Use of Plant Growth-Promoting Rhizobacteria in Tall Fescue and Bermudagrass Forage Systems

by

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Abstract

A 2-yr study was conducted evaluating plant growth-promoting rhizobacteria (PGPR) as an alternative N source for 'Russell' bermudagrass (*Cynodon dactylon*) and 'KY 31' tall fescue (*Lolium arundinaceum*) at two locations in Alabama. Fourteen, 3-m^2 plots were treated with High N (19 kg N ha^{-1}), Low N (10 kg N ha-1), Accomplish LM® (AMS), AMS + Low N (AMS + Fert), DH 44 (PGPR strain), Blend 20 (PGPR blend), and a negative control. Forage samples were taken every 4 weeks with a 0.1-m^2 quadrat then analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), total digestible nutrients (TDN), and dry matter (DM) using near infrared spectroscopy. Fertilizer applications were performed at the beginning of the growing season and then 30 d later. There was a treatment × harvest interaction on forage biomass and all nutritive value parameters, excluding CP, for both forage species ($P < 0.0001$). Across the growing season, High N had the greatest forage biomass compared to the control for both forage species. DH 44 had the lowest ADF in both forage species. For both forages, PGPR treated plots produced biomass and maintained forage nutritive value similar to that of commercial N fertilizer. In experiment 2, the PGPR strains making up Blend 20 and DH 44 were incubated with Sterling Blue, 2,4-D Amine, and Prowl H_2O® and evaluated for survival at 0, 24, 48, and 72 h after inoculation. There was an effect of PGPR, herbicide, PGPR × herbicide, and PGPR × herbicide × hour interactions on the PGPR concentration of the flasks after incubation with the herbicides ($P \leq 0.05$). Regardless of herbicide, both AP 7 and AP 18 had greater CFU counts than AP 282 and DH 44 at all time points ($P \leq 0.05$). DH 44 with all three herbicides had the lowest CFU ml^{-1} at all time points ($P \leq 0.05$). AP 7 mixed with Sterling Blue at 48 and 72 h were significantly greater ($P \leq 0.05$) than all other interactions, excluding AP 7 with Sterling Blue at 24 h as well as AP 18 with Prowl H_2O® at 72 h. A field demonstration was done using

botanical compositions to analyze the efficiency of each PGPR strain with all three herbicides, including controls ($n = 2$). There was an herbicide ($P < 0.0020$), PGPR × herbicide ($P < 0.0002$), and PGPR × herbicide × days after treatment (DAT) ($P < 0.0101$) interaction effect on the botanical composition. Sterling Blue and 2,4-D had the greatest percentage of weeds averaged across all DAT (74 vs. 76 %, respectively). The DH 44 control at all DAT had the lowest percentage of weeds (0 DAT: 59; 14: 49; 28 DAT: 53 %, respectively). As a result of these studies, DH 44 showed promising results as a bioherbicide in the field demonstration. More studies need to be conducted to determine the mechanistic ability of PGPR to act as a biofertilizer in forage-based systems.

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and coming back the next day for more. Oh, and for killing all the spiders that infested the basement while I quietly ran the other direction.

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List of Abbreviations

PGPR	Plant Growth-Promoting Rhizobacteria
ISR	Induced systemic resistance
SA	Salicylic Acid
JA	Jasmonic Acid
ETO	Ethylene
DM	Dry Matter
ADF	Acid detergent fiber
NDF	Neutral detergent fiber
CP	Crude Protein
IVTD	In vitro true digestibility
NUE	Nitrogen use efficieny
TDN	Total digestible nutrients
BG	Bermudagrass
TF	Tall fescue
N	Nitrogen
K	Potassium
CFU	Colony forming unit
TSA	Tryptic soy agar
TSB	Tryptic soy broth
iPGPR	intracellular plant growth-promoting rhizobacteria
ePGPR	extracellular plant growth-promoting rhizobacteria
ACC	Amino carboxylic acid

ABA	Abscisic acid
P	Phosphorus
DAT	Days after treatment
BSM	Bermudagrass stem maggot
Yr	Year
ROS	Reactive oxygen species
Fe	Iron
ADL	Acid detergent lignin
TIR1	Transport inhibition response 1
Aux/IAA	Auxin receptor complex
IAA	Indole-3-acetic acid
AFB	F box protein family
NCED	9-cis-epoxycartenoid deoxygenase
AP 7	*Bacillus pumilis*
AP 18	*Bacillus pumilis*
AP 282	*Bacillus sphaericus*
AMS	Accomplish® LM
AMS + Fert	Accomplish® LM + 10 kg N ha^{-1} ammonium sulfate
NIRS	Near infrared spectroscopy

I. Literature Review

Forage Production and Nutritive Value

Bermudagrass

Grasses in the *Cynodon* genus originated in Africa and are among the most commonly used perennial forages in the US (Hanna and Sollenberger, 2007). Bermudagrass (*Cynodon dactylon*) is well-adapted to moderately and well-drained soils, tolerant to grazing, has a canopy height ranging from 15 to 100 cm, and spreads via both rhizomes and stolons (Sollenberger, 2008). In the US, bermudagrass is planted on 10 to 12 million hectares (Vendramini et al. 2020) with several cultivars used in the Southeastern region. Typically, bermudagrass cultivars are grown from the mid-South through the Gulf Coast Region where average temperatures range from 27 to 35°C. Most bermudagrass cultivars are adapted to both grazing and hay production (Hill et al., 2001) and are highly responsive to nitrogen (N) and potassium (K) fertilization. Specific fertilization rates depend on management strategies (e.g., stockpiling, grazing, or hay production). A ratio of 4:1:2 is recommended for N to phosphorus (P) to K (Jackson et al., 1959), applied in two or more applications across the growing season (Ball et al., 2015). Root yield increases with fertilization, although low fertilization levels (100 kg N ha^{-1}) will meet the requirements for normal root growth (Wilkinson and Langdale, 1974). Under grazing systems, animals return up to 80% of the nutrients via animal excreta, improving yield and nutritive value. Which differs from hay production systems where forage and nutrients are exported from the field to an alternative location (Ball et al., 1996), increasing fertilizer needs.

Generally, herbage mass of bermudagrass ranges from 11,000 to 15,000 kg ha^{-1} (Lee, 2017) and peak of production occurs during the summer months (Ball et al., 2015). Defoliation events should occur every four to six weeks to optimize herbage mass and nutritive value, as well as allow for proper root carbohydrate replenishment, as warm-season forages store carbohydrates in in roots as starch to survive winter dormancy (Lee et al., 2017). Hybrid cultivars such as ‘Russell’, ‘Coastal’, and ‘Tifton 85’ have been developed to improve nutritive value and productivity (Hill et al., 2001). Hybrid cultivars produce little seed; therefore, they must be established by vegetative propagation using sprigs (Ball et al., 2002). Russell bermudagrass was found in the 1970s near Seale, AL, in an established ‘Callie’ field. It was then released as a cultivar in 1994 (Ball et al., 1996). It is a hybrid between common bermudagrass and Callie that soon became noticed once Callie was winterkilled in the field (Ball et al., 1996). Russell has higher yields and winter hardiness than Coastal, but has similar nutritive value (Corriher & Redmon, 2009).

Edwards (2000) evaluated forage mass and nutritive value responses of Russell, Coastal and Tifton 85 bermudagrasses amongst other cultivars. The values reported in this study are within the ranges to sustain cattle. The author reported seasonal forage masses of 13,810, 14,120 and 21,351 kg ha^{-1}for Russell, Coastal and Tifton 85, respectively, averaged across two years. Ball et al. (1996) reported crude protein (CP), neutral detergent fiber (NDF), and total digestible nutrients (TDN) concentrations in a test plot study of 118, 724 and 524 g kg^{-1}, respectively, for Russell, whereas Coastal had CP, NDF, and TDN concentrations of 120, 728, and 521 g kg^{-1}, respectively, in the same study. In a study conducted in Louisiana, Edwards (2000) reported NDF concentrations for Russell, Coastal, and Tifton 85 were 724, 693 and 745 g kg^{-1}, respectively. The ADF concentrations reported were 345, 328 and 355 g kg^{-1} for Russell, Coastal, and Tifton 85, respectively. The author obtained *in vitro* total digestibility (IVTD) of

Russell, Coastal, and Tifton 85 of 85 of 700, 728 and 707 g kg^{-1}, respectively. The CP concentrations were 125, 144, and 114 g kg^{-1} for Russell, Coastal and Tifton 85, respectively.

Bermudagrass Stem Maggot

In recent years, there has been increasing concern with bermudagrass stem maggot [BSM; *Atherigona reversura* Villeneuve (Diptera: Muscidae)] affecting susceptible bermudagrass cultivars. It was first noticed in 2010 by South Georgia hay producers (Baxter et al., 2017). An adult BSM will range between 3 and 3.5 mm in length, with females typically being larger than males (Baxter et al., 2014). The maggot will move through the plant to the last plant node and begin to burrow into the shoot to feed off the plant causing the leaves above to wither and die. Damage can be worse in fine-stemmed cultivars like Russell and 'Alicia' used for hay production (Hudson et al., 2013). The insect has a short life cycle (21 d), and its control requires timely hay harvest and use of pyrethroid insecticides, which may restrict its use in some forage systems due to cost. Baxter et al. (2019) evaluated the economic impact of BSM on bermudagrass cultivars. The author reported similar trends over two years with forage accumulation being unaffected by spraying pyrethroids during the first three harvests but an observed increased occurred in the last harvests between July and September. In the same study, BSM reduced the forage accumulation in four bermudagrass cultivars over four harvests in 2016. The authors reported the cost of using additional insecticides outweighed the slight benefits of increased forage accumulation. The full mechanism of damage of BSM is not well known and research is ongoing.

Tall Fescue

Tall fescue (*Lolium* arundinaceum) originated in Europe and was brought to the US in the late 1800s (Ball et al., 2015). Tall fescue is a bunch grass with an anchoring root system that grows from February to June and September to November in Alabama, making it one of the best forages for use as a fall-stockpiled forage (Ball et al., 2015). Nutritive value of tall fescue is greatest in the fall months and can maintain its nutritive value throughout the fall (Ball et al., 2015). In the southeastern US, tall fescue occupies over 14 million hectares (Young et al., 2014). KY 31tall fescue is the most widely used cultivar and can tolerate greater ambient temperatures and drought conditions than other cultivars. This is due to a symbiotic relationship with an endophytic fungus (Ball et al., 2019). While the fungus increases the plant's tolerance to environmental stressors, it also produces ergot alkaloids that can cause toxicity in livestock.

Tall fescue toxicosis was first reported in 1973 when three species of *Balansia* endophytic fungi were identified within a tall fescue plant (Bacon et al., 1975). Further toxicology studies have shown that these endophytic fungal species were toxic to cattle and horses, as well as have potential for ergot alkaloid synthesis (Porter et al., 1979). Recent research has shown that most KY 31 tall fescue plants are infected with the endophyte *Epichloë coenophialum* Bacon and Schardl. (Dillard et al., 2019). This fungal endophyte can result in high concentrations of ergot alkaloids accumulating in the base of the plant that are correlated with fescue toxicosis in livestock grazing them (Franzluebbers and Poore, 2021). Some management practices can be adopted to lessen the effects of the fungal endophyte, one being fall-stockpiling. Fall-stockpiling is a management practice by which forages are allowed to grow and accumulate later in the growing season for grazing when there is a forage deficit (Ball et al., 2015). The ergot alkaloid concentrations will dwindle in the winter months reducing the effects to livestock grazing the forage (Franzluebbers and Poore, 2021).

Forage breeding efforts have worked to develop endophyte-free cultivars and beneficial endophytes (Bouton et al., 2002). Drewnoski et al. (2007) compared the performance of infected KY 31 tall fescue, a novel-endophyte, ‘HiMag’ cultivar, and an endophyte-free cultivar, ‘Jesup’. KY 31 tall fescue accumulated the greatest forage mass (3,979 kg DM ha^{-1}) but was not different than the novel-endophyte fescue (3,828 kg DM ha^{-1}). However, both KY 31 and the HiMag were greater than the Jesup endophyte-free cultivar (3,509 kg DM ha^{-1}). In a 3-yr study, Abaye et al. (2009) reported forage yield for KY 31 endophyte-infected, KY 31 endophyte-free, ‘Quantum’ and ‘Lakota’ tall fescue cultivars seasonal averages of 3,811, 3,138, 3,419, and 2,914 kg DM ha^{-1}, respectively. Other studies have shown that endophyte-free tall fescue may be less productive and tolerant to environmental stress than endophyte-infected tall fescue (Arachevaleta et al., 1989; Bacon and Siegel, 1988). Drewnoski et al. (2007) reported the fall-stockpiled KY 31 endophyte-infected tall fescue to have 109 g kg^{-1} CP, 288 g kg^{-1} ADF, and 582 g kg^{-1} NDF concentrations compared with the HiMag novel-endophyte having 111 g kg^{-1} CP, 291 g kg^{-1} ADF, and 590 g kg^{-1} NDF concentrations, averaged across five growing seasons. In the same study, the author reported fall stockpiled Jesup endophyte-free tall fescue had 118 g kg^{-1} CP, 293 g kg^{-1} ADF, and 592 g kg^{-1} NDF concentrations. Franzluebbers and Poore (2021) conducted a study to evaluate tall fescue yield response and nutritive value to N fertilization inputs across the Southeast in 92 field trials over three years. The authors reported a median forage mass of 2,723 kg ha^{-1} when harvested at greater than 5-cm height. The CP ranged from 94 to 162 g kg^{-1} in the samples harvested ≥ 10-cm height. Concentrations of NDF and ADF ranged from 516 to 639 g kg^{-1} and 289 and 391 g kg^{-1}, respectively, of the samples harvested at heights greater than 10-cm.

Tall Fescue Toxicity

The endophytic fungus is present in majority of KY 31 tall fescue plants (Leuchtmann et al, 2014). Animals grazing on toxic, endophyte-infected tall fescue pastures were reported as having rough hair coats, reduced conception rates (Porter and Thompson, 1992), higher respiration rates (Obsorn et al., 1992), and reduced serum prolactin (Duckett et al., 2014) compared with animals grazing nontoxic, novel-endophyte tall fescue. Economic losses resulting from decreased growth and reproduction due to fescue toxicity have been estimated to be over $3.2 billion annually (Kallenbach, 2015). Under grazing conditions, Lacefield et al. (2003) reported the average daily gain (ADG) of 0.64 kg d^{-1} and animal gain per area per year (yr) of 415 kg ha^{-1} yr^{-1}on pastures with 90% endophyte-infected tall fescue. In tall fescue pastures with lower endophyte concentration (1% endophyte infected), the authors reported ADG of 0.99 kg d^{-1} and gain per acre of 518 kg ha $^{-1}$ yr^{-1}. Replacing toxic endophyte tall fescue with a novel-endophyte tall fescue may improve or maintain animal performance (Lacefield et al., 2003).

Horses are also affected by fescue toxicity. Pregnant mares grazing endophyte-infected tall fescue pastures in the last trimester of pregnancy can suffer from agalactia, abortion, red bag deliveries, or birth of still born foals (Schmidt et al., 1993). Mares grazing endophyte-infected tall fescue have been shown to have an increased gestation length by 20 to 27 days (Blodgett, 2001), do not show signs of approaching parturition, and have higher incidences of dystocia and retained placentas (Schmidt et al., 1993). Furthermore, after birth, most foals die or survive only for a few weeks, while suffering from abnormal maturation. Pregnant mares can graze infected tall fescue; however, it is recommended they be removed from the forage at least sixty days prior to parturition (Fribourg et al., 2009).

Plant Growth-Promoting Rhizobacteria

Plant growth promoting rhizobacteria (PGPR) are symbiotic bacteria that colonize the roots and seeds of plants, potentially enhancing plant growth (Kloepper, 1978: Kloepper, 1993) and can adapt to a variety of environments and metabolize varying compounds beneficial to plant grpwth (Bahattacharyya and Jha, 2012). In the rhizosphere, there can be over 1,000 colony forming units (CFU) of bacterial population and multiple microcolonies of bacteria populating up to 15% of the root surface (van Loon, 2007). Broadly, PGPR can be separated by extracellular (rhizospheric, ePGPR) and intracellular (endophytic, iPGPR) groups (Verma et al., 2019; Vessey 2003). Rhizospheric PGPR are known to produce secondary metabolites and live in the plant rhizosphere, whereas iPGPR live inside plant root cells in nodular structures (Verma et al., 2019). Endophytic PGPR are more specialized because they have direct access to organic compounds in the plant (Sivasakthi et al., 2014) resulting in them having less competition than ePGPR would. Often, competition between soil microbes and ePGPR can occur, compromising PGPR`s access to plant exudates. In order to provide benefits to the host plant, PGPR must survive inoculation, attach, and colonize the rhizosphere (Kloepper, 1993). Unless specified, for the remainder of the text PGPR will refer to ePGPR only.

