also determined that splitting applications resulted in higher yields in all but one of the fertilizer types (Burton and Jackson, 1962). Bermudagrass yield and quality are maximized when nitrogen is applied at rates greater than 400 kg/ha/yr (Overman et al., 1992) but

bermudagrass root requirements are met at lower levels of 100 kg/ha/yr (Wilkinson and Langdale, 1974). Fertilization recommendations vary based on the quality of the soil used in the forage system as well as location and soil type. However, the current recommendations are to use only amounts needed to produce required forage at rates of 33.6-224 kg/ha/yr for pasture and 224-672 kg/ha/yr for hay production (USDA-ARS, 2016). Phosphorus and potassium also play vital roles in productive forage and should be applied at a ratio of 4:1:2 nitrogen to phosphorus to potassium (USDA-ARS, 2016).

Hay production

Forage conservation, as either hay or silage, is an important part of livestock production and provides farmers with a high-quality alternative to pastures when managed correctly. Hay is one of the most widely grown crops in North America (Rohweder et al., 1978), and in Florida, total production is between 600,000 and 800,000 tons per year (Chambliss et al., 2006). Hay production is a method of forage conservation that requires the crop to be dried (cured) so that the crop is biologically inactive and cannot spoil due to microbial activity (Muck and Shinners, 2001). Hay crops should be at 12-15% moisture before baling, and hay baled between 18-20% moisture could result in heat and mold of the bale, especially in large bales (Chambliss et al., 2006). Modern hay systems are largely mechanized and harvested, dry hay can be packaged in the form of bales, stacks, cubes, and pellets. Bales come in many different sizes but come in three configurations: small square, large square, and large round (Muck and Shinners, 2001). To avoid weather damage after baling, hay should be stored off the ground and under a shelter.

Perennial forages such as bermudagrass, bahiagrass, and alfalfa are generally recommended for hay production instead of annuals to avoid repeated costs of establishment each year (Chambliss et al., 2006), but annuals such as sorghum-sudangrass and ryegrass can be

used effectively for hay (Bates, 2007). Within each class of forage, there can be variation in quality and nutritive value based on management practices. For high quality bermudagrass, nitrogen fertilization rates of 224-672 kg/ha/yr should be applied in split applications to increase nutrient use efficiency of the grass (USDA-ARS, 2016; Hansen et al., 2000). The first hay crop of the season should be harvested when the bermudagrass is approximately 45 cm tall, and harvests should take place 4-5 weeks after the first cutting for optimal quality hay (USDA-ARS, 2016; Hansen et al., 2000).

A forage's stage of maturity at harvest is crucial to producing high-quality hay but there are other factors that influence hay quality such as loss of dry matter and nutrients due to leaf shatter and environmental factors. In a three-year study evaluating the effects of weather, yield, and quality of fresh forage on drying rate, yield, and quality of hay, Hart and Burton (1967) reported considerable losses in dry matter and crude protein caused by rain during the drying process while losses were very small in good weather. After the first harvest, hay should be cut at 4-5 weeks of age if the grass can be cured without being rained on (Chambliss et al., 2006; Hart and Burton, 1967). If harvest is delayed beyond 6 weeks, quality declines and there is no further increase in yield (Chambliss et al., 2006).

PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR)

Background

The rhizosphere, the portion of the soil that surrounds and is influenced by the roots of plants, offers a place for proliferation of soil bacteria (van Loon, 2007). The roots of plants secrete metabolites (root exudates) that can be used as nutrients by the microbial population within the rhizosphere (Lugtenberg and Kamilova, 2009). Bacterial population densities in the

rhizosphere are greater than those in bulk soil (Lugtenberg and Kamilova, 2009) and up to 15% of the root surface may be covered by a variety of bacterial strains in the form of microbial colonies (van Loon, 2007).

Plant growth-promoting rhizobacteria (PGPR) are non-pathogenic, free-living, beneficial bacteria that colonize the seeds and roots of plants (Kloepper and Schroth, 1978). When in association with the host roots, PGPR can stimulate host plant growth either in roots or above ground growth. These bacteria are highly adaptable to a vast variety of environments, fast growth rates, and are biochemically versatile to metabolize a wide variety of compounds (Bhattacharyya and Jha, 2012). Bacterial strains must fulfill two out of the three following criteria to be classified as PGPR: aggressive colonization, stimulation of plant growth, and biocontrol (Weller et al., 2002; Vessey 2003). To be beneficial, PGPR must be able to compete well with other microbes for nutrients and areas of the root that can be colonized. Poor colonization can limit biocontrol efficiency and competition for nutrients. Microbes can benefit plants directly by using mechanisms that stimulate growth in the absence of pathogens or indirectly by using mechanisms that protect the plant against soil borne pathogens such as fungi (Lugtenberg and Kamilova, 2009).

Direct Mechanisms

The term biofertilizer is used by many people and is often used interchangeably with organic fertilizer. Organic fertilizers are defined as fertilizers containing organic compounds which directly or indirectly increase soil fertility. Biofertilizers are substances containing living microorganisms (bacteria or fungi) which colonize the rhizosphere, or the interior of the plant, and increase the supply or availability of nutrients to the host plant (Vessey, 2003). Only certain genera, species, and strains of theses microorganisms are beneficial to plants and are used in

biofertilizers. For PGPR in biofertilizers to effectively promote growth, there must be a symbiotic relationship between the plant host and bacteria. There are two types of relationships associated with PGPR: endophytic (capable of living within plant tissues) and epiphytic (capable of living on plant roots) (Vessey, 2003).

Nitrogen fixing bacteria form nodules on roots of plants and convert atmospheric nitrogen to ammonia, which can be utilized by the plant as a nitrogen source (Lugtenberg and Kamilova, 2009). The bacterium *Azospirillum*, for example, are free-living nitrogen fixers that increase root development, resulting in increased crop yield. Certain strains of PGPR produce siderophores which convert iron to a form that can be utilized by plant roots. Some PGPR strains increase the solubilization of phosphorus to make it available for plant uptake (Nelson, 2004).

Plant growth-promoting rhizobacteria can use nitrogen fixation as a means of directly stimulating plant growth, but nitrogen fixation is not the only mechanism utilized by rhizobacteria to directly increase growth. Some PGPR strains protect the roots and seeds of plants by degrading pollutants in the soil that could adversely affect plant growth. These bacteria (rhizoremediators) survive on root exudates, and live near the roots of plants (Lugtenberg and Kamilova, 2009). Certain PGPR strains, termed phytostimulators, synthesize phytohormones that stimulate growth. Other PGPR facilitate growth by decreasing plant stress. These bacteria decrease ethylene levels used as an indicator of plant stress (Vessey, 2003). Strains of PGPR may use one or more of these mechanisms in the rhizosphere to stimulate plant growth.

Indirect mechanisms

The direct effects of PGPR on plants often cause morphological or physiological changes that can also influence interactions with plant pathogens. Non-pathogenic rhizobacteria can antagonize pathogens through competition for nutrients, production of antibiotics, and secretion

of lytic enzymes (van Loon, 2007; Lugtenberg and Kamilova, 2009) resulting in biocontrol of pathogens. Other biocontrol mechanisms include: signal interference, predation, parasitism, and induced systemic response (ISR). Induced systemic response increases the plant's ability to defend itself from diseases, leading to a reduction in the rate of disease development and fewer diseased plants (van Loon, 2007).

Insect effects

In addition to aiding in resistance to crop disease, beneficial microbes can also interact and affect aboveground insects or herbivores through plant-mediated mechanisms. These mechanisms can lead to either positive or negative effects on the insect. Positive effects stem from the beneficial microorganism's ability to stimulate growth of the plant which translates to an increased food supply for herbivores. However, beneficial microorganisms can promote plant tolerance to herbivory by improving water and nutrient uptake which allows the plant to regrow plant tissue and biomass in the presence of herbivores (Pineda et al., 2010).