There are hundreds of genera of bacteria classified as rhizobacteria; however, *Pseudomonas*, *Bacillus*, *Azospirillum*, *Azotobacter*, *Arthrobacter*, *Achromobacter*, *Micrococcus*, *Enterobacter*, *Rhizobium*, *Agrobacterium*, *Pantoea*, and *Serratia* are now well known for their ability to promote plant growth (Verma et al., 2019). *Bacillus* is known for its versatility and multiple physiological characteristics to survive in extreme conditions (Shafi et al., 2017). *Bacillus* and *Paenibcaillus* are the most highly explored rhizobacteria, classified as ePGPR (Choudhary and Jhori, 2008), and are naturally present near the plant roots. *Bacillus* that are capable of solubilizing P can stimulate the growth of the plant by enhancing the uptake of N, P, K, and iron (Fe) (Sivasakthi et al., 2014), resulting in the increasing ability of the plant to utilize accumulated phosphates in the soil. *Rhizobia* and *Frankia* are the most well-known in the iPGPR group. Rhizobia are a large group of Gram-negative, aerobic, non-sporulating bacteria (Tak et al., 2017; Rao et al., 2018). Endophytic rhizobacteria are recently considered more effective than rhizospheric bacteria (Asaf et al., 2017). Some genera included in this category are *Bacillus, Enterobacter, Micrococcus, Pantoea, Psedomonas, Streptomyces,* and *Achromobacter* (Verma et al., 2013). Even though colonization of bacilli-bacteria is documented (Reva et al., 2002; Durham, 2013), a considerable amount related to colonization and application frequencies; persistence of biostimulants for plant growth remains unknown in perennial plant systems. Strains of rhizobacteria is a subtype of a microorganism, whereas a blend consists of two or more strains of rhizobacteria.

Plant growth-promoting rhizobacteria can act as a biocontrol agent through nutrient competition, induced systemic resistance (ISR), and antifungal metabolite production (Lutenberg and Kamilove, 2009). Plant growth above and below ground is affected by direct and indirect mechanisms of rhizobacteria. Several direct mechanisms exist; among them, atmospheric N fixation (Calvo et al., 2013), solubilization of P, hydrogen cyanide production, decreasing ethylene concentration (Vessy, 2003), and production of phytohormones such as, cytokinins, auxins, and gibberellins (Sivasakthi et al., 2014). Rhizobacteria can influence the plant indirectly by improving growth-limiting conditions by 1) production of antagonistic compounds or 2) inducing the host resistance to the plant pathogens (Sivasakthi et al., 2014).

Rhizobacteria can produce plant growth regulators such as auxins, ethylene, cytokinins, and gibberellins (Kudoyarova et al., 2019). These compounds modify the physiology of the plant by altering the principal mechanism of growth regulation and cell differentiation. Some of these plant growth regulators act in a similar way in microorganisms, an example being amino acid synthesis. The production and release of these compounds are dependent on 1) plant growth regulator concentration, 2) distance between rhizobacteria and the root surface, 3) diffusion of the plant growth regulator from the soil to the plant tissue, and 4) the competitiveness of the rhizobacteria colonization with high root exudation (Kudoyarova et al., 2019; Choudhary et al., 2015). The auxins released by the rhizobacteria will primarily affect the root system by increasing size, weight, branching number, and thereby the surface of the roots.

Induced systemic resistance is a result of physiological and biochemical reactions, and structural adaptations of plant cells to produce defensive substances (van Loon, 2007), which reduces plant disease. The non-harmful rhizobacteria will interact with the plant; however, the systemic response is not detected until challenged by pathogens (Choudhary et al., 2015). Once a pathogen has challenged the plant, reactive oxygen species (ROS) are released in necrotic areas, responsible for cell death. Biotic elicitors, molecules triggering ISR, are most commonly polysaccharides. During the ISR response, salicylic acid (SA) is not altered, but mediated by ethylene (ETO) and jasmonic acid (JA), two plant growth regulators acting as signal transductors instead of stress hormones (Choudhary et al., 2015; Glick, 2012). The ISR can remain active for long periods once the initial threat is activated.

The primary responsibility of ethylene is in the plants defense responses to diseases and stressors such as malnutrition, temperature extremes, salinity, and reduced light. Ethylene affects plant growth by promoting root initiation, inhibiting root elongation, and activating the synthesis of other hormones, and at increased concentrations, ethylene activates leaf abscission (Glick 2007). In horticulture systems, ethylene can cause a premature ripening of fruit. Endogenous levels of ethylene are increased in drought conditions and can negatively impact the growth of the plant (Bhattacharyya and Jha, 2012).

Ramamoorthy et al. (2001) discovered that there are rapid structural changes in the cell wall because of a defense mechanism that increased the thickness, lignification, and accumulation of phenolic compounds when the plant was treated with a PGPR strain. Rhizobacteria use hormones to increase the physical and mechanical strength of the plant cell wall as an adjustment to biochemical and physical reaction of environmental stressors (Labuschagne et al., 2010). Drought stress can negatively influence plant growth and production. Plant growth-promoting rhizobacteria can produce cytokinins which cause an accumulation of abscisic acid (ABA) in the foliage of the plant. This accumulation will cause a stomatal closure. The PGPR will compete with plant pathogens for nutrients and produce antibiotics and lytic enzymes which are important to the rhizosphere's health (van Loon, 2007; Odoh et al., 2017).

The plant root system secretes photosynthetic byproducts that the PGPR need to survive (Lutenburg and Kamilova, 2009), and some PGPR will fix atmospheric N, making it readily available to the plant. They can do this symbiotically or non-symbiotically. For symbiotic PGPR to succeed in claiming dominancy over the other soil microorganisms, they have to be able to compete for the available nutrients and space in the rhizosphere (Odoh et al., 2017). A symbiotic relationship is formed between the N-fixing PGPR nodule on the plant, resulting in the PGPR providing a soluble N form for plant growth and receiving photosynthetic byproducts for survival. This is common for some Rhizobium spp. but not all can fix atmospheric nitrogen. Some PGPR are free-living, meaning that they do not form nodules on root system, but remain in the rhizosphere proximity. These PGPR still fix atmospheric N and provide plant soluble N forms; however, they rely on other degrading mechanisms to receive nutrition (Nagargade et al., 2018). The PGPR use an enzyme called nitrogenase, which is a two component metalloenzyme incorporating dinitrogenase reductase, the iron protein, and dinitrogenase, the metal cofactor. Dinitrogenase reductase allows for the electrons to have a high reducing power while dinitrogenase uses the electrons to reduce the atmospheric N_2 to ammonia (Dean, 1992).

PGPR in Production Agriculture

Plant growth-promoting rhizobacteria was first researched on crops such as corn (*Zea mays*), cotton (*Gossypium hirsutum*), and soybeans (*Glycine max*). Kloepper (1978) first reported seeing positive results with application of 53 unspecified PGPR on radishes (*Raphanus sativus*) by using specific strains of rhizobacteria. Blatensperger et al. (1978) was the first to study PGPR on grasses where the work observed *Azospirllium* and *Azotobacter*, both nitrogen-fixing strains, in the top growth and N concentration of bermudagrass genotypes and their responses. This study resulted in no differences among root growth and total biomass production but did show the top growth stimulated an increase in N accumulation. Plants that were inoculated with PGPR strains such as *Paenibacillus polymyxa* and *Bacillus amyloliquefaciens* by soaking the plant roots or seeds overnight, reported a great resistance to different forms of biotic stress (Ngumbi and Kloepper, 2016). Auburn University's Department of Entomology and Plant Pathology selected PGPR strains based on their ability to increase root growth, top growth, and whole plant weight (Coy et al., 2014, Fike et al., 2017). When 'Burt-Davy Tifway' bermudagrass sprigs were treated with different PGPR blends, Coy et al. (2014) found that the root length of plants was 150% greater compared with the untreated plant roots. The first experiment conducted was a growth chamber trial that included twelve bacterial strains and six blends. Coy et al. (2014) noticed an increase in plant shoot weight by 236 to 345% compared with the untreated sprigs. In a greenhouse study, the authors observed eight blends that increased top growth by 150 to 197%

compared with the untreated sprigs. One particular blend, Blend 20, was observed to increase root length by 157%, root surface area by 173%, and root volume by 186% compared with the untreated sprigs. Blend 20 consists of three different PGPR strains in the *Bacillus* genre, *B. pumilus* (AP 7), *B. pumulis* (AP 18), and *B. sphaericus* (AP 287). DH 44 is a single strain of PGPR in the *Paenibcillus sonchi* (Groover et al. 2020). DH 44 was isolated from bermudagrass roots during a droughty period in Auburn, Alabama. In the study by Coy et al. (2014), Blend 20 both showed astonishing ability to increase root growth and biomass. When compared with the untreated sprigs, shoot weight was 109% greater and root weight was 364% greater when the sprigs were treated with Blend 20 (Coy et al., 2014). However, compared to Blend 20, the shoot weight was 9% greater and the root weight was 44% greater (Coy et al., 2014). Fike et al. (2017) and Gunter et al. (2018) performed studies with PGPR applied to Coastal bermudagrass. Both studies evaluated the nutritive value of Coastal bermudagrass hay fields treated with Blend 20 with a full-rate (56 kg N ha^{-1}) or a half-rate (28 kg N ha^{-1}) N fertilizer. Fike et al. (2017) did not observe a difference in the nutritive values [NDF, ADF, acid detergent lignin (ADL) concentration] of the bermudagrass. This study was conducted to determine if Blend 20 would increase the lignification of the plant. Since the nutritive quality was similar to plants fertilized with inorganic N, there seemed to be no evidence of increased lignification. In 2018, Gunter et al. investigated CP, DM digestibility (DMD), and nitrogen-use efficiency (NUE) in the Coastal bermudagrass samples collected during Fike et al. experiment. Blend 20 had the greatest digestibility and NUE; intermediate for full-rate N, least for half-rate N. No differences were noted in the fiber fractions when compared with the untreated control.

Adesemoye et al. (2009) reported that PGPR applied to tomato plants, reduced use of chemical fertilizer by 25%. Ker et al. (2012) reported 40% yield increase when PGPR inoculant was applied to switchgrass seeds. Griffin et al. (2020) evaluated the effects of PGPR application on fall-stockpiled Coastal bermudagrass. The author reported greater yields using Blend 20 with synthetic fertilizer (1,914 kg DM ha^{-1}) than DH 44 (1,271 kg DM ha^{-1}); however, Blend 20 with synthetic fertilizer did not differ from the synthetic fertilizer treatment (1,768 kg DM ha^{-1}). In the second year of the same study, there was a 15% yield increase compared with the first year. Both DH 44 and Blend 20 alone had the least CP concentration among the treatments. Between years, Blend 20 alone had greater NDF and ADF concentrations than control (736 vs 703 g kg^{-1} and 362 vs 342 g kg^{-1}) but did not differ from other treatments.

The use of microbes to influence plant and soil health have been investigated for enhancing stress tolerances to abiotic and biotic stressors in many row crops such as soybeans and corn; however, there are fewer studies in forages due to the lack of understanding the physiology and genetics associated (Kasim et al., 2013; Wang and Brummer, 2012). The use of a biostimulant containing viable microorganisms is an option to potentially allow for a reduction of N rates if PGPR can improve nutrient uptake and efficiency (Calvo et al., 2013), although where and how they colonize the host plant are critical factors (Vessey, 2003).

Herbicide Use in Forage Systems

In forage systems, the most controlled pests are weeds. When weeds are not controlled, they can spread in fields and reduce the desirable forage species overtime by out competing the desirable species for ground cover and light needed for photosynthesis. In the United States, forage pastures provide roughly $10 billion in production annually (USDA, 1998); however, with the presence of inedible weeds. the estimated monetary losses amount to roughly $2 billion (Pimentel, 1991). Approximately $23 billion annually is lost to non-native weeds in the US and roughly $3 billion has been depleted on herbicides aiming to control them (Pimentel, 2001).

Controlling weeds early in the growing season provides benefits such as improved forage quality over the first harvest and reducing stand loss from intense weed pressure throughout the season (Leep et al., 2003). According to the integrate pest management programs, there are several ways to control weed pests including mechanical, chemical, preventative, and cultural and sometimes more than one method is needed. (Dr. David Russell, personal communication). Proper management strategies are crucial to control weeds, and chemical applications are often the most efficient method. Herbicides can be categorized based on their activity as selective, targeting a specific weed species/type, or non-selective, targeting anything encountering the herbicide (Rana and Rana, 2019). Herbicides can also be categorized by when they are applied relative to the weed species life cycle. Pre-emergent herbicides are applied before the presence of weeds. These herbicides prevent the emergence of weed seedlings by 1) inhibiting the root growth, 2) inhibiting the shoot growth, or both (Hopper, 2016). Pre-emergent herbicides are termed "residual" because they remain in the soil on average 8 to 12 weeks after application when the soil microbial population starts to degrade them, and another herbicide application is required (Hopper, 2016). Post-emergent herbicides are foliar herbicides that should be applied directly to the weed after establishment. Post-emergent herbicides control broadleaf weeds more easily than their grassy counterparts (Reynolds et al., 2021). After weeds encounter these herbicides, the chemicals are absorbed into the foliage and translocated through the roots and phloem (Oisecka et al., 2011).

There are several labeled herbicides for use in bermudagrass fields, including pre- and post-emergent products. Choice of product depends on time of application, weed species to control, and weather conditions. Pre-emergent herbicides are usually applied in mid-February to mid-March aiming to start with a "clean" field for the summer growing season. Whereas post-emergent herbicides are applied as needed after weeds germinate and are often applied multiple times throughout the season (Russell, 2019). The induction of dependable herbicides has greatly influenced forage establishment and weed control in pastures, ultimately improving the productivity while reducing toxicity concerns in livestock (Hoveland, 2000).

Myresiotis et al., (2012) evaluated both *Bacillus subtilis* FZB24 and BG03 strains when inoculated in flasks with two herbicides, metribuzin and napropamide at one and ten times the recommended rate to determine biodegradation of the herbicides via PGPR. After being incubated for 72 h, the author reported *B. subtilis* FZB24 and GB03 degrading metribuzin by 14 to 18 and 8 to 12%, respectively. Whereas napropamide was degraded by *B. subtilis* FZB24 at 9 to 11%. When evaluating the bacterial growth in TSB medium in the same study, the author observed an initial spike in growth then a small decline in growth until the end of the study.

Pendimethalin

Prowl® H_2O (BASF Ag Products, Florham Park, NJ [Pendimethalin: N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine]) is a pre-emergent herbicide used to control broadleaf and grassy weed species in crop and non-crop areas. The active ingredient, pendimethalin, is a member of the dinitroaniline family of herbicides (Verma et al., 2018). Its mode of action is microtubule polymerization inhibition which prevents cells from undergoing mitosis and leads to cell death (Chrisoffolleti et al., 2016; Chen et al., 2021). Dinitroanilines will bind to the unpolymerized tubulin heterodimers halting the elongation of the microtubule and because the negative end of the microtubule continues to depolymerize naturally, the microtubules become increasingly shorter until complete dissociation (Chen et al., 2021). The family of dinitroanilines are extremely volatile and must be incorporated into the soil to reduce the volatilization loss (Ashworth et al., 2020). Once in the soil, dinitroanilines have a large binding affinity and a slow microbial degradation. Pendimethalin has been listed as a persistent bioaccumulative toxin by the U.S. Environmental Protection Agency (EPA, 2021). There have been studies evaluating pendimethalin degradation by soil microorganisms, toxicity, and its effects on the environment overall. There have been seven documented weed species reported to have resistance to dinitroaniline herbicide with the species residing primarily in Australia, Americas, and Japan (Heap, 2021).

2,4-D Amine

2,4-D Amine (Alligare®, LLC, Opelika, AL [2,4-Dichlorophenoxyacetic acid]) is a water dilutable herbicide used to suppress cypress surge (*Euphorbia cyparissias*), dogfennel (*Eupatorium capillifolium*), common mullein (*Verbascum thapsus*), and jimsonweed (*Datura stramonium*) and other broadleaf weeds. It is commonly applied to immature weeds in late spring but can also be applied throughout the summer at the appropriate plant stage of growth. It can be used as a post or pre-emergent herbicide with its mode of action being inhibiting the protein transport inhibition response 1 (TIR1) auxin receptors. This family enters the epidermal plant cells where it is absorbed into the symplast and migrated to the vascular system where it is translocated from the leaves to the stems and roots (Bovey, 2001). This herbicide belongs to the phenoxy or phenoxyacetic acid family making up one of the largest herbicide class groups. This chemical family is low cost, effective under low doses, and has good water solubility making the products appealing to producers (Nadin, 2007). The synthetic herbicides in this family were created to mimic a natural auxin, indole-3-yl-acetic acid, which plays a crucial role in the division, differentiation, and elongation of plant cells (Venkov et al., 2000), allowing them to obstruct the plant growth processes at high concentrations. Use of synthetic herbicides in this family pose many environmental concerns. They can be degraded biologically or in a photolytic mechanism where the compounds are exposed to varying environmental conditions (Sklivagou et al., 2012). If the photolytic mechanism occurs, the herbicide compounds can produce metabolites beholding different chemical and physical characteristics making the metabolites more toxic than the original compounds (Jankowska et al., 2004). Due to 2,4-D Amine having high water solubility and a low degradability by soil microorganisms, make it one of the major causes of water pollutants (Ángel-Sanchez et al., 2013).

Sterling Blue

Sterling Blue (WinField™ Solutions, St. Paul, MN [Diglycolamine salt of 3,6-dichloro-o-anisic acid]) is a water-soluble herbicide used to control and/or suppress numerous annual, biennial, and perennial broadleaf weeds. It is absorbed by plants through the root and its mode of action acts on auxins, a key group of phytohormones, leading to death. The mechanism and exact genes involved in the synthetic auxin involved in the phytotoxicity has remained a mystery for years due to the complex auxin signaling pathways. Specifically, diglycolamine interacts with TIR1 and AFB5 proteins causing a potential loss of auxin perception and lowered posttranslational regulation (Todd et al., 2020; Gleason et al., 2011). Products in the synthetic auxin herbicide class will interact to the auxin receptor complex (Aux/IAA) by binding to the Aux/indole-3-acetic acid (IAA) proteins responsible for transcriptional repressors of auxin-responsive genes (Gaines, 2020), resulting in expeditious transcription of those genes. McCauley et al. (2020) proposed the synthetic auxins would bind to the to the TIR1/AFB auxin receptor. This pathway could potentially result in the Aux/IAA ubiquination and destruction, where the auxin-responsive genes undergo rapid transcription. The gene, 9-cis-epoxycarotenoid deoxygenase (NCED), is a rate-limiting step in the production of ABA, and when it is up-regulated with the synthetic auxin, an accumulation of ABA occurs. The accumulation of ABA effects the abundance of photosynthesis-related genes by decreasing the transcription. As a result, plants will die from lack of photosynthetic activity (McCauley et al., 2020). It is suggested synthetic auxins are not targeting specific photosynthetic genes as other herbicide classes would, but rather causing a complete down-regulation of transcription for multiple genes involved in photosynthesis. Essentially, the metabolism of these herbicides will activate a biologically inactive molecule once in the plant and then detoxify said molecule. Plants try to maintain homeostasis of IAA; however, synthetic auxin products unbalance the homeostasis (Todd et al., 2020).