Plant-mediated effects of beneficial microorganisms on herbivores are species dependent and varies with single microbial species or with multiple species (Pineda et al., 2010). One study in *Arabidopsis* determined that the use of *Pseudomonas fluorescens* negatively affects the development of beet armyworm (*Spodoptera exigua*) (van Oosten et al., 2008). Plant genotype also plays a role in interactions between beneficial microorganisms and insects (Pineda et al., 2010). For example, *Rhizobium leguminosarum* in white clover (*Trifolium repens*) had a positive effect on performance of beet armyworm but when another cultivar that produced defense-related cyanogenic compounds was used the positive effect was neutralized. Through mutualism with the *Rhizobium* there was a surplus of nitrogen available that could be used for plant growth as well as production of defense compounds (Kempel et al., 2009).

Plant hormones salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) play major roles in plant defense signaling pathways which are involved in induced plant defense against pathogens and herbivory (van Oosten et al., 2008). Specifically, the JA-signaling pathway is important in the emission of complex volatile blends which the plants will emit when under attack of an herbivore, and the volatiles will attract the natural enemies of the herbivore. Beneficial microorganisms which induce JA responses will affect the composition or rate of emission of those volatiles.

Previous work

Previous research on PGPR has been geared towards agronomic crops such as corn, soybean, and cotton. Some of the first studies of the effect of PGPR on crops were performed using potatoes, sugar beets, and radishes. Kloepper and Schroth (1978) reported evidence indicating growth stimulation of radishes caused by specific strains of rhizobacteria. The authors named were the first to use plant growth-promoting rhizobacteria and PGPR. In this study and those that have followed, bacteria thought to be growth-promoting are screened and selected for their ability to increase root growth, above ground biomass, or total plant weight (Kloepper and Schroth, 1978; Coy et al., 2014). The mechanisms (nitrogen fixation, siderophores, phosphorus solubilization) of these strains can also be characterized. Candidate strains can either be used alone or in combination with other bacteria in a solution as a blend or bacterial consortium.

Though grasses can be considered a forage, pasture, or turf crop, limited research has focused on how PGPR affects the growth of grasses. One of the first studies on the effect of PGPR on grasses (Baltensperger et al., 1978) examined whether inoculation of various genotypes of bermudagrass with *Azospirillum* and *Azobacter* (nitrogen fixing bacterial strains) would increase growth and nitrogen content under greenhouse conditions. They also evaluated if

genotypes of bermudagrass responded differently to inoculation. There were no interactions between bermudagrass genotypes and inoculum. Mean crown and root production and total dry matter were not different as a result of inoculation, but there was a difference in response to inoculation among genotypes. Baltensperger et al. (1978) determined that the increase in top growth from inoculation caused an increase in total nitrogen accumulation.

Ker et al. (2012) hypothesized that Switchgrass (*Panicum virgatum* L.) seeds inoculated with a mixed culture of 8 bacterial strains would increase switchgrass production across a range of soils and environmental conditions. Authors found that inoculation of seeds using PGPR increased overall yield compared to uninoculated plots due to increased stand density. In both fertilized (100 kg N/ ha) and unfertilized plots (0 kg N/ ha), inoculation caused an increase in the number of tillers (the plant shoot that springs from the root or the original shoot) per area which led to higher yields compared to uninoculated switchgrass plants, and plants were taller due to inoculation. It is also important to note that inoculated plants (with and without fertilizer input) and uninoculated plants treated with 100 kg N/ ha had increased stand density and yield compared to unfertilized, uninoculated switchgrass plots (Ker et al., 2012). A combination of fertilizer input and inoculation increased yield by 123%, and Ker et al. (2012) concluded that there is a potential yield increase of 40% from PGPR inoculum alone.

Coy et al. (2014) evaluated bacterial blends isolated at Auburn University's Department of Entomology and Plant Pathology to determine their effects on growth promotion of hybrid, turf-type bermudagrass. This study was conducted using two experiments, one of which used growth chamber conditions, and the other experiment used greenhouse conditions similar to those used by Baltensperger et al. (1978). In the growth chamber experiment 12 bacterial blends were evaluated, and six blends, including Blend 20, enhanced shoot weight by 236 to 345%

compared to the control. In the greenhouse experiment, eight blends were evaluated. Blends 8, 18, 19, and 20 enhanced weight of top growth by 158 to 197% relative to control. Blend 20 increased root length by 157%, and Blends 19 and 20 significantly increased root surface area by ≥ 173% and root volume by 186% relative to the untreated plants. Coy et al. (2014) concluded that Blends 19, 20, MC 2 and MC 3 should be further investigated for their use in pasture systems.

Future application

There is growing concern for the overuse of inorganic fertilizers used in crop systems and their harmful impact on the environment. Fertilizers are essential to modern agriculture to produce high quality and higher yielding crops. There is a potential for the use of PGPR in agriculture to decrease the amount of inorganic fertilizer necessary for crop production. A study by Adesemoye et al. (2009) looked at reduced rates of inorganic fertilizer coupled with PGPR or PGPR plus arbuscular mycorrhiza fungi (AMF) compared to full rates of fertilizer for plant growth, yield, and nutrient uptake of tomato (*Solanum lycopersicum*) plants. They also determined the minimum level of fertilizer reduction that could be used with inoculants. Results of this study indicated that PGPR or PGPR plus AMF can improve the nutrient use efficiency of fertilizers. Adesemoye et al. (2009) determined that when inoculants are applied fertilizer use can be reduced to 75% of the typical rate while achieving similar growth responses. The results also demonstrated that inoculants could not replace chemical fertilization altogether but could decrease the overall input of fertilizer used without sacrificing plant productivity (Adesemoye et al., 2009). There are commercially available products containing specific PGPR strains which are intended for specific crops (Bhattacharyya and Jha, 2012). The interest in these products is greatly increasing, and it has been suggested that the global market for biofertilizers has been

projected to reach over $2.2 billion by 2018. Between 2013 and 2018, the biofertilizer markets are expected to have a growth rate of 12.5% annually (Calvo et al., 2014). Success of these products is dependent on rhizosphere management which requires further consideration of soil and crop systems as well as inoculant formulation and delivery methods (Nelson, 2004). Overall, PGPR offers a promising solution to sustainable, environmentally-friendly crop production.

MATERIALS AND METHODS

Sod harvest and establishment

On March 24, 2016, Coastal bermudagrass sod was mechanically harvested in 30.48 × 45.72-cm slabs from a hay field grown and maintained by a commercial hay grower in Midway, AL. The sod was transported to a 72-m^2 gravel patio covered in landscaping fabric behind the Pesticide Research Laboratory at Auburn University in Auburn, AL. Sod slabs were washed free of native soil, and the remaining grass mats were transplanted into 62 0.09-m^2 pots. Each pot contained 27.21 to 31.75 kg of sandy loam field soil taken from E.V. Smith Research Center in Milstead, AL. During the 68-d establishment period, ammonium sulfate (PRO fertilizer, 21-0-0, Harrell's Inc., Lakewood, FL) was applied weekly for four wks, at a rate of 650 mg/ 0.09 m^2 (56 kg/ha total for 4 wks). Overhead irrigation, accumulating to 1.27 cm of water, was applied post fertilizer application. Weeds were removed by hand weekly for 3 wks from May 6, 2016 to May 27, 2016. Overhead irrigation of 1.27 cm water was applied 3 days per week (15 minutes).

Treatment structure and assignment

Treatments were arranged in a factorial design with a control (water only), PGPR, 56 kg/ha of N (full rate), and 28 kg/ha of N (half rate) applied at different time intervals. There were 13 total treatments (Table 1), each assigned a letter A-M. Treatment A was the control. The PGPR treatments were represented by letters B-E with B applications on d 28, 56, 91, and 119; C applications on d 28; D applications on d 56, and E applications on d 91. Full rate of N treatments were represented by letters F-I with F applications on d 28, 56, 91, and 119; G applications on d 28; H applications on d 56; and I application on d 91. Letters J-M represented

Table 1. Treatment assignment of control, PGPR, full rate N, or half rate N to Coastal bermudagrass in 0.09m^2 pots.

Untreated Control

	Block 1	Block 2	Block 3	Block 4	Day				
Letter	Pot#				0	28	56	91	119
A	14	52	42	18	-	-	-	-	-

PGPR Treatment

	Block 1	Block 2	Block 3	Block 4	Day				
Letter	Pot#				0	28	56	91	119
B	50	54	16	43	X	X	X	X	-
C	15	39	40	17	X	X	-	-	-
D	34	6	56	3	X	-	X	-	-
E	12	9	21	4	X	-	-	X	-

Nitrogen (50lb/acre)

	Block 1	Block 2	Block 3	Block 4	Day				
Letter	Pot#				0	28	56	91	119
F	49	10	26	1	X	X	X	X	-
G	47	5	57	45	X	X	-	-	-
H	30	28	23	8	X	-	X	-	-
I	51	29	24	44	X	-	-	X	-

Nitrogen (25lbs/acre)

	Block 1	Block 2	Block 3	Block 4	Day				
Letter	Pot#				0	28	56	91	119
J	33	53	25	2	X	X	X	X	-
K	32	37	55	62	X	X	-	-	-
L	13	11	22	19	X	-	X	-	-
M	31	38	7	20	X	-	-	X	-

All pots were harvested on d-23, 56, 91, and 119
No treatments were applied to the Untreated Control
Letter corresponds to type of treatment and when treatment was administered
X denotes when pots were treated

half rate of N treatments with J applications on d 28, 56, 91, and 119; K applications on d 28; L applications on d 56; and M applications on d 91.

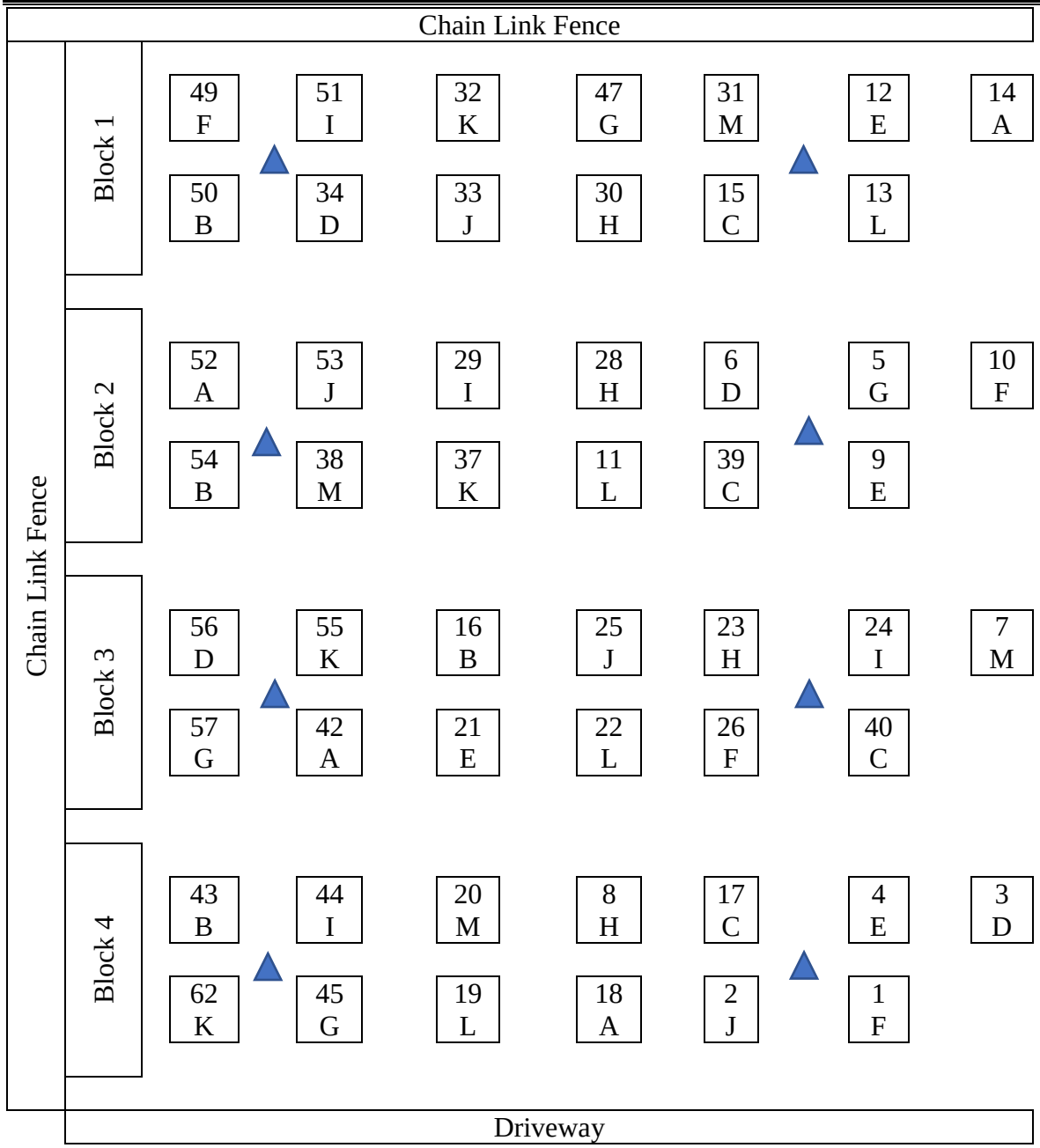
Before the end of the establishment period, pots were assigned a number 1 to 62 then subjectively given a percent pot coverage score. Pots were divided into quarters and scored based on how much bare soil could be seen in each quarter. The percentage of bare soil seen was subtracted by 100 percent to get the percent pot coverage. The percent coverage score helped eliminate pots that did not sufficiently establish compared with others in the 68-d period. Ten pots with less than 75% coverage were eliminated from the study. The remaining 52 pots were then arranged into 4 blocks based on proximity to 4 rows of overhead irrigation heads with each block containing 13 pots (Figure 1). Blocks were arranged using a randomized complete block design, and each block represented a replicate. Each pot was randomly assigned to 1 of 13 treatments, resulting in 4 pots per treatment.

Bacterial strains and inoculation preparation

A PGPR inoculant, containing three bacterial strains (*Bacillus pumilus* AP 7, *Bacillus pumulis* AP 18, and *Bacillus sphaericus* AP 282) was used in this study (Blend 20, Auburn University Department of Entomology and Plant Pathology). Bacterial strains were transferred from cryovials maintained at -80°C for long-term storage to plates of tryptic soy agar (TSA). After incubation at 28°C for 48 to 72 h, bacteria were scraped from TSA plates with inoculating loops, and transferred to either new TSA plates, or the bacterial growth was collected into plastic centrifuge tubes (50 ml, VWR, Radnor, PA) which contained 40 ml of sterile water, and vigorously shaken to evenly distribute bacterial cells.

Bacterial populations in the suspension were determined by serial 10-fold dilutions of each bacterial suspension into sterile water blank tubes (20 ml Glass Culturable, VWR, Radnor,

Figure 1. Layout of 0.09m^2 pots containing Coastal bermudagrass and assigned treatment of control, PGPR, full rate N, or half rate N.



Triangles represent irrigation heads used for assigning blocks

PA) to a final dilution of 10^{-5}. Bacterial populations were determined by plating 50 µl of the serially diluted bacterial suspensions onto TSA plates, incubating the plates for 24 to 48 h then counting the number of bacterial colonies that grew on each plate. Once concentrations in the prepared suspensions of each strain were determined, the populations of all strains were used to make a bacterial stock solution. Stock solutions were prepared by the addition of 1 L of equal parts of each bacterium to achieve a blend with a final concentration of 1×10^7 colony forming units (cfu)/ml of each strain.

Treatment application

June 1, 2016 was designated d-0 and was considered the first day of the trial. On d 0, all control, PGPR, full rate N, and half rate N pots received initial treatments. Bacteria, water, and fertilizer treatments were applied before 0900 hr CDT when winds were < 5 mph. Control and PGPR applications were made with a plastic, trigger-type hand sprayer, and each treatment had its own sprayer. Applications were made every 28 to 35 d immediately following forage harvest, according to treatment structure. Either 50 ml of Blend 20 or distilled water were applied. Fertilized plants were treated with ammonium sulfate at a rate of 56 kg/ha of N (2,,590 mg/0.09 m^2) or 28 kg/ha of N (1,295 mg/0.09 m^2). Bacteria, control, and fertilizer treatments were moved into the root zone by 1.27 cm of overhead irrigation (15 min). Overhead irrigation of 1.27 cm was applied to each pot three times per week beginning at 0715 hr CDT. Supplemental irrigation was stopped between d 56 and 91 due to excessive rainfall, and was resumed after d 91 for the remainder of the study.