Through direct and indirect mechanisms, PGPR may have a role in forage systems to increase production by releasing growth hormones to the plant. Some PGPR have been shown to reduce disease instances and herbivorous pests in other cropping systems. The objective of this study was to determine the effects of *Paenibacillus sonchi* DH 44, Blend 20, and Accomplish® LM (commercial PGPR product) with and without synthetic fertilizer on forage biomass and nutritive value of KY 31 tall fescue and Russell bermudagrass. To determine the ability to tank-mix PGPR and herbicides, a second study was conducted to determine the survivability of the PGPR in a suspension with three common herbicides. A field study was conducted to evaluate the botanical composition of plots treated with PGPR, herbicides or PGPR and herbicides on bermudagrass.

II. Influence of Plant Growth-Promoting Rhizobacteria on Yield and Nutritive Value of ‘Russell’ Bermudagrass and ‘KY 31’ Tall Fescue

Introduction

In the US, forages support animal production such as cattle, horses, goats, and sheep, and many others. Forage and rangeland production are one of the leading cropping systems. In the Southeast US, bermudagrass and tall fescue dominate the pasture systems and occupy millions of hectares. During some periods of the year, typically the winter months, additional forage is needed to sustain animal production resulting the utilization of hay.

Bermudagrass (*Cynodon dactylon*) is a warm-season perennial forage that is widely utilized in the Southeastern United States. It is well adapted to moderately and well-drained soils, tolerant to grazing, with a canopy height ranging from 15 to 100 cm, and having both rhizomes and stolons (Sollenberger, 2008). Hybrid bermudagrass cultivars such as ‘Russell’, ‘Coastal’, and ‘Tifton 85’, are also known for their responsiveness to nitrogen (N) and potassium (K) fertilization. Tall fescue (*Lolium arundinaceum*) is a bunch grass that grows from February to June and September to November in Alabama (Ball et al., 2015). In the southeastern United States, tall fescue occupies over 14 million hectares (Young et al., 2014). KY 31 tall fescue is the most used cultivar tolerating higher temperatures and droughty conditions (Ball et al., 2019), but it presents issues with ergot alkaloid synthesis resulting in fescue toxicosis in livestock.

There is an increasing cost of inorganic N fertilizers that affects the management of forage production systems. The major source of N to forages and plants is primarily commercial manufactured N fertilizers (Ball et al., 2015). Losses of N in fertilized grasses can be as high as 50% (Frank and Guertal, 2013). Nitrogen can be lost by leaching, volatilization, and denitrification resulting in releasing nitrous oxide, a greenhouse gas that has raised environmental concerns (Ball et al., 2015; NRC 1993).

Plant growth-promoting rhizobacteria (PGPR) are symbiotic rhizobacteria that colonize roots and seeds of plants to enhance plant growth (Kloepper, 1978; Kloepper 1993). These rhizobacteria are non-pathogenic, soil-inhabiting rhizobacteria that have a symbiotic relationship with the host plant. In this symbiotic relationship, the plant root system secretes photosynthetic byproducts that PGPR utilize (Lutenburg and Kamilova, 2009), while PGPR fix atmospheric N to supply to the plant. These rhizobacteria can benefit the host plant by increasing drought and insect tolerance, nutrient uptake, and increasing top and root growth (Vessey, 2003; Nelson, 2004).

Majority of the studies conducted evaluating PGPR on agronomic row crops and turfgrass systems with less emphasis on forage-type grasses. Coy et al. (2014) studied sixteen rhizobacterial strains on 'Burt – Davy Tifway' bermudagrass, a turf-type bermudagrass. As a result of this research, Blend 20 (a blend of *Bacillus* strains developed by Auburn University's Department of Entomology and Plant Pathology) was specifically chosen for further studies due to its ability to increase shoot and root growth greater than the other blends/strains studied. Fike et al. (2017) evaluated the nutritive value of Coastal bermudagrass hay that was treated with Blend 20 or fertilizer, and the reported concentrations of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were not different than bermudagrass fertilized with synthetic N. Other studies in stockpiled-forage systems by Griffin et al. (2020) and Gunter et al. (2018) provide evidence that PGPR can increase bermudagrass growth similar to a synthetic fertilizer.

Plant growth-promoting rhizobacteria applied to bermudagrass results in similar dry matter yields with comparable nutritive quality to forages utilizing N fertilization (Coy et al., 2014; Fike et al., 2017). Using the tall fescue and bermudagrass forage systems in Alabama, the objective of this experiment was to evaluate PGPR as a biostimulant to increase forage yield while maintaining or improving the nutritive value of Russell bermudagrass and KY 31 tall fescue.

Materials and Methods

Research Site

A 2-yr study (2019 and 2020) was conducted with bermudagrass or tall fescue to evaluate the influence of PGPR on the yield and nutritive quality. An established stand of Russell bermudagrass (BG) in Lawrence County, Alabama (34.430992, -87.495809) was managed as a hay production system with harvests every 28 d. This location consists predominantly of an Allen sandy loam (Fine-loamy, siliceous, semiactive, thermic Typic Paleudults) soil type with high organic matter and a pH of 6.4. An established stand of KY 31 tall fescue (TF) was used in Montgomery County, Alabama (32.196121, -86.267604). This field was managed as a fall stockpiled system with soils in the Sumter silty clay (Fine-silty, carbonatic, thermic Rendollic Eutrudepts) with a pH of 7.7. Weather data at both locations (Figures 1 and 2) was recorded using the National Oceanic and Atmospheric Administration (2021) data from stations closest to each location.

At each location, fourteen 3-m^2 plots were mowed to 5-cm stubble height prior to application in each year. Each plot was surrounded by a 3-m^2 alley to prevent drift and/or research activity. In Year 1 and 2, the TF stand was mowed on July 9 and September 28, respectively. In Year 1 and 2, the BG stand was initially mowed on June 20 and May 15 and, due to high forage mass accumulation, the BG stands were also mowed after every forage sampling event.

Figure 1: A) Total precipitation, cm, during the 2019 and 2020 growing seasons in Lawrence County, AL. B) Average daily temperature, °C, during the 2019 and 2020 growing seasons in Lawrence County, AL.

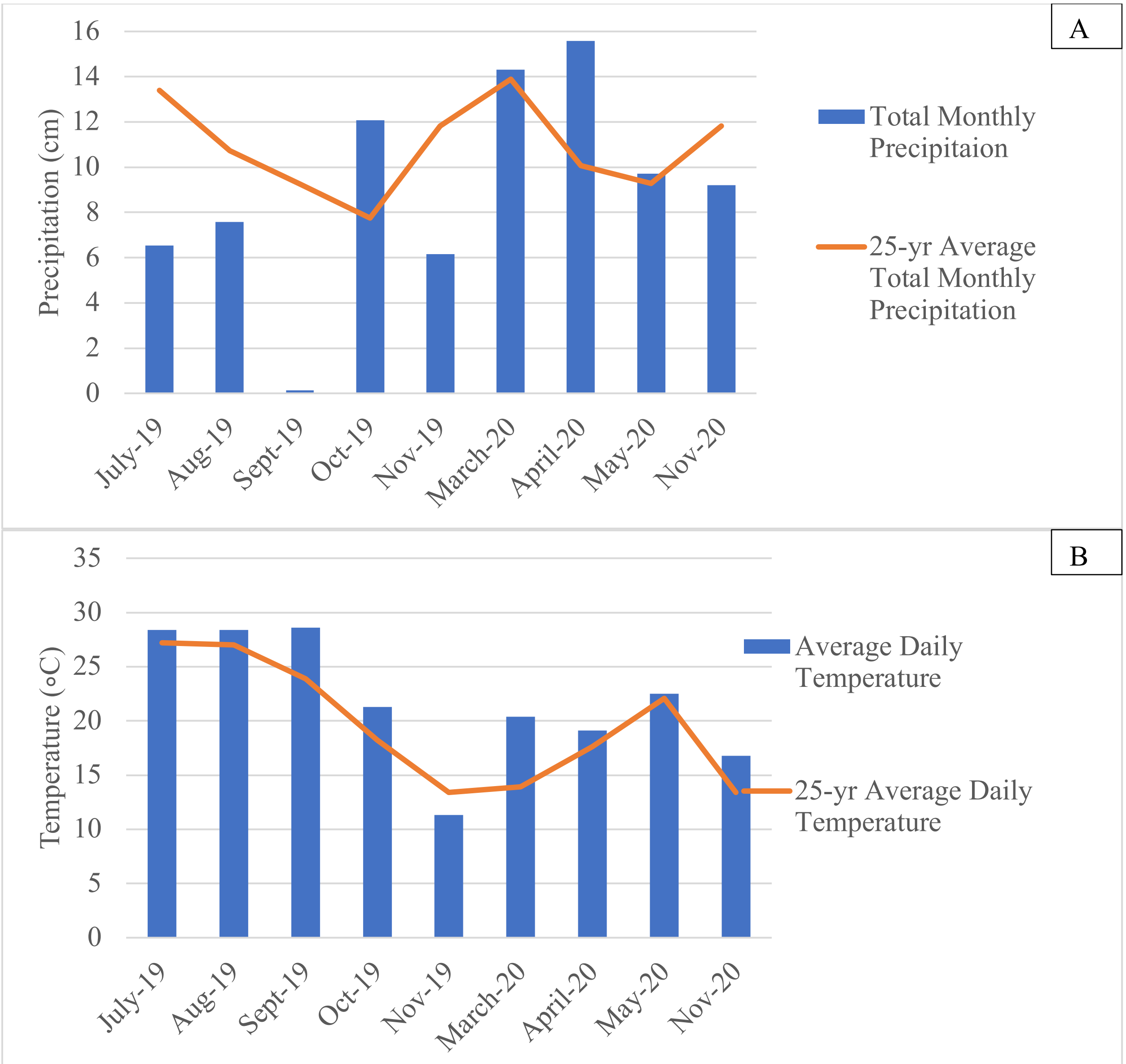

Figure 2: A) Total precipitation, cm, during the 2019 and 2020 growing seasons in Montgomery County, AL. B) Average daily temperature, °C, during the 2019 and 2020 growing seasons in Montgomery County, AL.

Production of PGPR

Blend 20 treatment [AP7: (*Bacillus pumilus*), AP18: (*B. pumilus*), and AP 282: (*B. sphaericus*)] and DH 44 (*Paenibacillus sonchi*) were selected based on the previous evaluation of growth promotion in bermudagrass by the Auburn University Department of Entomology and Plant Pathology (Coy et al., 2014; Griffin et al., 2020; Groover et al., 2020). Bacterial cultural methods were similar to those used in these previous studies. In brief, bacterial strains were transferred from cryovials maintained at -80°C for long-term storage on plates of tryptic soy agar (TSA). Strains of Blend 20 and DH 44 were incubated at 28°C for up to 24 h and 72 h, respectively. The PGPR were then scraped from the TSA plates with sterile inoculating loops and transferred to either a new TSA plate, or collected into 50- or 250-ml plastic centrifuge tubes (VWR, Radnor, PA) containing autoclaved deionized (DI) water. Bacterial cells were distributed evenly throughout the solution using a vortex machine and then soaked in an unstirred water bath at 80°C to heat-shock vegetative cells. Subsequently, the bottles remained in the bath for 20 minutes before they were taken out, allowed to cool, and stored at room temperature (28°C).

Bacterial populations in the suspension were determined by serial 10-fold dilutions of each bacterial suspension into blank tubes containing sterile DI water to a final dilution of 1×10^{-6}. Bacterial populations were determined by plating 50 µl of each serially diluted bacterial suspensions onto TSA plates. Plates were incubated for 12 – 24 hours for Blend 20 strains and 72 h for DH 44. Colony forming units (CFU) were then determined by counting the number of bacterial colonies that grew on each plate. After each prepared suspension’s concentrations were determined, the populations of all strains were used to make the bacterial stock solution. Stock suspensions were prepared by adding bacterial suspension and distilled water to reach the final concentration (CFU ml^{-1}) of 1×10^{7} of each strain.

Treatment Application

Plots (n = 2/treatment) were randomly assigned to treatments that included a negative control, two rates of synthetic fertilizer (High and Low), DH 44, Blend 20, Accomplish® LM (Loveland Products, Inc®, Greeley, CO; AMS), and AMS + Low N fertilizer. For treatments including synthetic fertilizer, ammonium sulfate (Profertilizer® 21-0-0, Harrell's Inc., Lakewood, FL) was applied at 19 (High) and 10 (Low) kg N ha^{-1}, respectively. Accomplish® LM was used at the labelled rate of 8.4 ml mixed with water to reach the consistent volume of 3.8 L. For Blend 20 and DH 44, each strain was applied in a suspension totaling 3.8 L per plot at a concentration of 1×10^7.

In Year 1 and 2, all treatments were applied on July 9 and September 28, and March 27 and October 5, at the TF site, respectively. Similarly, the BG site fertility applications were performed on June 20 and August 14 , and May 15 and July 24 for Year 1 and 2, respectively. The commercial fertilizer treatment was increased in the second year to better align with common producer practices for bermudagrass hay systems, resulting in the application of 38 kg N ha^{-1} and 20 kg N ha^{-1} in the High and Low N treatments. Three backpack sprayers (one for each PGPR treatment and one for AMS) were used to apply PGPR and AMS to each plot.

Forage mass and nutritive value

In Year 1, plots were harvested every 4 weeks from July 19 to September 13 for BG and August 6 to November 26 for TF using hand clippers and a 0.1-m^2 quadrat. In Year 2, plots were harvested every 4 weeks from June 19 to Oct 20 for BG and March 27 to May 28 as well as November 2 and November 30 for TF, respectively. Three randomized samples were taken per plot at both locations. Bermudagrass plots were mowed to 5-cm stubble height after each harvest to mimic the management on a hay production system. The TF plots were only mowed to a 5-cm stubble height prior to each treatment application to mimic a stockpiled system. Forage samples were weighed wet at the Auburn University Department of Animal Sciences Ruminant Nutrition Laboratory (Auburn, AL) and then placed in a forced-air oven at 50°C for 72 h. Samples were then weighed to yields and ground to pass a 1-mm screen using a Wiley Mill (Thomas Scientific, Philadelphia, PA). Forage nutritive value of all samples was determined using Near Infrared Reflectance Spectroscopy (NIRS) at Auburn University Soil and Forage Testing Laboratory (Auburn, AL) using a Unity SpectraStar 2500 XL (Unity Scientific, Milford, MA) with equations developed by National Forage Testing Association (NAFTA). Each year, 10% of the total sampled were randomly selected from each species and analyzed via wet chemistry to validate results. Samples that were selected for wet chemistry were analyzed for DM, neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP). Forage DM concentrations were determined according to the procedures of AOAC International (1990). Concentrations of NDF, ADF, and ADL were determined using the methods described by van Soest et al. (1991). Concentrations of NDF and ADF were analyzed using ANKOM 2000® fiber analysis system (Ankom Technology Coporation, Fairport, NY). Nitrogen concentration was determined using the Kjeldhal procedure (AOAC, 1995). Crude protein concentration was obtained by multiplying the N concentration by 6.25.

Statistical Analysis

Data were analyzed using PROC GLIMMIX of SAS 9.4 (SAS Institution, Cary, NC). Mean comparisons of forage mass and nutritive value were conducted using Fisher-protected least square means, and all effects and interactions were considered significant at $\alpha = 0.05$. Forage mass was summed across harvests to provide an average harvest forage mass. Fertility treatments were considered a fixed effect with harvest date considered a repeated measure. Each forage type was analyzed according to this plan. In order to analyze treatments over time, year was not considered in the analysis. The statistical model included treatment, harvest date, and treatment × harvest interaction.

Results

'Russell' Bermudagrass

Forage Biomass

There was not a significant effect on forage biomass for fertility treatments ($P > 0.1584$). A treatment × harvest interaction occurred for forage biomass ($P < 0.0001$). In Oct-20, DH 44, High N, AMS + Fert, and Blend 20 were greater than the control by 57, 52, 25 and 13%, respectively ($P = 0.0035$, $P = 0.0070$, $P = 0.0026$, respectively); however, AMS + Fert and Blend 20 were not significantly different ($P = 0.1733$, $P = 4929$, respectively). The same treatments in Jul-20 were less than the same treatments in Oct-20 (DH 44: 70%; High N: 53%; AMS + Fert: 38%; Blend 20: 28%, respectively). AMS and AMS + Fert in Jun-20 was less than AMS + Fert in Jul-20 ($P = 0.0366$, $P = 0.0256$, respectively). AMS and AMS + Fert in Jul-20 and Aug-20 were less than AMS in the Oct-20 harvest ($P = 0.0306$, $P = 0.0460$, respectively).