Forage harvesting, sampling, and laboratory analyses

Plant heights were taken at 0700 hr on d 0, 28, 56, 91, 119 using a grazing ruler. All forage from each pot was harvested to a 5.08-cm stubble height using hand clippers on d 28, 56,

91, and 119 at 0730 hr. Samples were placed into plastic, zip-closure bags and transported in a cooler to Auburn University's Department of Animal Sciences Nutrition Laboratory for analysis.

Forage samples were weighed prior to being dried in a 50°C oven for 48 to 72 h. Samples were dried to a constant weight, and air-equilibrated samples were reweighed and ground to pass a 1-mm screen in a Wiley Mill (Thomas Scientific, Philadelphia, PA). Forage concentration of DM was determined per procedures of AOAC (1990). Concentrations of NDF, ADF, and ADL were determined sequentially according to the procedures of Van Soest et al. (1991). Neutral detergent fiber and ADF were analyzed using an ANKOM 2000 (Ankom Technology Corporation, Fairport, NY). Lignification was calculated by dividing ADL by NDF to determine the percent lignification of the cell wall. Biomass values were calculated by multiplying the wet weight of sample by the DM value to get g biomass for each 0.09-m^2 pot.

Statistical analyses

Biomass and forage nutritive quality data were analyzed as a randomized complete block design. Each block represented a replicate (4 replicates per treatment), and pot was the experimental unit. Data were treated as repeated measures using the MANOVA procedure of JMP 12.0.1 (2015 SAS, Inc.). The significance was set at $P < 0.05$ for all analyses. The statistical model included sampling date as the independent variable, and block and treatment were included as model effects for forage mass, lignification, and forage concentrations of NDF, ADF, and ADL. Individual sampling date × treatment interactions were analyzed using the LSMeans contrast procedure of JMP 12.0.1 (2015 SAS, Inc). Contrasts performed on whole treatment groups included: control versus PGPR, control versus full rate N, control versus half rate N, PGPR versus full rate N, PGPR versus half rate N, and full rate N versus half rate N. Contrasts of individual treatments included: A versus B, A versus F, A

versus J, B versus F, B versus J, and F versus J.

RESULTS

Supplemental irrigation was removed between harvests on d 56 and d 84 to compensate for sufficient rain early in the growing season. A few pots were noticeably affected by the environmental conditions. Harvest interval was increased from 28 to 35 d from the harvest on d 56 due to noticeable deterioration in plant health, which was possible caused by drought conditions. The 28-d harvest interval resumed after the sample collection on d 91 of the experiment.

Biomass. Mean biomass yield (DM basis) per 0.09 m^2 (Table 2) was influenced ($P < 0.05$) by sampling date and was characterized by sampling date × treatment and sampling date × block interactions. The PGPR-treated grass produced similar biomass to the control, and both the control and PGPR-treated grass produced less ($P < 0.05$) biomass per 0.09 m^2 at all sampling dates relative to the grass treated with the full rate of N (Table 3). The half rate of N treatments produced more ($P < 0.05$) biomass per 0.09 m^2 at d 56, 91, and 119 than the control. For total biomass produced, the control was not different from the PGPR treatments, but more ($P < 0.05$) total biomass was produced by the full rate of N and the half rate of N compared to the control. The full rate of N and half rate of N treatments produced more ($P < 0.05$) biomass than PGPR treatments at all sampling dates, and total biomass yield. Full rate of N treatments produced greater ($P < 0.05$) biomass than half rate of N treatments at d 56 and d 91, and produced more ($P < 0.05$) total biomass than the half rate of N treatments.

When evaluating individual treatments, there were no differences between the control and plants treated with PGPR following each harvest for all sampling dates and total biomass. Grass treated with full rate of N following each harvest produced more ($P < 0.05$) biomass at all

sampling dates and total biomass relative to the control. The grass treated with the half rate of N following each harvest produced greater total biomass and greater biomass ($P < 0.05$) on d 56, 91, and 119 relative to the control. Plants treated with full rate of N following each harvest and half rate of N following each harvest resulted in greater ($P < 0.05$) biomass production relative to plants treated with PGPR after each harvest at each sampling date and resulted in greater ($P < 0.05$) total biomass yield. Full rate of N plants treated after each harvest were not different from half rate of N plants treated after each harvest, but grass treated with full rate of N after each harvest resulted in greater ($P < 0.05$) biomass production on d 56 than plants treated with half rate of N after each harvest.

Table 2. Effect of PGPR, full rate of N (56 kg N ha), or half rate of N (28 kg N/ha) treatment on biomass yield (DM basis) of Coastal bermudagrass in 0.09-m^2 pots.

		Mean biomass g/0.09 m^2				
Treatment Type	Day treated	28 d	56 d	91 d	119 d	Total
Control	-	37.12	9.77	9.75	6.08	62.72
PGPR	28, 56, 91	31.78	9.08	7.67	4.92	53.44
PGPR	28	31.68	11.13	8.35	5.62	56.79
PGPR	56	36.73	10.48	9.76	7.26	64.23
PGPR	91	48.38	12.77	8.59	4.92	74.65
Full rate N	28, 56, 91	61.21	31.76	35.20	20.34	148.50
Full rate N	28	38.70	23.69	15.59	8.19	86.17
Full rate N	56	83.31	17.11	28.57	7.01	136.01
Full rate N	91	56.39	13.59	10.22	15.17	95.37
Half rate N	28, 56, 91	55.49	21.44	23.97	16.35	117.24
Half rate N	28	42.48	24.15	13.45	7.27	87.34
Half rate N	56	69.49	12.74	18.25	6.32	106.80
Half rate N	91	49.58	14.01	9.52	10.75	83.86
±SE	-	± 7.66	± 2.22	± 2.17	± 1.79	± 9.45

Table 3. Orthogonal contrasts comparing biomass yield (DM basis) for PGPR, full rate of N (56 kg N ha), or half rate of N (28 kg N/ha) treated Coastal bermudagrass in 0.09-m^2 pots.

Contrast[a]	28 d	56 d	91 d	119 d	Total
C1: Control versus PGPR	*P*= 0.9982	*P*= 0.6601	*P*= 0.6343	*P*= 0.8440	*P*= 0.9669
C2: Control versus Full rate N	*P*= 0.0114*	*P* < 0.0001*	*P* < 0.0001*	*P* < 0.0021*	*P* < 0.0001*
C3: Control versus Half rate of N	*P*= 0.0526	*P* < 0.0018*	*P*= 0.0103*	*P*= 0.0479*	*P* < 0.0015*
C4: PGPR versus Full rate of N	*P* < 0.0002*	*P* < 0.0001*	*P* < 0.0001*	*P* < 0.0001*	*P* < 0.0001*
C5: PGPR versus Half rate of N	*P* < 0.0031*	*P* < 0.0001*	*P* < 0.0001*	*P* < 0.0011*	*P* < 0.0001*
C6: Full rate of N versus Half rate of N	*P*= 0.3040	*P*= 0.0342*	*P* < 0.0003*	*P*= 0.0554	*P*= 0.0117*
C7: A versus B	*P*= 0.6244	*P*= 0.8280	*P*= 0.5002	*P*= 0.6496	*P*= 0.4916
C8: A versus F	*P*= 0.0322*	P< 0.0001*	P < 0.0001*	P < 0.0001*	P < 0.0001*
C9: A versus J	*P*= 0.0983	*P*= 0.0007*	P < 0.0001*	*P*= 0.0002*	*P*= 0.0002*
C10: B versus F	*P*= 0.0099*	P< 0.0001*	P < 0.0001*	P < 0.0001*	P < 0.0001*
C11: B versus J	*P*= 0.0348*	*P*= 0.0003*	P < 0.0001*	P < 0.0001*	P < 0.0001*
C12: F versus J	*P*= 0.6003	*P*= 0.0022*	*P*= 0.0008	*P*= 0.1240	*P*= 0.0247*

* Denotes significance between treatments from contrasts (*P* < 0.05).

[a] PGPR, full rate N, and half rate N refer to all pots within that treatment group. Letters indicate individual treatments: A(control), B (PGPR treated d 28, 56, 91), F (full rate N treated d 28, 56, 91), and J (half rate N treated d 28, 56, 91).

Dry Matter. There was a sampling date effect ($P < 0.05$) for concentration of forage DM (Table 4). When evaluating treatment types, no differences were seen between the control treatments and the PGPR, full rate of N, or half rate of N treatments (Table 5). Full rate of N treated plants contained greater ($P < 0.05$) DM % relative to PGPR treatments on all sampling dates except d 28 and 56. There were no differences between PGPR and half rate of N treatments or between full rate of N and half rate of N treatments at any sampling date. When evaluating individual treatments, no differences were observed between control and full rate of N treatments treated after each harvest, control and half rate of N treatments treated after each harvest, or full rate of N and half rate of N treatments treated after each harvest. The control, full rate of N treated after each harvest, and half rate of N treated after each harvest were all lower ($P < 0.05$) in DM % than plants treated with PGPR after each harvest at d 28 only.

Table 4. Effect of PGPR, full rate of N (56 kg N ha), or half rate of N (28 kg N/ha) treatment on DM (%) of Coastal bermudagrass in 0.09-m^2 pots.

		Mean DM %			
Treatment Type	Day treated	28 d	56 d	91 d	119 d
Control	-	43.83	38.92	40.93	36.02
PGPR	28, 56, 91	49.64	38.24	46.42	36.34
PGPR	28	43.80	39.62	40.84	34.92
PGPR	56	41.88	38.48	40.19	39.46
PGPR	91	42.35	39.77	42.43	37.65
Full rate N	28, 56, 91	40.56	37.87	40.34	38.32
Full rate N	28	41.07	39.09	40.80	38.04
Full rate N	56	39.60	36.46	51.84	38.05
Full rate N	91	42.20	37.94	45.47	34.36
Half rate N	28, 56, 91	43.02	37.05	47.25	37.65
Half rate N	28	43.17	38.52	42.50	36.99
Half rate N	56	43.27	35.92	46.54	40.96
Half rate N	91	40.59	38.08	44.42	35.30
±SE	-	± 1.69	± 1.36	± 3.91	± 2.05

Table 5. Orthogonal contrasts comparing DM (%) of PGPR, full rate of N (56 kg N ha), or half rate of N (28 kg N/ha) treated Coastal bermudagrass in 0.09-m^2 pots.

Contrast[a]	28 d	56 d	91 d	119 d
C1: Control versus PGPR	*P*= 0.7584	*P*= 0.9444	*P*= 0.7266	*P*= 0.644
C2: Control versus Full rate N	*P*= 0.1228	*P*= 0.4819	*P*= 0.4047	*P*= 0.6134
C3: Control versus Half rate N	*P*= 0.6053	*P*= 0.3209	*P*= 0.3373	*P*= 0.4628
C4: PGPR versus Full rate N	*P*= 0.0049*	*P*= 0.2245	*P*= 0.443	*P*= 0.9654
C5: PGPR versus Half rate N	*P*= 0.1186	*P*= 0.0969	*P*= 0.3336	*P*= 0.6652
C6: Full rate N versus Half rate N	*P*= 0.1731	*P*= 0.643	*P*= 0.8393	*P*= 0.7155
C7: A versus B	*P*= 0.0197*	*P*= 0.7237	*P*= 0.3269	*P*= 0.9135
C8: A versus F	*P*= 0.1779	*P*= 0.5874	*P*= 0.9156	*P*= 0.4342
C9: A versus J	*P*= 0.7353	*P*= 0.337	*P*= 0.2604	*P*= 0.5784
C10: B versus F	*P*= 0.0005*	*P*= 0.8495	*P*= 0.2783	*P*= 0.4999
C11: B versus J	*P*= 0.0085*	*P*= 0.5415	*P*= 0.8821	*P*= 0.6544
C12: F versus J	*P*= 0.3086	*P*= 0.6732	*P*= 0.2192	*P*= 0.8194

* Denotes significance between treatments from contrasts ($P < 0.05$).

[a] PGPR, full rate N, and half rate N refer to all pots within that treatment group. Letters indicate individual treatments: A(control), B (PGPR treated d 28, 56, 91), F (full rate N treated d 28, 56, 91), and J (half rate N treated d 28, 56, 91).

Concentration of NDF. Mean concentrations of NDF (%, DM basis) were influenced ($P < 0.05$) by sampling date and interactions of sampling date × treatment, and sampling date × block (Table 6). When evaluating treatment types, the control was not different from PGPR treatments. The full rate of N and half rate of N treatments had a lesser ($P < 0.05$) concentration of NDF than the control on only d 56 (Table 7). The PGPR treatments had greater ($P < 0.05$) concentrations of NDF on d 56 and d 119 when compared to the full rate of N and half rate of N treatments, but no differences were observed at any other sampling dates. There were no differences seen between the full rate of N and half rate of N treatments at any sampling date.

Individual treatments were also compared for differences in concentrations of NDF. No differences were observed between the control and plants treated with PGPR after each harvest. The plants treated with full rate of N after each harvest had lesser ($P < 0.05$) concentrations of NDF on d 28, 56, and 119 than the control. On d 56 and d 119 grass treated after each harvest with half rate of N had lesser ($P < 0.05$) concentrations of NDF than the control. Plants treated with full rate of N after each harvest had lesser ($P < 0.05$) concentrations of NDF on d 56, 91, and 119 than plants treated with PGPR after each harvest, but no differences were observed at any sampling date between plants treated with half rate of N after each harvest and plants treated with the full rate of N after each harvest. On d 56 only, plants treated with half rate of N after each harvest had a lesser ($P < 0.05$) concentration of NDF than plants treated with PGPR after each harvest.

Table 6. Effect of PGPR, full rate of N (56 kg N ha), or half rate of N (28 kg N/ha) treatment on concentration of NDF (%, DM basis) of Coastal bermudagrass in 0.09-m^2 pots.

		Mean NDF %			
Treatment Type	Day treated	28 d	56 d	91 d	119 d
Control	-	77.22	80.00	76.34	80.15
PGPR	28, 56, 91	77.00	79.07	77.13	79.98
PGPR	28	75.88	79.17	74.61	80.22
PGPR	56	77.22	78.35	76.08	80.21
PGPR	91	76.45	79.82	77.46	79.82
Full rate N	28, 56, 91	75.55	76.24	74.31	77.24
Full rate N	28	76.70	77.76	75.14	79.20
Full rate N	56	76.88	78.13	74.68	79.71
Full rate N	91	75.93	78.35	76.78	78.39
Half rate N	28, 56, 91	76.38	76.93	75.41	77.77
Half rate N	28	76.85	76.46	76.82	78.59
Half rate N	56	76.56	79.14	73.67	80.80
Half rate N	91	75.98	78.74	76.76	77.82
±SE	-	± 0.51	± 0.57	± 0.86	± 0.78

Table 7. Orthogonal contrasts comparing concentration of NDF (%, DM basis) of PGPR, full rate of N (56 kg N ha), or half rate of N (28 kg N/ha) treated Coastal bermudagrass in 0.09-m^2 pots.