Nutritive Value

There was not a treatment × harvest interaction for CP concentration ($P > 0.0691$, Figure 4). The CP concentration was also not affected by fertility treatment (115 g kg^{-1}; $P > 0.1050$, Table 1).

The neutral detergent fiber was not significantly affected by fertility treatment (672 g kg^{-1}; $P > 0.1379$). There was a treatment × harvest interaction effect on NDF concentration ($P < 0.0001$, Figure 5). In Jun-20, all treatments were significantly greater than the control, excluding AMS + Fert (AMS: 24%, Blend 20: 22%, DH 44: 25%, High N: 26%, Low N: 22%; $P = 0.0001$, respectively). All treatments in the Jun-20 harvest were less than all treatments in the Aug-20 and Oct-20 harvests ($P \leq 0.0500$). Furthermore, AMS in Jul-20 was less than the Aug-20 harvest ($P = 0.0200$).

Acid detergent fiber was not affected by fertility treatments ($P > 0.0520$); however, it was affected by a treatment × harvest interaction ($P < 0.0019$, Figure 6). High N, Low N, and AMS + Fert in the fourth harvest resulted in the lowest ADF concentrations at 28.1, 28.8, and 29.1%, respectively, and was less than the control by <10% but was not significantly different ($P = 0.3679$). AMS + Fert in Aug-20 was greater in ADF concentration than AMS + Fert in the Oct-20 harvest ($P = 0.0377$). Within the Aug-19 harvest, Blend 20 was greater in ADF concentration than all treatments ($P \leq 0.0500$), excluding, AMS and AMS + Fert. Concentrations of ADF for AMS and AMS + Fert treated plots in Jun-20 were significantly less than AMS + Fert treated plots in Jul-20 ($P = 0.0378$, $P = 0.0327$, respectively) as well as the Aug-20 harvest ($P = 0.0046$, $P = 0.0002$, respectively) and AMS + Fert in Oct-20 ($P = 0.0238$). Plots treated with DH 44 in the Jun-20 harvest was less than the Aug-20 harvest ($P = 0.0204$). In the Jun-20 harvest, High N was less than the Jul-20, Aug-20, and Oct-20 harvests ($P = 0.0065$, $P = 0.0016$, $P = 0.0246$, respectively). Low N in the Jul-20 harvest was less than Aug-20 ($P = 0.0050$, respectively). There were no differences among treatments in the last harvest ($P > 0.0500$).

Fertility treatments did not have a significant effect on TDN concentration of bermudagrass (616 g kg^{-1}; $P > 0.1698$). However, there was a treatment × harvest interaction effect on TDN concentration ($P < 0.0001$, Figure 7). The fertility treatments in Jun-20 resulted in the greatest TDN concentrations (High N: 69, Low N: 68, AMS + Fert: 68, AMS: 68, DH 44: 67, Blend 20: 66%). In the same harvest, High N, Low N, and AMS + Fert, were all <10% greater than the control but were not significantly different ($P = 0.8635$, $P = 0.9650$, $P = 0.7848$, respectively). Furthermore, Low N, Blend 20, and AMS in the Sept-19 harvest had the lowest TDN concentrations among harvests (56, 55, and 54%, respectively). AMS in the Jul-19 harvest was greater than AMS in Sept-19 ($P = 0.0200$); however, it was less than all other harvests, excluding Aug-19, ($P \leq 0.0500$). AMS + Fert in the Jul-19 harvest was greater than both AMS and AMS + Fert in Aug-19 ($P = 0.0192$, $P = 0.0364$, respectively). Low N in the Jul-19 harvest (60%) had a greater TDN concentration than both Aug-19 and Sept-19 (57%, $P = 0.0461$,5 6%, $P = 0.0150$, respectively). In the Aug-19 and Sept-19 harvests, all treatments were less than all treatments in the Jun-20 through Oct-20 harvests ($P \leq 0.0500$). AMS in the Jun-20 harvest had a greater TDN concentration than AMS in the Aug-20 harvest ($P = 0.0040$). Concentrations of TDN for plots treated with DH 44 in the Jun-20 harvest was greater than the Aug-20 harvest ($P = 0.0187$).

Table 1: Mean harvest yield and nutritive value of ‘Russell’ bermudagrass during the 2019 and 2020 growing seasons in Lawrence County, AL.

Treatment	Yield	CP	NDF	ADF	TDN
	Kg DM ha^{-1}	-----------g DM kg^{-1}-----------			
AMS	941^{a}	108^{b}	685^{a}	321^{ac}	612^{ab}
AMS + Fert	891^{ab}	117^{a}	662^{b}	326^{a}	618^{ab}
B20	859^{ab}	119^{a}	676^{ab}	324^{ab}	614^{ab}
DH44	940^{a}	112^{ab}	677^{ab}	320^{ac}	618^{ab}
High N	989^{a}	118^{a}	674^{ab}	311^{bc}	623^{a}
Low N	849^{ab}	116^{ab}	673^{ab}	313^{bc}	623^{a}
Control	791^{b}	112^{ab}	657^{b}	326^{a}	609^{b}
SEM[‡]	52.2232	0.2885	0.7208	0.4039	0.4173

[†]AMS = Accomplish® LM; AMS + Fert = Accomplish ® LM + Low N; B20 = Blend 20; High N = 38 kg N ha^{-1}; Low N = 20 kg N ha^{-1}; control = no fertility added.
[‡]SEM = standard error of the mean.
[a,b,c]Means within a column followed by a common letter are not different ($P > 0.05$).

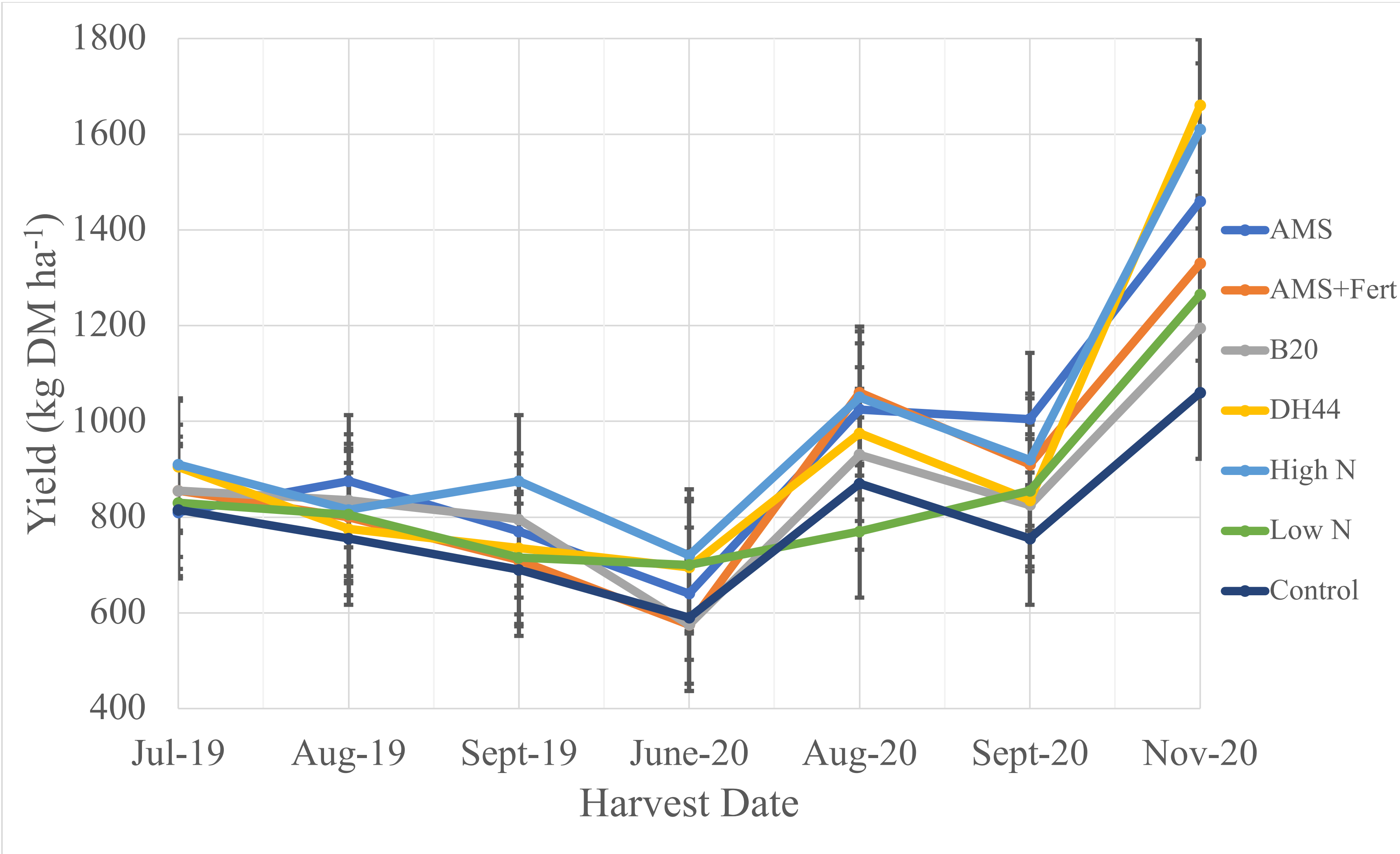

Figure 3: Forage biomass (kg DM ha^{-1}) of 'Russell' bermudagrass during the 2019 and 2020 growing seasons in Lawrence County, AL. The treatments are as follows: AMS = Accomplish® LM; AMS + Fert = Accomplish® LM + Low N; B20 = Blend 20, PGPR blend; DH44, PGPR strain; High N = 38 kg N ha^{-1}; Low N = 20 kg N ha^{-1}; Control = no fertility added.

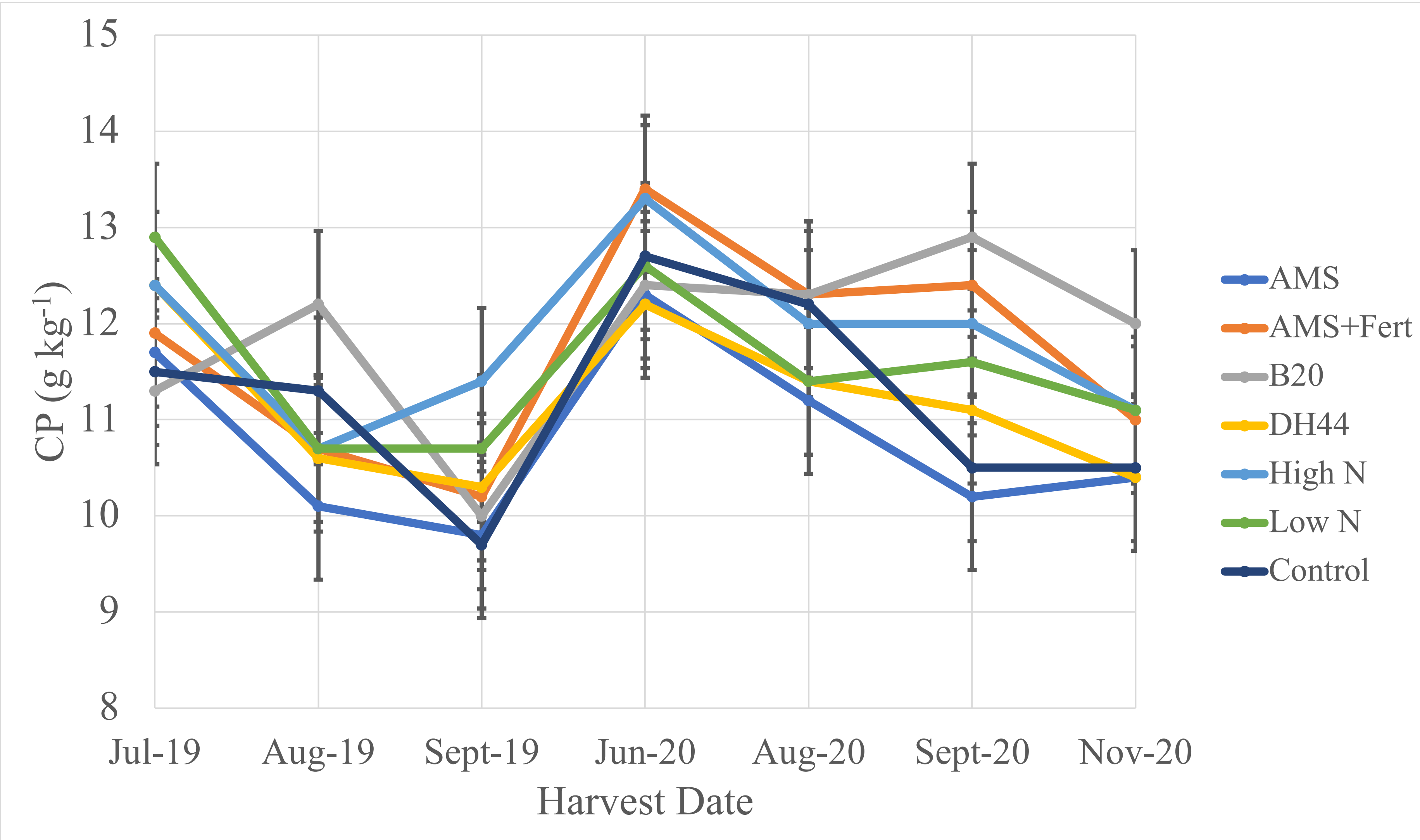

Figure 4: Concentration of CP (g kg^{-1}) of 'Russell' bermudagrass during the 2019 and 2020 growing seasons in Lawrence County, AL. The treatments are as follows: AMS = Accomplish® LM; AMS + Fert = Accomplish® LM + Low N; B20 = Blend 20, PGPR blend; DH44, PGPR strain; High N = 38 kg N ha^{-1}; Low N = 20 kg N ha^{-1}; Control = no fertility added.

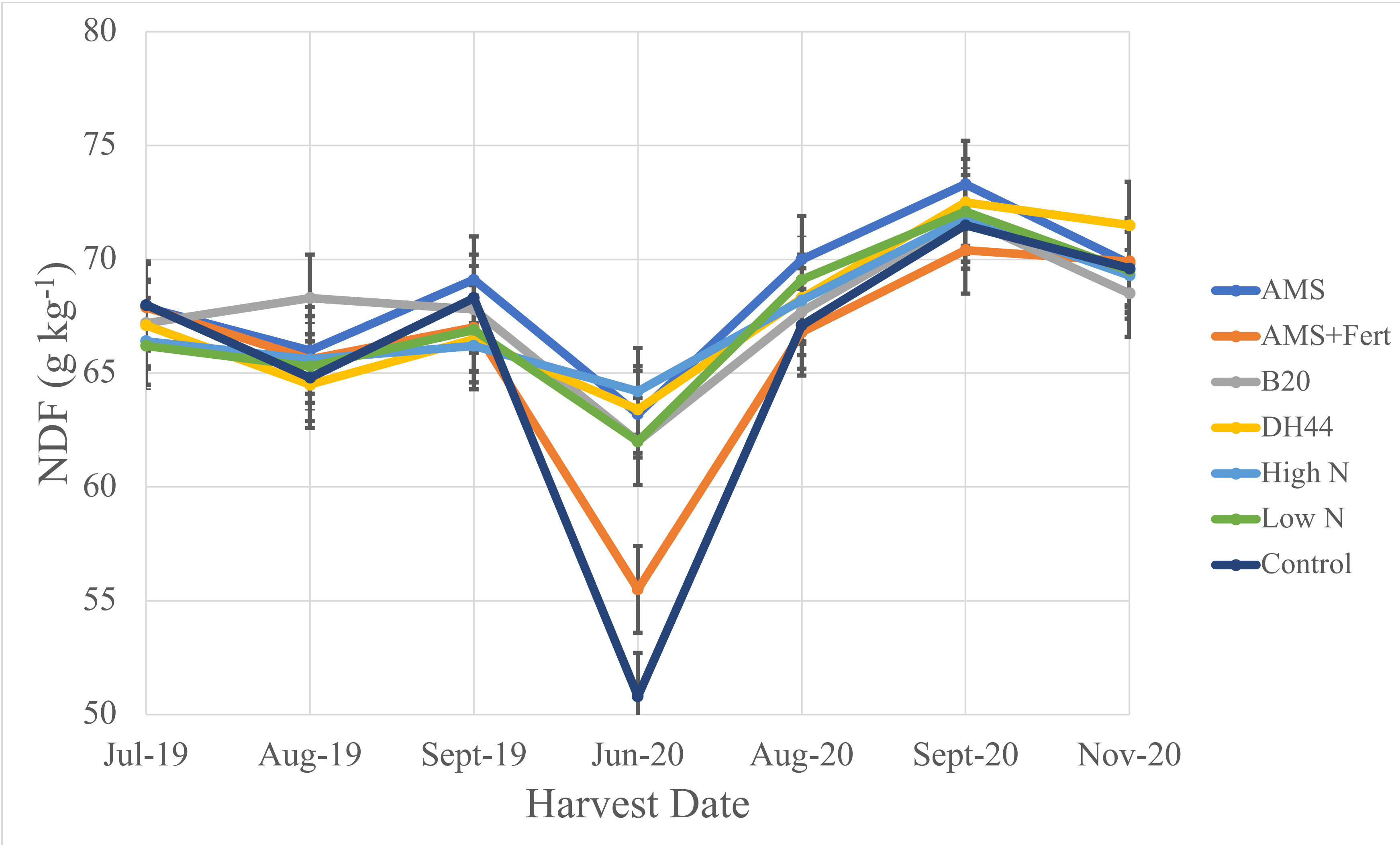

Figure 5: Concentration of NDF (g kg^{-1}) of 'Russell' bermudagrass during the 2019 and 2020 growing seasons in Lawrence County, AL. The treatments are as follows: AMS = Accomplish® LM; AMS + Fert = Accomplish® LM + Low N; B20 = Blend 20, PGPR blend; DH44, PGPR strain; High N = 38 kg N ha^{-1}; Low N = 20 kg N ha^{-1}; Control = no fertility added.