Contrast[a]	28 d	56 d	91 d	119 d
C1: Control versus PGPR	*P*= 0.3130	*P*= 0.1686	*P*= 0.9856	*P*= 0.9167
C2: Control versus Full rate N	*P*= 0.1018	*P*= 0.0007*	*P*= 0.2566	*P*= 0.0912
C3: Control versus Half rate N	*P*= 0.1812	*P*= 0.0016*	*P*= 0.4892	*P*= 0.1167
C4: PGPR versus Full rate N	*P*= 0.3074	*P*= 0.0008*	*P*= 0.0810	*P*= 0.0141*
C5: PGPR versus Half rate N	*P*= 0.5943	*P*= 0.0030*	*P*= 0.2889	*P*= 0.0229*
C6: Full rate N versus Half rate N	*P*= 0.6218	*P*= 0.6332	*P*= 0.4778	*P*= 0.8412
C7: A versus B	*P*= 0.7628	*P*= 0.2571	*P*= 0.5187	*P*= 0.8783
C8: A versus F	*P*= 0.0261*	*P* <0.0001*	*P*= 0.1040	*P*= 0.0122*
C9: A versus J	*P*= 0.2503	*P*= 0.0005*	*P*= 0.4519	*P*= 0.0379*
C10: B versus F	*P*= 0.0515	*P*= 0.0013*	*P*= 0.0260*	*P*= 0.0178*
C11: B versus J	*P*= 0.3932	*P*= 0.0119*	*P*= 0.1662	*P*= 0.0531
C12: F versus J	*P*= 0.2586	*P*= 0.4050	*P*= 0.3709	*P*= 0.6337

*Denotes significance between treatments from contrasts ($P < 0.05$).

[a] PGPR, full rate N, and half rate N refer to all pots within that treatment group. Letters indicate individual treatments: A(control), B (PGPR treated d 28, 56, 91), F (full rate N treated d 28, 56, 91), and J (half rate N treated d 28, 56, 91).

Concentration of ADF. Mean concentration of ADF (%, DM basis) was affected ($P < 0.05$) by sampling date, and for which there were sampling date × treatment interactions (Table 8). Treatment types were compared for differences in concentrations of ADF. The control treatment was not different from the PGPR or the half rate of N treatments at any sampling date. The control and full rate of N treatments differed on d 56 and d 91 at which time the full rate of N treatments had a lesser ($P < 0.05$) concentration of ADF on d 56 than the control, but the control had a lesser ($P < 0.05$) concentration of ADF on d 91 than the full rate of N treatments (Table 9). On d 28, 56, and 119, PGPR treatments were not different from full rate of N or half rate of N treatments. However, the PGPR treatments had lesser ($P < 0.05$) concentrations of ADF on d 91 when compared to the full rate of N and half rate of N treatments. Full rate of N treatments were not different from half rate of N treatments except on d 91, at which time half rate of N treatments had lesser ($P < 0.05$) concentrations of ADF than full rate of N treatments.

When comparing individual treatments, the control was not different from plants treated with PGPR after each harvest. On d 56, the plants treated with full rate of N after each harvest had a lesser ($P < 0.05$) concentration of ADF than untreated plants; however, on d 91 untreated plants had a lesser ($P < 0.05$) concentration of ADF than plants treated with full rate of N after each harvest. The control differed from plants treated with half rate of N after each harvest only on d 56 at which time the concentration of ADF was less ($P < 0.05$) with the half rate of N treatment. Grass treated with PGPR after each harvest did not differ from grass treated with full rate of N after each harvest or half rate of N after each harvest on d 28, 56, and 119; however, on d 91 the concentration of ADF in the PGPR-treated grass was lower ($P < 0.05$) than both full rate and half rate of N treated plants. On only d 91 the concentration of ADF in plants treated with

full rate of N after each harvest was greater ($P < 0.05$) than in plants treated with half rate of N after each harvest.

Table 8. Effect of PGPR, full rate of N (56 kg N ha), or half rate of N (28 kg N/ha) treatment on concentration of ADF (%, DM basis) of Coastal bermudagrass in 0.09-m^2 pots.

		Mean ADF %			
Treatment Type	Day treated	28 d	56 d	91 d	119 d
Control	-	31.93	33.80	32.82	32.75
PGPR	28, 56, 91	32.00	33.16	32.33	32.86
PGPR	28	31.54	32.75	32.39	32.87
PGPR	56	32.15	33.49	32.95	33.04
PGPR	91	31.96	34.16	33.07	33.71
Full rate N	28, 56, 91	31.92	32.41	34.48	32.98
Full rate N	28	31.58	32.00	32.99	32.60
Full rate N	56	33.15	33.98	34.17	34.39
Full rate N	91	32.26	32.88	33.56	32.30
Half rate N	28, 56, 91	31.77	32.36	33.28	32.34
Half rate N	28	32.57	32.33	33.28	32.72
Half rate N	56	32.24	33.55	32.69	33.66
Half rate N	91	32.53	33.56	33.40	32.06
±SE	-	± 0.37	± 0.43	± 0.32	± 0.31

Table 9. Orthogonal contrasts comparing concentration of ADF (%, DM basis) of PGPR, full rate of N (56 kg N ha), or half rate of N (28 kg N/ha) treated Coastal bermudagrass in 0.09-m^2 pots.

Contrast[a]	28 d	56 d	91 d	119 d
C1: Control versus PGPR	*P*= 0.9689	*P*= 0.3969	*P*= 0.7076	*P*= 0.3032
C2: Control versus Full rate N	*P*= 0.4747	*P*= 0.0460*	*P*= 0.0103*	*P*= 0.3804
C3: Control versus Half rate N	*P*= 0.4098	*P*= 0.0830	*P*= 0.3503	*P*= 0.8711
C4: PGPR versus Full rate N	*P*=0.2362	*P*= 0.0640	*P* <0.0001*	*P*= 0.8062
C5: PGPR versus Half rate N	*P*= 0.1757	*P*= 0.1523	*P*= 0.0431*	*P*= 0.0639
C6: Full rate N versus Half rate N	*P*= 0.8610	*P*= 0.6576	*P*= 0.0086*	*P*= 0.1048
C7: A versus B	*P*= 0.8983	*P*= 0.2941	*P*= 0.2916	*P*= 0.8062
C8: A versus F	*P*= 0.9887	*P*= 0.0269*	*P*= 0.0008*	*P*= 0.6124
C9: A versus J	*P*= 0.7620	*P*= 0.0219*	*P*= 0.3243	*P*= 0.3631
C10: B versus F	*P*= 0.8871	*P*= 0.2232	*P* <0.0001*	*P*= 0.7933
C11: B versus J	*P*= 0.6669	*P*= 0.1929	*P*= 0.0455*	*P*= 0.2502
C12: F versus J	*P*= 0.7728	*P*= 0.9309	*P*= 0.0124*	*P*= 0.1605

* Denotes significance between treatments from contrasts ($P < 0.05$).

[a] PGPR, full rate N, and half rate N refer to all pots within that treatment group.
Letters indicate individual treatments: A(control), B (PGPR treated d 28, 56, 91), F (full rate N treated d 28, 56, 91), and J (half rate N treated d 28, 56, 91).

Concentration of ADL. There were multiple interactions ($P < 0.05$) for concentrations of ADL (%, DM basis) including: sampling date × treatment and sampling date × block interactions (Table 10). Mean concentration of ADL was also influenced by sampling date ($P < 0.05$). No differences were observed for ADL concentrations between the control and PGPR and half rate of N treatments (Table 11). The control differed from the full rate of N treatments at only d 91 at which time ADL concentrations for the control were less ($P < 0.05$) than ADL concentrations in the full rate of N treatments. The PGPR treatments had lesser ($P < 0.05$) concentrations of ADL on d 28 and d 91 than the full rate of N treatments; however, ADL concentrations were less ($P < 0.05$) in full rate of N treatments on d 119 than the PGPR treatments. Acid detergent lignin concentrations were not different between PGPR and full rate of N treatments on d 56. The PGPR treatments did not differ from half rate of N treatments except on d 119 when ADL concentrations were less ($P < 0.05$) in half rate of N treatments than in PGPR treatments. Full rate of N treatments did not differ from half rate of N for ADL concentrations except on d 91 when ADL concentrations were less ($P < 0.05$) in half rate of N treatments.

Contrasts of individual treatments followed a similar pattern to treatment type contrasts. The control did not differ from plants treated with PGPR after each harvest or half rate of N after each harvest at any sampling date. On d 91, ADL concentration for untreated plants was less ($P < 0.05$) than concentration of ADL in plants treated with full rate of N after each harvest. Plants treated with PGPR after each harvest had lesser ($P < 0.05$) concentrations of ADL than those treated with the full rate of N after each harvest on only d 91, and all other sampling dates did not differ. Concentrations of ADL were only different between grass treated with PGPR after each harvest and plants treated with the half rate of N after each harvest on d 119 when concentrations were less ($P < 0.05$) in plants treated with the half rate of N after each harvest

than plants treated with PGPR after each harvest. Plants treated with full rate of N after each harvest and half rate of N after each harvest only differed on d 91; concentrations of ADL in plants treated with half rate of N were less ($P < 0.05$) than in those treated with the full rate of N after each harvest.

Table 10. Effect of PGPR, full rate of N (56 kg N ha), or half rate of N (28 kg N/ha) treatment on concentration of ADL (%, DM basis) of Coastal bermudagrass in 0.09-m^2 pots.

		Mean ADL %			
Treatment Type	Day treated	28 d	56 d	91 d	119 d
Control	-	4.75	4.86	4.60	4.43
PGPR	28, 56, 91	4.79	4.77	4.58	4.68
PGPR	28	4.78	4.61	4.59	4.73
PGPR	56	4.70	4.93	4.71	4.63
PGPR	91	4.83	5.31	4.88	4.88
Full rate N	28, 56, 91	4.99	4.99	5.86	4.41
Full rate N	28	4.84	4.69	4.75	4.30
Full rate N	56	5.36	5.14	5.24	4.58
Full rate N	91	4.95	4.88	4.74	4.40
Half rate N	28, 56, 91	4.55	4.40	4.75	4.08
Half rate N	28	4.89	4.85	4.68	4.52
Half rate N	56	5.20	4.92	4.78	4.54
Half rate N	91	4.89	4.87	4.91	4.08
±SE	-	± 0.18	± 0.24	± 0.13	± 0.18

Table 11. Orthogonal contrasts comparing concentration of ADL (%, DM basis) of PGPR, full rate of N (56 kg N ha), or half rate of N (28 kg N/ha) treated Coastal bermudagrass in 0.09-m^2 pots.

Contrast[a]	28 d	56 d	91 d	119 d
C1: Control versus PGPR	*P*= 0.8821	*P*= 0.8758	*P*= 0.5246	*P*= 0.1386
C2: Control versus Full rate N	*P*= 0.1573	*P*= 0.8229	*P*= 0.0005*	*P*= 0.9570
C3: Control versus Half rate N	*P*= 0.5066	*P*= 0.6954	*P*= 0.2129	*P*= 0.5108
C4: PGPR versus Full rate N	*P*= 0.0478*	*P*= 0.9148	*P*< 0.0001*	*P*= 0.0178*
C5: PGPR versus Half rate N	*P*= 0.4151	*P*= 0.3884	*P*= 0.3296	*P*= 0.0014*
C6: Full rate N versus Half rate N	*P*= 0.2296	*P*= 0.3332	*P*= 0.0002*	*P*= 0.3412
C7: A versus B	*P*= 0.8574	*P*= 0.7793	*P*= 0.9229	*P*= 0.3193
C8: A versus F	*P*= 0.3458	*P*= 0.7125	*P*< 0.0001*	*P*= 0.9361
C9: A versus J	*P*= 0.4207	*P*= 0.1776	*P*= 0.4015	*P*= 0.1572
C10: B versus F	*P*= 0.4438	*P*= 0.5174	*P*< 0.0001*	*P*= 0.2826
C11: B versus J	*P*= 0.3261	*P*= 0.2819	*P*= 0.3502	*P*= 0.0189*
C12: F versus J	*P*= 0.0850	*P*= 0.0891	*P*< 0.0001*	*P*= 0.1811

* Denotes significance between treatments from contrasts ($P < 0.05$).

[a] PGPR, full rate N, and half rate N refer to all pots within that treatment group. Letters indicate individual treatments: A(control), B (PGPR treated d 28, 56, 91), F (full rate N treated d 28, 56, 91), and J (half rate N treated d 28, 56, 91).

Lignification. Lignification was calculated by dividing ADL by NDF to determine percent lignification of the cell wall. Data were evaluated using the MANOVA procedure of JMP, but no separate contrasts comparing treatments were assessed. A sampling date effect on lignification of the cell wall (%, DM basis) was seen (Table 12), which was expected. No other effects or interactions were observed. Most treatments followed a pattern similar to % DM in which lignification decreased from d 28 to d 56, then increased from d 56 to d 91, and then decreased again from d 91 to d 119. During spring green up (prior to d 28) the grass was allowed to grow for a longer period relative to the harvest intervals during the study, and the harvest interval increase from 28 to 35 d between d 56 and d 91, which may have played a role in the pattern of higher lignification values on d 28 and d 91.

Table 12. Effect of PGPR, full rate of N (56 kg N ha), or half rate of N (28 kg N/ha) treatment on lignification (%, DM basis) of Coastal bermudagrass in 0.09-m^2 pots.

		Mean Lignification %			
Treatment Type	Day treated	28 d	56 d	91 d	119 d
control	-	6.37	6.66	6.56	5.93
PGPR	28, 56, 91	6.41	5.55	6.42	5.23
PGPR	28	6.31	6.94	6.01	5.62
PGPR	56	6.28	6.18	6.02	5.90
PGPR	91	6.09	5.97	6.50	5.68
Full rate N	28, 56, 91	6.21	6.73	6.29	5.72
Full rate N	28	6.31	6.33	6.34	5.83
Full rate N	56	6.64	6.49	6.73	5.72
Full rate N	91	6.34	5.78	6.56	5.23
Half rate N	28, 56, 91	6.54	5.86	6.29	5.61
Half rate N	28	6.43	6.17	6.61	5.39
Half rate N	56	6.53	5.90	6.53	6.04
Half rate N	91	6.52	6.14	6.44	5.61
±SE	-	± 0.25	± 0.34	± 0.32	± 0.22

DISCUSSION

The present study is one of the first reports on the use of PGPR in a forage grass system and the first to investigate the influence of PGPR inoculation on nutritive quality of forage bermudagrass. Nearly all of the published PGPR research has been conducted using food and fiber crops such as corn, rice, and cotton.

In this study, biomass was increased using the full rate of N treatment compared with the untreated control, PGPR, and half rate of N treatments. Due to the exceptional response of bermudagrass to N fertilization (Ball et al., 2015), an increase in herbage yield is expected with increasing N rates (Overman et al., 1992). However, in this study, no differences were observed between PGPR treatments and the control, which may have been due to discontinuing N application after the establishment period. When comparing PGPR treatments to either full or half rate of N treatments, PGPR produced less biomass than the N fertilization treatments. These results are consistent with Ker et al., 2012 who found that fertilized switchgrass plants had greater stand density and yield than plants that did not receive a fertilizer treatment. They also determined the inoculated plants (with and without fertilizer) had increased stand density and yield relative to untreated, uninoculated plants. Adesemoye et al. (2008) showed that PGPR alone will not substitute for conventional fertilizers, but may reduce use of conventional fertilizers.

Productivity of hay systems (systems in which forage is cut and removed from the field) is highly dependent on fertilizer inputs. When the forage is removed, virtually all the above ground nutrients are removed. It is estimated that 125.2 kg N is removed per 13.44 metric tons/ha of bermudagrass yield (Ball et al., 2015). Results indicate that PGPR should not be used

as a sole means of fertilization for bermudagrass hay, which is consistent with reports by Adesemoye et al. (2009).

In this study, concentration of DM was effected by sampling date on d 28 of the experiment. Dry matter concentration was greater in the PGPR treated plants compared to the control, full rate of N, and half rate of N treated plants. This increase in DM concentration may be due to morphological changes elicited by PGPR as a direct mechanism for growth. Concentrations of DM during this experiment decreased between d 28 and d 56 then increased between d 56 and d 91, and decreased again from d 91 to d 119. The pattern of changes observed in DM concentrations could be due to maturity of the grass when harvested as well as environmental challenges. The grass was allowed a 68-d establishment period from March to June, which allowed for primary growth to occur for a longer period of time, resulting in a more mature forage at d 28 compared with other sample dates. As forage increases in maturity, DM concentration increases and digestibility decreases (Blaser, 1962). The increase in harvest interval after d 56 was due to irrigation removal which caused observable drought stress from certain pots.