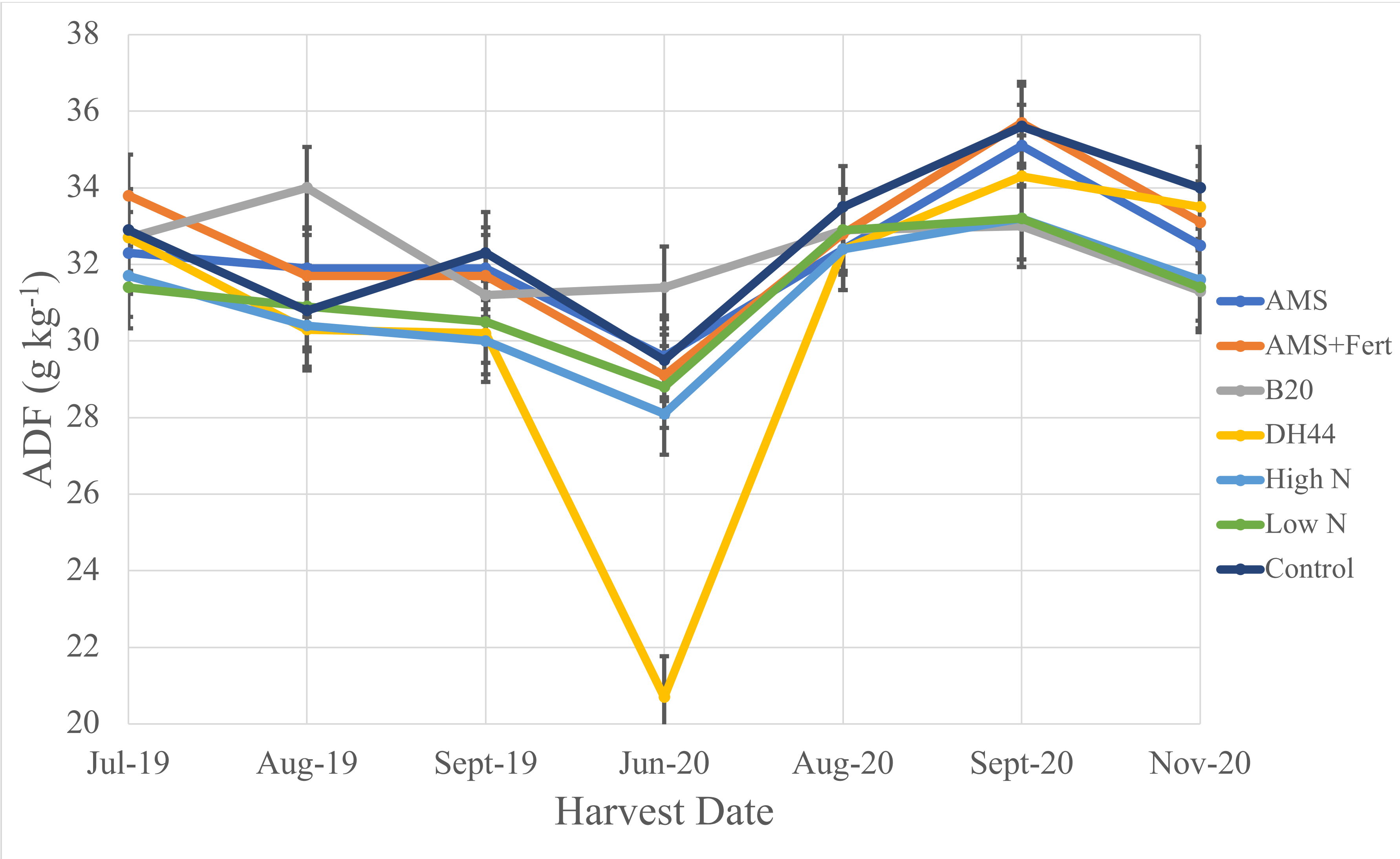

Figure 6: Concentration of ADF (g kg^{-1}) of 'Russell' bermudagrass during the 2019 and 2020 growing seasons in Lawrence County, AL. The treatments are as follows: AMS = Accomplish® LM; AMS + Fert = Accomplish® LM + Low N; B20 = Blend 20, PGPR blend; DH44, PGPR strain; High N = 38 kg N ha^{-1}; Low N = 20 kg N ha^{-1}; Control = no fertility added.

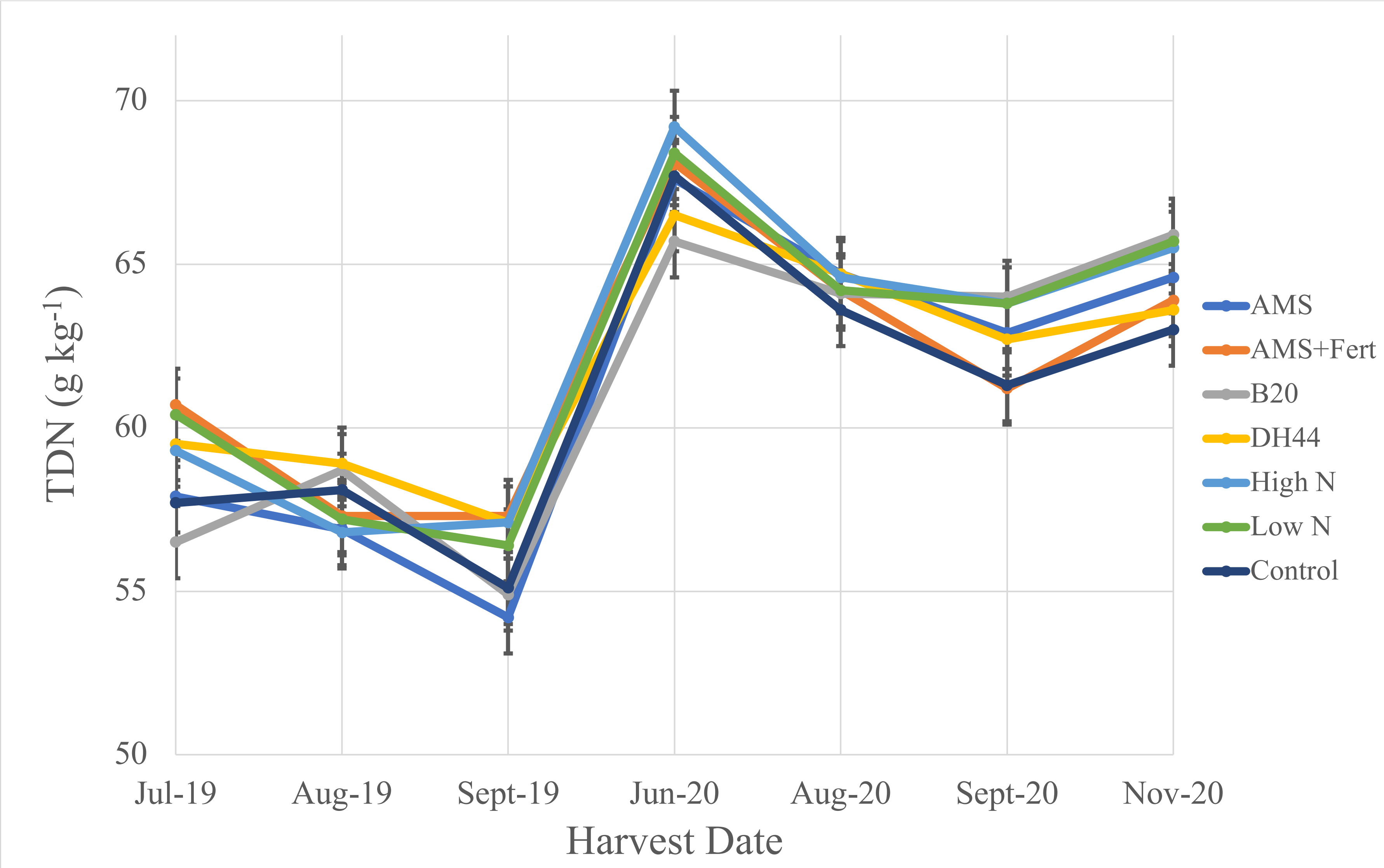

Figure 7: Concentration of TDN (g kg^{-1}) of 'Russell' bermudagrass during 2019 and 2020 growing season in Lawrence County, AL. The treatments are as follows: AMS = Accomplish® LM; AMS + Fert = Accomplish® LM + Low N; B20 = Blend 20, PGPR blend; DH44, PGPR strain; High N = 38 kg N ha^{-1}; Low N = 20 kg N ha^{-1}; Control = no fertility added.

'KY 31' Tall Fescue

Forage Biomass

Fertility treatments did not have a significant effect on forage biomass of tall fescue (717 kg DM ha^{-1}, $P = 0.2550$). There was a treatment × harvest interaction effect forage biomass ($P < 0.0001$, Figure 8). High N, AMS + Fert, and Low N resulted in the greatest yield in Apr-20 (1,250, 1,070, 1,050 kg DM ha^{-1}, respectively). High N had a 22% increase in yield over the control in the same harvest. In the Sept-19 harvest, AMS was greater yielding than AMS + Fert in Nov-19 ($P = 0.0091$) and early Oct-19 ($P = 0.0368$). In the Sept-19 harvest, Low N was greater than in the Nov-19 harvest ($P = 0.0409$). Tall fescue treated with DH 44 in Mar-20 was less than the Apr-20 harvest ($P = 0.0004$). AMS in the late Oct-19 harvest was greater than AMS + Fert in the Nov-19 ($P = 0.0236$). Blend 20, in the Mar-20 harvests, was less than Blend 20 in the Apr-20 harvest ($P = 0.0116$). In the Mar-20 harvest, AMS + Fert was greater than AMS and AMS + Fert in the May-20 harvest ($P = 0.0002$, $P = 0.0001$, respectively). High N in the Mar-20 harvest was greater than in May-20 ($P = 0.0080$), but less than the Apr-20 harvest ($P = 0.0054$). All treatments in the Apr-20 harvest were greater than all treatments in the May-20 ($P \leq 0.0500$). Within the May-20 harvest, AMS was greater yielding than AMS + Fert ($P = 0.0368$Tall fescue treated with AMS + Fert in May-20 resulted in the least forage biomass at 355 kg DM ha^{-1} but was not significantly different than Blend 20 in the same harvest, 565 kg DM ha^{-1} ($P = 0.0560$).

Nutritive Value

Fertility treatments did not have a significant effect on CP concentration of tall fescue (112 g kg^{-1}; $P > 0.1913$). However, there was a treatment × harvest interaction effect on CP concentration ($P < 0.0001$, Figure 9). The control in Mar-20 resulted in the greatest CP concentration (14.8%) but was not different than High N and Blend 20 in the same harvest (14.4

and 14.1%, respectively). In the Aug-19 harvest, AMS was greater in CP concentration than AMS in early and late Oct-19 (*P* = 0.0038, *P* = 0.0380, respectively). Blend 20 was greater in Aug-19 than the early Oct-19 and Nov-19 (*P* = 0.0007, *P* = 0.0125, respectively). Concentrations of CP for DH 44, High N, and Low N treated tall fescue in Aug-19 was greater than the early Oct-19 (*P* = 0.0180, *P* = 0.0283, *P* = 0.0019, respectively). Blend 20 and Low N in the Sept-19 harvest was greater than in early Oct-19 (*P* = 0.0152, *P* = 0.0330, respectively). In early Oct-19, AMS + Fert was lower in CP concentration than AMS + Fert in the late Oct-19 harvest (*P* = 0.0016). Blend 20, DH 44, High N, and Low N treated tall fescue in early Oct-19 had lower CP concentrations than the late Oct-19 (*P* = 0.0005, *P* = 0.00011, *P* = 0.0288, *P* = 0.0002, respectively). Within the early Oct-19 harvest, AMS was less than AMS + Fert (*P* = 0.0294). AMS + Fert in the early Oct-19 harvest was greater in CP concentration than AMS and AMS + Fert in Nov-19 (*P* = 0.0189, *P* = 0.0206, respectively). Blend 20 and Low N were greater in CP concentration in early Oct-19 compared with Nov-19 (*P* = 0.0096, *P* = 0.0094, respectively). All treatments, excluding DH 44 and High N, in the Nov-19 harvest were less than all treatments in the Mar-20 harvest ($P \leq 0.0500$). In the Mar-20 harvest, all treatments, excluding the Low N, were greater than all treatments in the Apr-20 and May-20 ($P \leq 0.0500$).

There was not a treatment effect on NDF concentration of tall fescue (625 g kg^{-1}; $P >$ 0.3978). There was a treatment × harvest interaction effect on neutral detergent fiber ($P <$ 0.0001, Figure 10). Treatments in the Mar-20 harvest resulted in the lowest NDF concentrations with all treatments being <10% greater than the control (50%). Whereas AMS + Fert, Blend 20, AMS, and Low N in early Nov-20 resulted in the greatest NDF concentration among harvest dates (72, 71, 70, 70 %, respectively). AMS + Fert in Aug-19 was greater in NDF concentration than AMS in Nov-19 (*P* = 0.0291). Concentration of NDF with DH 44 treated tall fescue was greater in Aug-19 and Sept-19 compared with the Nov-19 ($P = 0.0086$, $P = 0.0113$, respectively). In the Sept-19 harvest, AMS and AMS + Fert had greater NDF concentrations than AMS in the Nov-19 harvest ($P = 0.0269$, $P = 0.0388$, respectively). Tall fescue treated with High and Low N in Sept-19 resulted in a greater NDF concentration compared with the Nov-19 ($P = 0.0007$, $P = 0.0310$, respectively). In the early Oct-19 harvest, all treatments had a greater NDF concentration than the same treatments in Nov-19 ($P \leq 0.0500$). In the late Oct-19 harvest, AMS had a greater NDF concentration than AMS and AMS + Fert in Nov-19 ($P = 0.0020$, $P = 0.0069$, respectively). Concentration of NDF for tall fescue treated with AMS + Fert was greater in the late Oct-19 harvest compared with AMS and AMS + Fert in the Nov-19 harvest ($P = 0.0224$, $P = 0.0040$, respectively). DH 44 had a greater NDF concentration in late Oct-19 compared with Nov-19 ($P = 0.0419$); however, it had a lower concentration in the Nov-19 harvest ($P = 0.0371$). In late Oct-19, High N had a greater concentration than the Nov-19 ($P = 0.0070$). In Mar-20 and Apr-20, all treatments, excluding Blend 20 and the control, had lower concentrations of NDF than the same treatments in May-20 ($P \leq 0.0500$). AMS + Fert in the Mar-20 harvest had a lower concentration than AMS in the Apr-20 harvest ($P = 0.0413$). Concentrations of NDF was lower for tall fescue treated with High N in Mar-20 compared with the Apr-20 harvest ($P = 0.0150$).

Fertility treatment influenced ADF concentration of tall fescue ($P < 0.0162$). AMS and Blend 20 treated tall fescue resulted in greater ADF concentrations compared with the control ($P = 0.0306$, $P = 0.0194$, respectively), DH 44 ($P = 0.0042$, $P = 0.0024$, respectively), and High N ($P = 0.0235$, $P = 0.0146$, respectively) by <10%.

There was a treatment × harvest interaction effect on ADF concentration for tall fescue ($P < 0.0001$, Figure 11). Treatments in Nov-19 and Mar-20 resulted in the least ADF concentrations for tall fescue. The control in Mar-20 was <10% lower than all fertility treatments in Mar-20

(29%) but was not significantly different. Whereas in Nov-19, all fertility treatments were <10% lower than the control (30%) but were not significantly different. Blend 20 had a lower concentration in the Aug-19 and Sept-19 harvests compared with the early Oct-19 ($P = 0.0329$, $P = 0.0323$, respectively). DH 44 resulted in a greater ADF concentration in Aug-19 and Sept-19 compared with Nov-19 ($P = 0.0201$, $P = 0.0454$, respectively). Concentration of ADF for tall fescue treated with High N was greater in the Sept-19 harvest compared with the Nov-19 ($P = 0.0344$). All treatments in the early and late Oct-19 (excluding the control, DH 44, and Low N) had a greater ADF concentration than the same treatments in the Mar-20 harvest ($P \leq 0.05$). DH 44 and Low N treated tall fescue in the early Oct-19 harvest had greater ADF concentrations compared with Nov-19 harvest ($P = 0.0010$, $P = 0.0223$, respectively). In the late Oct-19 harvest, AMS had a greater ADF concentration compared with the Nov-19 harvest ($P = 0.0025$). All treatments Mar-20 harvest had lower ADF concentrations than the same treatments in May-20 ($P \leq 0.0500$). In the Mar-20 harvest, AMS + Fert, DH 44, and High N treated tall fescue resulted in lower ADF concentrations than in Apr-20 ($P = 0.0362$, $P = 0.0344$, $P = 0.0178$, respectively).