Concentrations of NDF did not differ between full rate of N and half rate of N treatment types, and the control did not differ from the PGPR treatments. Full rate of N and half rate of N treatment types had lesser concentrations of NDF than the PGPR treatments. Mean values (across all treatments) for this experiment ranged from 73.67 to 80.80% NDF (DM basis). Reported concentrations are similar to those observed by Mandebvu et al. (1999) and Sturgeon et al. (2000) who reported concentrations of 66.4 % (DM basis) for ‘Coastal’ pastures for growing steers and 83.29% (DM basis) for ‘Coastal’ hay fed to horses, respectively. While the ranges of

NDF values reported in this study and previous studies are similar, the influence of NDF concentration on forage quality as it related to animal performance warrants further discussion.

Neutral detergent fiber is a key variable in determining overall fibrosity of forages and is an important component in forage selection. It is known that NDF has a negative relationship with energy and intake by the animal (Ball et al., 2015) with intake being dependent on structural volume or bulk (cell wall content) (Van Soest, 1994)

Acid detergent fiber is also a good indicator of fiber content in forages and is used in determining overall forage quality. In this study, ADF concentration in PGPR treatments did not differ from the control at any sampling date, but had lesser concentrations of ADF on d 91 than full rate of N and half rate of N treatments. On all other sampling dates, PGPR treatments did not differ from full rate of N and half rate of N treatment. Acid detergent fiber is a measure of the lignified or undigestible portions and accounts for the cellulose and lignin fractions of the cell wall (Ball et al., 2015). Concentration of ADF is negatively correlated to digestibility of a feedstuff; therefore, it is recommended that producers select forages with lower ADF content (Ball et al., 2015).

Average concentrations of ADF (across all treatments) ranged from 31.54 to 34.48 % (DM basis) which lay between ADF concentrations reported by Mandebvu et al. (1999) and Sturgeon et al. (2000) who reported concentrations of 29.2% (DM basis) for ‘Coastal’ pasture grown for growing steers and 39.97 % (DM basis) for ‘Coastal’ hay fed to mature horses, respectively.

The lignin content oxidized from ADF (ADL) is another important quality parameter due to its negative relationship to digestibility in forages and its rapid development in maturing warm-season perennial grasses (Ball et al., 2015). Highly lignified forages will cause a decrease

in dry matter intake due to these forages having a high retention time in the rumen and their rate of digestion is slower than forages that are less lignified (Ball et al., 2015). In this study, concentration of ADL did not differ between the control and PGPR treatments and half rate of N treatments. Plant growth-promoting rhizobacteria treatments had lesser concentrations of ADL on d 28 and d 91 than the full rate of N treatments, but did not differ from half rate of N treatments except on d 119 when ADL concentration was lower in half rate of N treatments. Individual treatment comparisons mimicked the results from treatment type evaluation.

Average ADL concentrations (across all treatments) ranged from 4.08 to 5.86 %. These average values are less than values determined by Sturgeon et al. (2000) who reported a 'Coastal' bermudagrass hay ADL concentration of 7.31% (DM basis), and LaCasha et al. (1999) who reported an ADL concentration of 7% (DM basis) for 'Coastal' hay harvested 35 d after the previous harvest and fed to yearling horses. Mandebvu et al. (1999) reported a mean cultivar ADL concentration of 5.4% for 'Coastal' pasture grown for growing steers, which is more comparable to results obtained in this study. Mean lignin concentrations for bermudagrass reported by Mandebvu et al. (1999), showed an increased in lignin as the forage matured from 2 wk (4.8%) to 7 wk (6.2%).

CONCLUSIONS

The results of this study indicate that the use of PGPR will not adversely change the nutritive value of ‘Coastal’ bermudagrass. Reported values fall within ranges reported in previous literature which can be used as a guideline for determining quality of the forage evaluated in this study. Concentrations of NDF, ADF, and ADL were not biologically different among treatments which is a good indicator that application of Blend 20 (isolated by Auburn University) does not increase the fiber content of ‘Coastal’ bermudagrass.

Further investigation of the effect of PGPR inoculants on nutritive quality and palatability of forage grass should be performed. Field studies should be included as a means of determining effects of PGPR on forage grasses on a larger scale, and used to find the ideal rate of N fertilization used in conjunction with PGPR to achieve highly productive and high quality forage. Forage palatability is one of the most important factors when choosing a forage because it affects an animal’s intake. Forages treated with PGPR should be evaluated to determine any changes in palatability which might adversely affect intake and animal performance. Additional studies of the effect of single PGPR strains on forage production may also prove to be important for future use of PGPR in the management of forage systems.

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APPENDIX

Appendix Table 1. Mean plant heights (cm)

Pot #	Treatment	d-0	d-28	d-56	d-91	d-119
1	F	20.64	26.92	25.40	26.42	18.80
2	J	19.05	21.84	20.32	21.34	19.81
3	D	17.46	23.62	16.51	15.75	11.68
4	E	16.83	19.56	17.53	17.53	12.95
5	G	15.56	23.88	24.38	25.65	14.48
6	D	19.69	23.88	20.57	19.81	13.97
7	M	20.96	27.69	22.10	14.99	17.53
8	H	28.58	32.00	23.88	23.37	14.73
9	E	23.50	26.92	21.59	14.22	10.16
10	F	24.77	30.73	31.50	24.38	19.30
11	L	19.37	27.18	18.03	19.05	12.95
12	E	22.23	27.43	21.59	16.26	11.43
13	L	25.40	32.77	24.89	20.57	12.70
14	A	17.15	19.30	21.34	14.48	10.16
15	C	17.78	23.37	22.61	14.73	14.22
16	B	17.15	20.57	16.76	16.76	14.73
17	C	17.46	22.10	17.53	14.73	11.68
18	A	18.73	21.34	20.32	18.29	12.19
19	L	20.96	26.92	20.57	22.10	12.45
20	M	21.91	28.96	18.29	17.27	13.46
21	E	18.42	23.11	20.07	13.97	9.40
22	L	17.78	25.91	18.80	19.30	11.94
23	H	21.59	34.80	25.40	26.92	16.00
24	I	20.32	28.70	20.83	18.54	16.00
25	J	20.96	30.48	24.89	21.34	17.78
26	F	21.59	31.50	27.69	24.89	20.32
28	H	26.04	34.54	20.32	24.38	16.51
29	I	17.46	23.11	21.59	12.95	15.49
30	H	17.15	28.45	22.86	24.89	13.97
31	M	19.69	24.28	22.86	14.99	16.26
32	K	19.05	26.92	24.38	17.02	10.67
33	J	19.37	28.19	24.64	21.34	15.75
34	D	16.51	23.37	20.32	16.26	9.91
37	K	14.61	21.84	21.59	15.75	9.65
38	M	16.51	25.55	21.59	13.72	12.19
39	C	17.46	23.11	20.83	12.70	9.14
40	C	19.05	23.37	15.49	15.75	10.41
42	A	19.37	24.38	21.34	13.97	9.14
43	B	19.05	22.10	18.80	16.51	10.41

Appendix Table 1 cont'd. Mean plant heights (cm)

Pot #	Treatment	d-0	d-28	d-56	d-91	d-119
44	I	21.59	29.97	19.81	17.02	17.53
45	G	20.00	22.61	21.08	21.08	12.19
47	G	16.19	24.89	25.91	18.29	14.73
49	F	17.78	22.61	24.38	28.96	21.08
50	B	17.15	21.34	19.30	14.48	10.16
51	I	16.51	24.89	20.07	17.27	19.56
52	A	21.27	23.62	24.38	20.32	15.49
53	J	18.10	23.11	22.61	22.61	18.29
54	B	17.78	21.84	19.05	12.19	9.40
55	K	14.29	23.37	20.83	19.81	13.97
56	D	16.51	21.59	17.27	15.24	9.65
57	G	16.51	20.32	22.86	13.34	10.16
62	K	19.69	22.10	21.08	17.02	13.21