There was not a treatment effect on TDN concentration of tall fescue (616 g kg^{-1}; $P > 0.1718$). However, there was a treatment × harvest interaction effect on ADF ($P < 0.0001$, Figure 12). All fertility treatments in Mar-20 resulted in the greatest TDN concentrations. The control (69%) was <10% greater than all fertility treatments, excluding High N (69%). Tall fescue treated with AMS in early and late Nov-20 had the lowest TDN concentrations (55 and 56%) but the control was <10% greater in both harvests. In the Sept-19 harvest, AMS had a greater TDN concentration than late Oct-19 ($P = 0.0400$). Whereas Blend 20 and DH 44 treated tall fescue in Spet-19 had a greater TDN concentration compared with the early Oct-19 ($P = 0.0307$, $P = 0.0113$, respectively). In the early Oct-19 harvest, tall fescue treated with AMS and DH 44 had lower TDN concentrations than the Nov-19 ($P = 0.0271$, $P = 0.0100$, respectively). Within the late Oct-19 harvest, AMS (57%) had a lower TDN concentration than AMS + Fert (63%, $P = 0.0394$). All treatments in the Mar-20 harvest had a greater TDN concentration compared with the same treatments in the early and late Nov-20 harvests ($P \leq 0.0500$). AMS and AMS + Fert treated tall fescue in the Mar-20 harvest resulted in greater TDN concentrations compared with the May-20 harvest ($P = 0.0162$, $P = 0.0474$, respectively). Tall fescue treated with High N in Mar-20 had a greater TDN concentration compared with both the Apr-20 and May-20 harvests ($P = 0.0221$, $P = 0.0023$, respectively).

Table 2: Mean harvest yield and nutritive value of 'KY 31' tall fescue during the 2019 and 2020 growing seasons in Montgomery County, AL.

Treatment	Yield	CP	NDF	ADF	TDN
	Kg DM ha^{-1}		-----------g DM kg^{-1}-----------		
AMS	729^{ab}	107^{c}	632	363^{a}	606^{c}
AMS + Fert	726^{ab}	112^{abc}	632	356^{ab}	615^{abc}
B20	687^{b}	108^{bc}	630	364^{a}	607^{bc}
DH44	686^{b}	117^{ab}	614	346^{b}	624^{ab}
High N	762^{a}	118^{a}	618	349^{b}	620^{abc}
Low N	732^{ab}	111^{abc}	630	355^{ab}	615^{abc}
Control	699^{ab}	112^{abc}	621	350^{b}	625^{a}
SEM‡	24.1587	0.2921	0.7179	0.4187	0.6017

†AMS = Accomplish® LM; AMS + Fert = Accomplish® LM + Low N; B20 = Blend 20; High N = 19 kg N ha^{-1}; Low N = 10 kg N ha^{-1}, control = no fertility added.
‡SEM = standard error of the mean.
[a,b,c]Means within a column followed by a common letter are not different ($P > 0.05$).

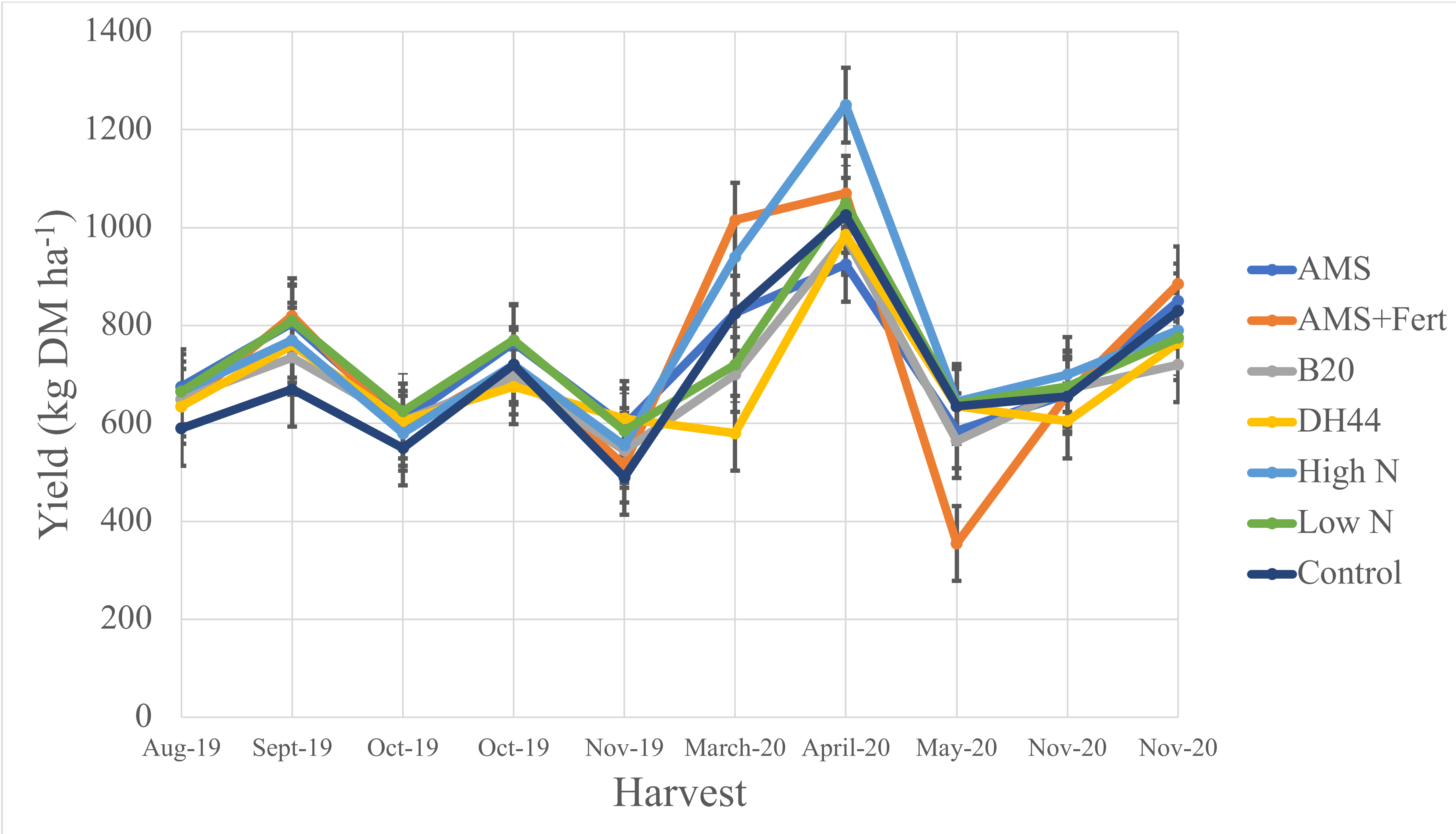

Figure 8: Forage biomass (kg DM ha^{-1}) of 'KY 31' tall fescue during the 2019 and 2020 growing seasons in Montgomery County, AL. The treatments are as follows: AMS = Accomplish® LM; AMS + Fert = Accomplish® LM + Low N; B20 = Blend 20, PGPR blend; DH44, PGPR strain; High N = 19 kg N ha^{-1}; Low N = 10 kg N ha^{-1}; Control = no fertility added.

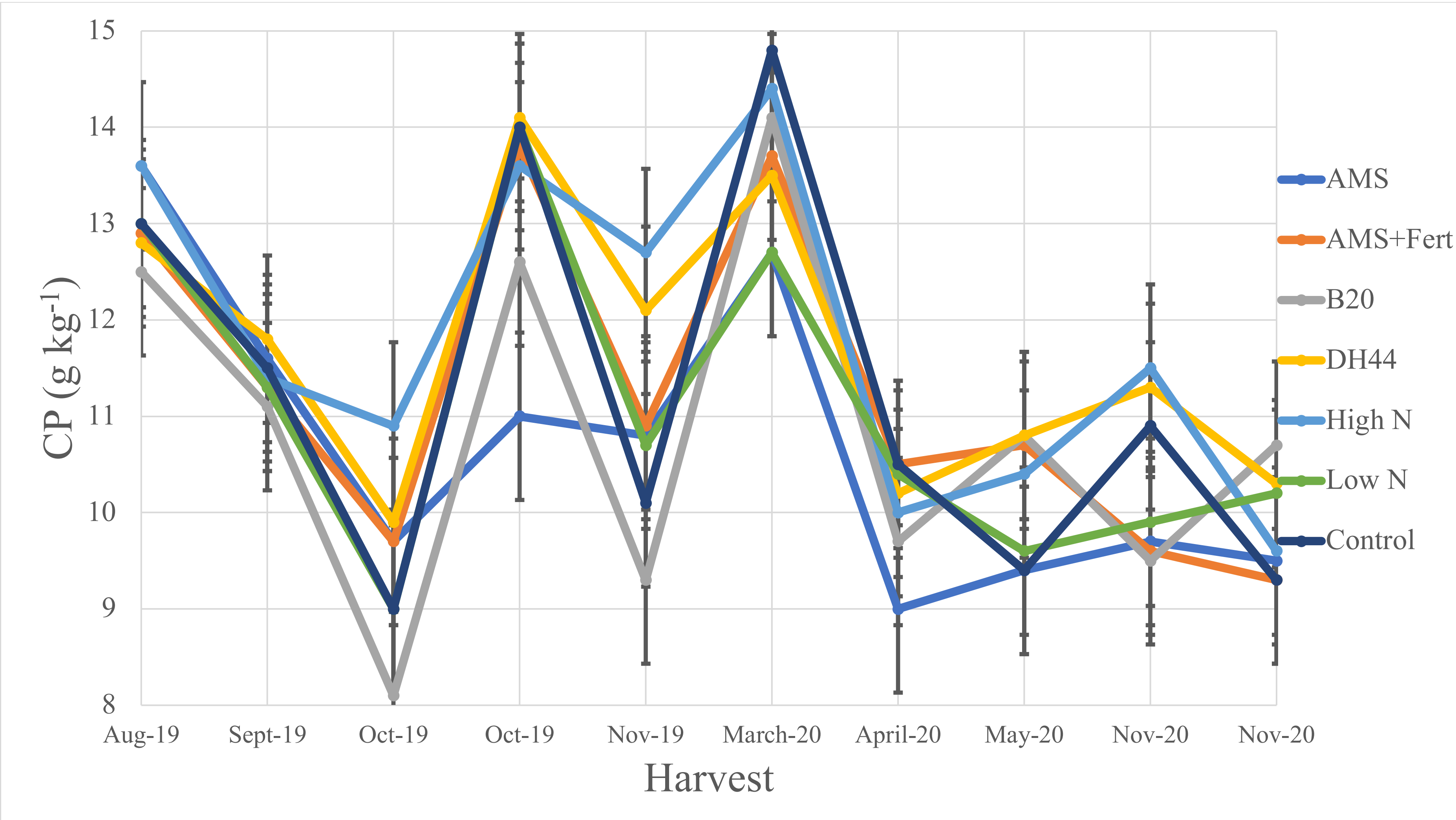

Figure 9: Concentration of CP (g kg^{-1}) of 'KY 31' tall fescue during the 2019 and 2020 growing seasons in Montgomery County, AL. The treatments are as follows: AMS = Accomplish® LM; AMS + Fert = Accomplish® LM + Low N; B20 = Blend 20, PGPR blend; DH44, PGPR strain; High N = 19 kg N ha^{-1}; Low N = 10 kg N ha^{-1}; Control = no fertility added.

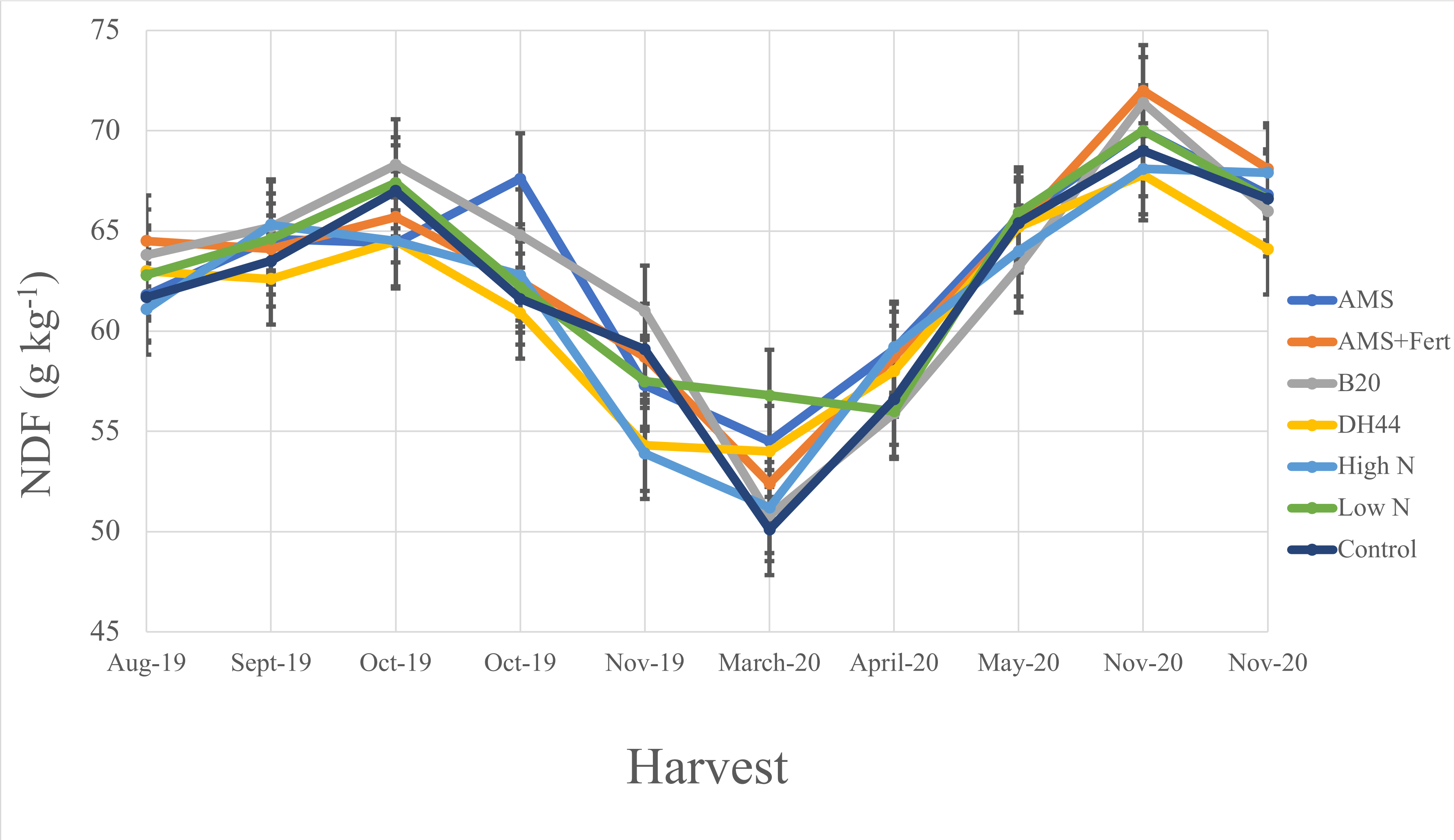

Figure 10: Concentration of NDF (g kg^{-1}) of 'KY 31' tall fescue during the 2019 and 2020 growing seasons in Montgomery County, AL. The treatments are as follows: AMS = Accomplish® LM; AMS + Fert = Accomplish® LM + Low N; B20 = Blend 20, PGPR blend; DH44, PGPR strain; High N = 19 kg N ha^{-1}; Low N = 10 kg N ha^{-1}; Control = no fertility added.

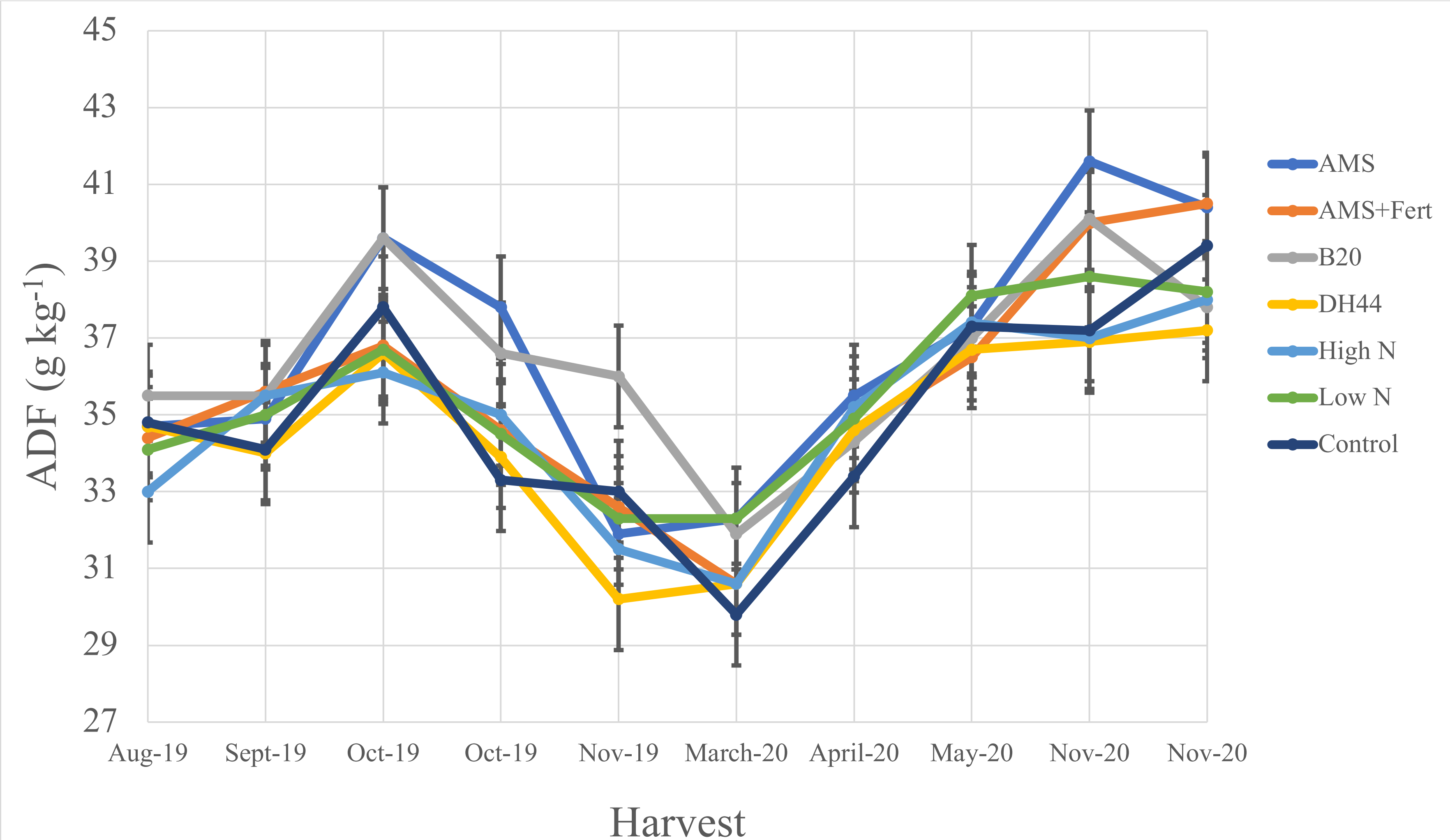

Figure 11: Concentration of ADF (g kg^{-1}) of 'KY 31' tall fescue during the 2019 and 2020 growing seasons in Montgomery County, AL. The treatments are as follows: AMS = Accomplish® LM; AMS + Fert = Accomplish® LM + Low N; B20 = Blend 20, PGPR blend; DH44, PGPR strain; High N = 19 kg N ha^{-1}; Low N = 10 kg N ha^{-1}; Control = no fertility added.

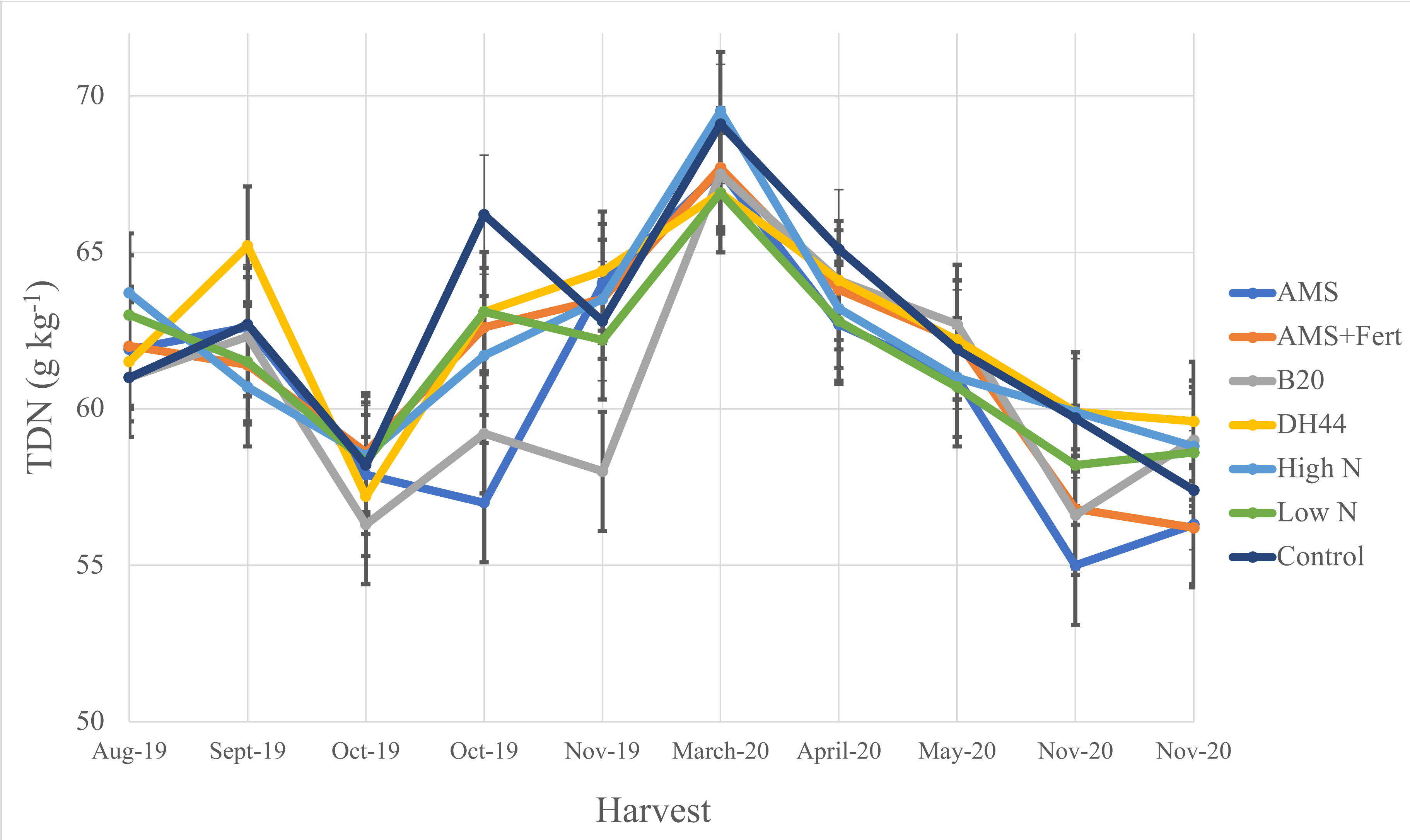

Figure 12: Concentration of TDN (g kg^{-1}) of 'KY 31' tall fescue during the 2019 and 2020 growing seasons in Montgomery County, AL. The treatments are as follows: AMS = Accomplish® LM; AMS + Fert = Accomplish® LM + Low N; B20 = Blend 20, PGPR blend; DH44, PGPR strain; High N = 19 kg N ha^{-1}; Low N = 10 kg N ha^{-1}; Control = no fertility added.

Discussion

Bermudagrass

Bermudagrass is responsive to N fertilization (Ball et al., 2015); therefore, an increase in yield with N-fertilized treatments was expected relative to the non-treated control. An increase in yield for harvest means in the second year was observed, largely as a result of a greater N application rate in the second year. The forage biomass yields in this study were reported as a harvest mean. All the treatments in October 2020 had the greatest biomass, averaging 1,369 kg DM ha^{-1}, with DH 44 yielding the most, 1,660 kg DM ha^{-1}. The yields reported by Griffin et al. (2020) ranged from 1,477 to 1,665 kg ha^{-1} averaged across three harvests and two years for stockpiled bermudagrass. These numbers were similar to the yields in the current study as a hay production system. The temperature for the area followed the 30-year average for all harvest months excluding October 2020 during which it was significantly lower. Hart et al. (1969) determined that less mature forages had greater deterioration from weathering during winter dormancy. The temperatures in the first year were greater than those in the second year. The harvest months in the first summer had less precipitation than the 30-year average and those in the second summer, which along with the temperature patterns could explain the greater yields in the second-year harvests. There were greater drought conditions during the first three harvest months which could explain, in part, the differences observed in the yields.

Hendricks et al. (2020) reported CP concentrations of Tifton 85 bermudagrass ranging from 90 to 154 g kg^{-1}. Hill et al. (1997) reported Coastal bermudagrass as having 146 g kg^{-1} CP concentration which is greater than the current study with Russell. Griffin et al. (2020) reported a CP concentration of Blend 20 and DH 44 treated stockpiled Coastal bermudagrass compared with the control (105 g kg^{-1}) that were less than the values in the current study. Averaged across all harvests, the Blend 20 treatment had the greatest CP concentration, 119 g kg^{-1} compared with AMS, which had the least, 108 g kg^{-1}. The CP concentrations in this study would meet the daily needs of a dry, pregnant beef cow (70 to 80 g kg^{-1}), a lactating beef cow (100 to 120 g kg^{-1}) and a 295 kg growing beef steer [100 to 110 g kg^{-1}, Ball et al., 2015]. Treatments in June of 2020 had the greatest CP concentration averaging 127 g kg^{-1}. Temperatures in this month were less than the temperatures in the previous year. Maturity stages of the forages in this harvest were less than that of later harvests because the plant is nearing reproductive stage and exiting the vegetative stage which could explain, in part, these results. Lalman et al. (2000) observed highly soluble N in standing forages is more susceptible to leaching during long periods of greater precipitation. The last four harvests in this study were exposed to greater levels of precipitation than the first three.

Ball et al. (1996) reported Russell having 691 g kg^{-1} NDF concentration in a test plot study. Griffin et al. (2020) reported NDF values ranging from 710 to 745 g kg^{-1} in stockpiled Coastal bermudagrass, whereas Fike et al. (2017) reported values ranging from 737 to 808 g kg^{-1} in a simulated hay production system. Hendricks et al. (2020) reported NDF concentrations for Tifton 85 ranging from 406 to 610 g kg^{-1}. Values in the current study were greater than those reported by Fike et al. (2017), but similar to those reported by Griffin et al. (2020). Concentration of NDF of all treatments in June 2020 was the least, averaging 602 g kg^{-1}. Differences among harvests in NDF concentration could be explained by the varying plant maturity stages among harvests. June nears the beginning of the growing season for bermudagrass, and the plant had not reached maturity prior to that harvest.

Acid detergent fiber is the lignocellulose fraction of the cell wall in the plant, being a negative correlation to the digestibility of the plant (Ball et al., 2015). In a test plot study by Ball et al. (1996), Russell bermudagrass had an ADF concentration of 329 g kg^{-1}. Fike et al. (2017) reported ADF concentrations ranging from 315 to 345 g kg^{-1} for Coastal bermudagrass hay when treated with PGPR or N fertilizer. Griffin et al. (2020) reported values for stockpiled Coastal bermudagrass treated with Blend 20, DH 44, and synthetic fertilizer of 362, 355, and 356 g kg^{-1}, respectively. Values in the current study were less than those reported by Griffin et al. (2020) but similar to those reported by Fike et al. (2017), which is expected since the first two studies evaluated stockpiled bermudagrass which is more mature at the time of sampling than bermudagrass hay. The June 2020 harvest had the lowest ADF concentrations averaging 296 g kg^{-1}. Differences among harvests could be explained by the maturity of the forage at the different harvest dates.

The TDN value is the sum of the digestible protein, fiber, nitrogen-free extract, and fat multiplied by 2.25. It is usually expressed as a percent or mass instead of a caloric energy measurement (Ensminger and Olentine, 1978). Averaged across all harvests, the treatment High N had the greatest TDN concentration compared with the control (623 vs. 609 g kg^{-1}, respectively). Kering et al. (2011) reported the TDN concentration of bermudagrass in a seasonal mean across seven years averaging 600 g kg^{-1}, which is similar to the results of the current study. Hendrick et al. (2020) reported a TDN ranging from 619 to 675 g kg^{-1} for Tifton 85 bermudagrass. The author also concluded that TDN increased linearly with increased N fertilization levels. The TDN concentrations in the current study would meet the requirements of a dry, pregnant beef cow (500 g kg^{-1}), lactating beef cow (600 g kg^{-1}), and a 204 kg growing beef steer (Ball et al., (2015). The greater the NDF and ADF concentrations in the plant results in less total digestible nutrients due to a higher concentration of the cell wall constituents responsible for the rigidity of the plant. Since the harvest in June of 202 had the least NDF and ADF concentrations, it resulted in the most total digestible nutrients. Crude protein is a factor in the total digestible nutrients equation causing it to increase or decrease depending on the CP concentration of the plant.

Tall Fescue

Ocumpaugh et al. (1977) reported stockpiled tall fescue having a maximum yield of 1,000 kg DM ha^{-1} for the first year. High N had the greatest forage biomass among treatments at 762 kg DM ha^{-1} for a harvest mean, which would be average compared to the seasonal yields found in literature. Freeman et al. (2019) reported tall fescue yielding 2,855 kg DM ha^{-1} averaged across three years. The yield in the current study was greatest for all treatments in April 2020, averaging 1,041 kg DM ha^{-1} for a harvest mean. The November 2019 and May 2020 harvests had the least yield, which could be explained by the harvests timing falling at the end of the growing seasons in the fall and spring, respectively. Tall fescue spring growth begins in mid- to late-February and starts to fall off in early May, whereas fall growth begins in mid-August and continues through late-November. However, if fall stockpiled, tall fescue can provide forage for grazing throughout December and January if managed appropriately. There was a significantly greater amount of precipitation in the second year of this study, potentially influencing the yield of the last harvests. The precipitation in the first year was consistently less than the 25-year average for all harvest months. The average monthly temperature was lower in the last five harvests compared with the first five harvests but followed the trend of the 25-year average. During the first couple of harvests, there was competition in the stand from warm-season forages and weeds. This could have impacted the yield and nutritive value of some harvests.

Averaged across all harvests, High N had the greatest CP concentration and AMS with the least compared with the control (High N: 118; AMS: 107 vs. 112 g kg^{-1}). Freeman et al. (2019) reported the CP concentration of fall-stockpiled tall fescue at 144 g kg^{-1} averaged across three years from 18 privately owned farms in North Carolina. Franzluebbers et al. (2021) reported CP concentrations ranging from 90 to 110 g kg^{-1}. The control in the March 2020 harvest had the greatest CP concentration, 148 g kg^{-1}, but was not different than all treatments in the August 2019, second harvest in October 2019, and March 2020 harvests ($P > 0.05$), which results could be explained, in part, by the plant maturity at each harvest. As the forage matures, the CP concentration decreases (Ball et al., 2015).

Averaged across all harvests, AMS + Fert had the greatest NDF concentration and DH 44 had the least compared with the control (AMS+ Fert: 632; DH 44: 614 vs. 621 g kg^{-1}). Franzluebbers et al. (2021) reported the NDF concentration of fall-stockpiled tall fescue pastures ranging from 516 to 659 g kg^{-1}. Gerrish et al. (1994) reported the NDF concentration averaged across three years of fall-stockpiled tall fescue being 564 g kg^{-1} with zero N applied. However, there was not a difference among all treatments. All treatments in the first harvest in November 2020 harvest had the greatest NDF concentration averaging 697 g kg^{-1} but was not different than all treatments, excluding DH 44 in the second harvest in November 2020 ($P > 0.0500$). Treatments in the March 2020 harvest had the least NDF concentration, averaging 528 g kg^{-1}. The concentrations of NDF in the current study were consistent with the concentrations reported in previous studies. Temperatures during the first four harvests were greater than those of the last five, which could explain the differences in NDF concentrations among harvest dates. Higher temperatures lead to an increase in lignification of the plant (Ball et al., 2015) which causes the concentration of NDF to increase with more lignin content. The maturity level of the forage was greater in the last two harvests in November 2020 compared with those in the March 2020 harvest resulting in greater cell wall contents such as lignin, cellulose, and hemicellulose.

Harvest mean concentrations of ADF were greatest for Blend 20 at 364 g kg^{-1} and least for DH 44 346 g kg^{-1}, compared with the control, 350 g kg^{-1}. Freeman et al. (2019) reported the ADF concentration of fall-stockpiled tall fescue being 310 g kg^{-1} averaged across three years. Franzluebbers et al. (2021) reported the tall fescue ADF concentration ranging from 289 to 424 g kg^{-1}. Gerrish et al. (1994) reported the ADF of fall-stockpiled tall fescue averaged across three years at 387 g kg^{-1} with zero N applied. Cool-season perennial grasses like tall fescue will decline in nutritive value as the forage maturity advances in the spring (Ball et al., 2015).

The second harvest in October 2019 had the greatest TDN concentration among treatments, averaging 676 g kg^{-1} a harvest, which was similar to those reported by Franzluebbers et al. (2021), ranging from 529 to 702 g kg^{-1}. Freeman et al. (2019) reported the TDN of fall-stockpiled tall fescue pastures being 678 g kg^{-1} averaged across three years. All treatments in the March 2020 harvest, had the greatest TDN concentration averaging 679 g kg^{-1}, which was expected because of the NDF and ADF values were more favorable in these harvests. Considering the weather during the growing seasons, harvests in the first year were subject to greater temperatures and less precipitation than the second year. Tall fescue is usually more digestible than bermudagrass due to the forage having more leafy material than bermudagrass (Ball et al., 2015). The digestibility of cool-season perennials is greatest in the spring months and deteriorates later in the summer months (Ball et al., 2015) as observed in the current study. The TDN concentrations in the current study would meet the requirements of a dry, pregnant beef cow (500 g kg^{-1}), lactating beef cow (60 g kg^{-1}), and a 204 kg growing beef steer (Ball et al., (2015).

Griffin et al. (2020) found that Blend 20 outperformed DH 44 in the yield of Coastal bermudagrass under a fall-stockpiled small plot study. The current study found that DH 44 and Blend 20 had similar mean harvest yields and nutritive values to the commercial fertilizer for bermudagrass. However, the harvest means of DH 44 and Blend 20 were not significantly different than the control for forage biomass, with a <10% increase in yield over the control for all fertility treatments. In bermudagrass, there was no advantage to adding fertilizer to the Accomplish® LM treatment for forage biomass; however, it did increase the crude protein. Therefore, applying it with a greater N rate could potentially cause an increase in yield. DH 44 was originally found during an unusually warm summer in Alabama. DH 44 may perform more adequately in higher temperatures but to further studies are needed to confirm. The PGPR used in this study increased forage yield while maintaining the nutritive quality, making them a viable option for as a biostimulator to both bermudagrass and tall fescue. Additional work should also compare higher rates of N fertilization to determine its viability as a biostimulator for hay production systems. Additional studies should determine the efficiency of the PGPR with the natural return of nutrients from grazing animals and viability in the system through nutrient return mechanisms.

III. Analyzing Plant Growth-Promoting Rhizobacteria Presence When Mixed with Three Common Herbicides

Introduction

In forage systems, the presence of pests, such as weeds, can influence forage production and nutritive value. Proper management strategies are crucial to controlling pests, and pesticides are often the most efficient method. In both hay and grazing systems, the most controlled pests are weeds. Weeds can suppress forage growth due to competition for ground cover, nutrients, water and light used for photosynthesis. The most common management practice to controlling weeds are the use of chemical applications. An advantage to using chemical weed control includes eliminating the early-season weed competition (Leep et al., 2003). The history of using chemicals to control weeds have been reported as early as 1944 when Hammer and Tukey reported using high concentrations of 2,4-D to control broadleaf plants in cereal grains (Rana and Rana, 2019).

In a study by Johnson (1993), pendimethalin controlled large crabgrass (*Digitaria sanguinalis*) by 90% in common bermudagrass stands but only ~53% in tall fescue stands in summer months. The author attributed these large differences to the varying degrees of competition in the common bermudagrass stand versus the tall fescue stand. Brosnan et al. (2014) reported pendimethalin effects on turf-type bermudagrass taking 53 days to reach 50 % hybrid bermudagrass cover. Butler et al. (2006) conducted a study on the yields of Coastal bermudagrass with various herbicides. When 2,4-D ester was applied at a rate of 2.31 kg a.i. ha^{-1}, Coastal bermudagrass had a total yield of 7,690 kg DM ha^{-1} compared with the control at 7,930 kg DM ha^{-1} in one year. In both bermudagrass pastures and hayfields, many weeds can be controlled with well-timed chemical applications (Newman et al., 2014).

Herbicides can be categorized as selective, targeting a specific weed species or type, or non-selective, targeting anything that encounters the chemical (Rana and Rana, 2019). Prowl® H_2O (pendimethalin; BASF Ag Products, Florham Park, NJ) is a dinitroaniline herbicide commonly used as a pre-emergent in bermudagrass hay systems to control a broad spectrum of weeds by microtubule polymerization inhibition, preventing cells from undergoing mitosis, ultimately killing the weed (Tredaway, 2019). Another common herbicide used is 2,4-D Amine (Alligare®, LLC, Opelika, AL), which inhibits the protein transport inhibition response 1 (TIRI1) auxin receptors, causing the plant stems to twist and leaves to curl within hours (Rana and Rana, 2019). Lastly, Sterling Blue® (Diglycolamine, WinField™ Solutions, St. Paul, MN), is a water-soluble herbicide used to suppress perennial and annual broadleaf weeds by invading the weed's roots, translocating throughout the vascular system, and accumulating in areas of active growth, interfering with growth hormones, auxins, and resulting in the death of the weed.

Another way to control weeds is using bioherbicides. Bioherbicides are compounds typically derived from microorganisms such as *Rhizobium.* Rhizobacteria, known as PGPR, can induce systemic resistant in the plant by producing antibiotics and cytokines that activate the plant's defense system to produce phytohormones. This method tends to have milder effects on the environment than commercially made, synthetic chemicals because the bioherbicides are generally naturally occurring in the area they are being used (Rana and Rana, 2019).

There have been studies conducted evaluating PGPR's ability to suppress weed pressure and diseases compared with synthetic herbicides, mostly in row crop production; however, there is little information known about interactions in the soil microbiome. Some of these studies showed a reduction in weed presence and diseases in row crops. In 2018, the global market for biostimulants was estimated to be $2 billion (USD) (Calvo et al., 2014). With increasing curiosity, there is an expected increase in use of PGPR products for biofertilizers, biopesticides, and phytostimulants.

Expansion of studies determining the herbicide-PGPR interaction in forage systems could create opportunities for solutions to environmental stressors that dominate in forage production systems. The ability for herbicides and PGPR to be tank-mixed and applied simultaneously will reduce the amount of time, labor, and fuel costs for producers. Minimizing the length of time forage is exposed to weed pressure will reduce plant stress, assisting in overall plant production. Ability to apply herbicide and biofertilizer simultaneously could positively impact the overall production of the forage and reduce labor for producers. More specifically, studying the relationship between PGPR and herbicides can further improve the understanding of the application of these rhizobacteria. The objective of this study was to determine whether the PGPR that promote growth in bermudagrass forage would survive when mixed with three different herbicides in solution.

Materials and Methods

PGPR Production

Blend 20 [AP7: (*Bacillus pumilus*), AP18: (*Bacillus pumilus*), and AP 282: (*Bacillus sphaericus*)] and DH 44 (*Paenibacillus sonchi*) were selected based on the previous evaluation of growth promotion in bermudagrass by the Auburn University Department of Entomology and Plant Pathology (Coy et al., 2014, Groover et al., 2020) and Auburn University Department of Animal Sciences (Griffin et al., 2020). Tryptic soy agar (TSA) was mixed using 18 g agar (Difco™ Agar Technical Solidifying Agent, Sparks, MD) and 20 g of tryptic soy broth (VWR; Bacto™ Tryptic Soy Broth without Dextrose, Becton, Dickinson and Company, Sparks, MD) and then autoclaved. Bacterial strains were transferred from cryovials maintained at -80°C for long-term storage on plates of tryptic soy agar (TSA) using an inoculating loop. Strains of Blend 20 and DH 44 were incubated at 28°C for up to 24 h and 72 h, respectively. Then, rhizobacteria colonies were scraped from the TSA plates with inoculating loops and transferred to either a new TSA plate, or collected into plastic centrifuge tubes (50 and 250 ml, VWR, Radnor, PA) autoclaved DI water (Ash et al., 2009). Bacterial cells were distributed evenly throughout the solution using a vortex machine and then soaked in an unstirred water bath (VWR, Radnor, PA) at 80°C to heat-shock vegetative cells. Subsequently, the bottles remained in the bath for 20 minutes before they were taken out, allowed to cool, and then stored at room temperature (28°C) These methods were adapted from previous studies that Coy et al., (2014), Fike at al., (2017), and Griffin et al., (2020) used to prepare the inoculation.

Bacterial populations in the suspension were determined by serial 10-fold dilutions of each bacterial suspension into blank tubes (15 ml tubes, VWR, Radnor, PA) containing sterile-water to a final dilution of 10^{-6}. Bacterial populations were determined by plating 50 µl of each serially diluted bacterial suspensions onto TSA plates. Plates were incubated for 12 to 24 hours for Blend 20 strains and 72 h for DH 44. Colony forming units (CFUs) were then determined by counting the number of bacterial colonies that grew on each plate. After each prepared suspension's concentrations were determined, the populations of all strains were used to make the bacterial stock solution. Stock suspensions were prepared by adding bacterial suspension and distilled water to reach the final concentration of 1×10^7 colony forming units (CFU) ml^{-1} of each strain.

Experiment 1

Laboratory Inoculation of PGPR and Herbicides

This experiment was a completely randomized design with a 3 × 4 factorial with three herbicide treatments and four PGPR strains each replicated four times. Prowl® H_2O (pendimethalin; BASF Ag Products, Florham Park, NJ), Sterling Blue® (Diglycolamine, WinField™ Solutions, LLC, St. Paul, MN), and 2,4-D Amine® (Alligare®, LLC, Opelika, AL) herbicides were selected based on their herbicide class and liquid mode of application. DH44 and strains comprising Blend 20 (*B. pumilis* AP 7, *B. pumilis* AP 18, and *B. sphaericus* AP 282) were analyzed independently. Tryptic soy broth (TSB) was utilized as a medium for the PGPR growth. The TSB was mixed at 20 g per liter of deionized water (VWR; Bacto™ Tryptic Soy Broth without Dextrose, Becton, Dickinson and Company, Sparks, MD). Once the solution was homogenous, 70 ml were pipetted into 125 ml Erlenmeyer flasks covered with aluminum foil and then autoclaved at 120°C for 15 minutes. Once the flasks were cooled to room temperature, 35 ml of the PGPR strain were pipetted into the flask and gently stirred by hand to mix thoroughly; then the respective herbicide treatment was added, and the flask gently stirred again.

Each herbicide was mixed at the following rate: Prowl H_2O®: 1.3 µl; Sterling Blue®: 1.4 µl; 2,4-D Amine®: 2.8 µl. Flasks were placed onto an incubating orbital shaker (VWR, Radnor, PA) at 28°C at a speed of 150 rpm. Serial dilutions were conducted at 0, 24, 48, and 72 h. At each time point 1 ml of stock suspension was taken from each flask and put into a 15 ml glass tube with 9 ml of autoclaved deionized water. With six total dilutions, the 4th through 6th were plated for the AP strains and the 3rd through the 6th were plated for DH 44. Plated dilutions were incubated at 28°C for 24 h for AP strains and 72 h for DH 44, and the CFU were then recorded. The countable plate was determined as the plate between 25 and 250 CFU, and the concentrations were calculated. Methodology was adapted from Myresiotis et al. (2015).

Experiment 2

Field Application of PGPR and Herbicides

Research Site and Treatments

A 1-year field demonstration was conducted at Auburn University North Auburn Research Unit bermudagrass pastures (32.689765, -85.500129) in Auburn, AL. Herbicide treatments were determined based on their herbicide classifications. Herbicides chosen were Prowl® H_2O (pendimethalin; BASF Ag Products, Florham Park, NJ) as a pre-emergent, Sterling Blue® (Diglycolamine, WinField™ Solutions, LLC, St. Paul, MN), and 2,4-D Amine® (Alligare®, LLC, Opelika, AL) both as a post-emergent. Pre-and post-emerge treatments were applied on March 13 and April 30, 2020, respectively. Treatments included each herbicide with each PGPR, Blend 20 or DH 44, a positive control of Blend 20 and DH 44 alone, or a negative control of Prowl® H_2O, Sterling Blue®, or 2,4-D Amine® alone.

Treatment Application

Each herbicide was applied at a rate per 1-m^2. Backpack sprayers were used to apply the herbicide-PGPR mixture to each plot. Each backpack sprayer was cleaned with a dish soap-bleach mixture between different PGPR-herbicide treatments. The herbicides were weighed and mixed in 15 ml tubes for 24 h prior to application and then mixed directly with the PGPR at the application site. Weed ratings were taken at application then every two weeks after until harvest (before, during, and end, respectively). After the last weed rating on April 10 2020 for pre-emergents and May 28 for post-emergents, plots were harvested and taken back to the Auburn University Ruminant Nutrition Laboratory (Auburn, AL) lab for botanical separations. Weights of weeds and bermudagrass were determined.

Statistical Analysis

Data were analyzed using Proc GLIMMIX of SAS version 9.4 (SAS Institute, Cary, NC). Data were considered significant at $P \leq 0.05$. Experiment 1 was a completely randomized design with four replications where incubation time was considered a repeated measure. Experiment 2 was a completely randomized design with two replications where PGPR and herbicide as fixed effects and each sampling date a repeated measure. Mean separation was achieved using Fisher's-protected Least Significant Difference.

Results

Experiment 1: Laboratory Inoculation of PGPR and Herbicides

There was an herbicide effect on the concentration of the flasks ($P < 0.0001$, Table 3). Flasks treated with both 2,4-D Amine and Prowl® H_2O resulted in lower bacterial populations overall than flasks treated with Sterling Blue ($P = 0.0001$, $P = 0.0002$, respectively) but still had increased bacterial growth from the initial inoculation. There was also a PGPR effect on the concentration of the flasks ($P < 0.0001$, Table 3). Flasks inoculated with AP 18 were greater than flasks inoculated with AP 282 ($P = 0.0001$) and DH 44 ($P = 0.0001$) which could be due to differences in bacterial growth rate and characteristics. Flasks inoculated with AP 282 had lower populations than flasks inoculated with AP 7 ($P = 0.0001$). Flasks inoculated with AP 7 had greater populations than flasks inoculated with DH 44 ($P = 0.0001$).

There was a PGPR × herbicide interaction effect on the concentration of PGPR ($P < 0.001$, Table 3). Flasks treated with AP 18 and 2,4-D Amine had greater populations than flasks inoculated with AP 7 and both 2,4-D Amine and Prowl® H_2O ($P = 0.0048$, $P = 0.009$, respectively). Flasks inoculated with AP 18 and Prowl® H_2O resulted in greater populations than flasks inoculated with AP 18 and Sterling Blue ($P = 0.0123$). Flasks inoculated with AP 7 and both 2,4-D and Prowl® H_2O were less than flasks inoculated with AP 7 and Sterling Blue ($P = 0.0001$, $P = 0.0001$, respectively).

There was a PGPR × herbicide × time effect on the concentration of the PGPR inoculated flasks ($P < 0.0001$, Figure 13-15). The dilutions at 0 h from flasks inoculated with AP 18 and 2,4-D Amine had lower populations of bacterial growth than the same treatment at 72 h dilutions ($P = 0.0001$), as well as flasks with AP 18 and Prowl® H_2O at 48 and 72 h dilutions ($P = 0.0285$, $P = 0.0001$, respectively). AP 18 and 2,4-D Amine inoculated flasks at 24 and 48 h dilutions had less populations than AP 18 with 2,4-D Amine (P = 0.0003, P = 0.0016, respectively). At 72 h, flasks inoculated with AP 18 and 2,4-D Amine had greater populations than dilutions at 0 (P = 0.0001, P = 0.0001, respectively), 24 (P = 0.0003, P = 0.0003, respectively), and 48 h (P = 0.0064, P = 0.0005, respectively) from flasks inoculated with AP 18 and Prowl® H_2O and Sterling Blue. The same treatment also had greater populations than AP 18 and Sterling Blue at 72 h (P = 0.0001). AP 18 and Prowl® H_2O inoculated flasks at 0 h dilutions, had a lower population than the same treatment at 48 and 72 h (P = 0.0294, P = 0.0001, respectively). However, at 24 and 48 h dilutions AP 18 and Prowl® H_2O inoculated flasks were lower in population than AP 18 and Prowl® H_2O at 72 h (P = 0.0001, P = 0.0007, respectively). Furthermore, AP 18 with Prowl® H_2O inoculated flasks at 72 h dilutions had greater populations than AP 18 and Sterling Blue at 0, 24, 48, and 72 h dilutions (P = 0.0001). Dilutions from all incubation times for flasks inoculated with AP 7 and 2,4-D Amine had less populations than flasks treated with AP 7 and Sterling Blue at 0 (P = 0.0001), 24 (P = 0.0001), 48 (P = 0.0001), and 72 h (P = 0.0001). Flasks inoculated with AP 7 and Prowl® H_2O had greater populations than flasks treated with AP 7 and Sterling Blue at all time points (P = 0.0008, P = 0.0001, P = 0.0001, P = 0.0001, respectively). At 0 h dilutions, the population of AP 7 with Sterling Blue treated flasks were less than the populations of the same treatment at 48 and 72 h (P = 0.0048, P = 0.0101, respectively).

Table 3: Average concentrations, in CFU/ml, for PGPR, herbicide, and PGPR × herbicide interactions.

	Herbicide‡				
PGPR†	S. Blue	2,4-D	Prowl		
	----------CFU/ml----------			**Mean**	**SEM±**
AP7	7.61E+08	1.15E+08	1.28E+08	3.35E+08[a]	2.56E+07
AP18	1.71E+08	2.94E+08	3.30E+08	2.65E+08[b]	2.56E+07
AP282	2.68E+06	2.05E+06	2.46E+05	2396875[c]	2.56E+07
DH44	2.93E-06	1.05E+06	5.87E+05	546667[c]	2.86E+07
Mean	2.34E+08[x]	1.03E+08[y]	1.15E+08[y]		
SEM±	2.22E+07	2.42E+07	2.22E+07		

[a,b,c] Means within a column followed by a common letter are not different (*P* > 0.05).
[x,y,z] Means within a row followed by a common letter are not different (*P* > 0.05).
†AP 18, AP 7, AP 282, and DH 44
‡Sterling Blue (1.4µl 0.25 sq. ft^{-1}), Prowl® H_2O (1.3 µl 0.25 sq. ft^{-1}), 2,4-D Amine (2.8 µl 0.25 sq. ft^{-1})
±SEM = standard error of the mean.

Experiment 2: Field Application of PGPR and Herbicides

There was a PGPR × herbicide × days after treatments (DAT) interaction effect on weed composition ($P < 0.0101$, Figures 16 and 17). DH 44 mixed with 2,4-D Amine and Prowl® H_2O treated plots reduced weed composition by 45 and 51% over the 28 d period; however, when mixed with Sterling Blue weed composition was only reduced by <10%. Plots treated with DH 44 only reduced weed composition by 21% in the first two weeks before weed composition increased by 13% the last two weeks. Plots treated with Blend 20 and 2,4-D Amine, Sterling Blue, and Prowl® H_2O reduced weed composition by 34, 56, and 17% over the 28 d period. Plots treated with Blend 20 and Prowl® H_2O started with a lower weed composition rating at 0 DAT (62%). Blend 20 control plots had a weed composition reduction of <10% over the 28 d period.

At 0 DAT, Blend 20 with 2,4-D Amine had a greater weed presence than the Blend 20 control at 0, 14, and 28 DAT ($P = 0.0405$, $P = 0.0300$, $P = 0.0115$, respectively) as well as Blend 20 with 2,4-D Amine at 14 and 28 DAT ($P = 0.0140$, $P = 0.0413$, respectively). Blend 20 with 2,4-D Amine at 0 DAT also had a greater weed composition than Blend 20 with Prowl® H_2O at 0, 14, and 28 DAT ($P = 0.0099$, $P = 0.0010$, $P = 0.0010$, respectively) and Blend 20 with Sterling Blue at 28 DAT ($P = 0.0042$). Compared with the 2,4-D Amine control at 28 DAT, Blend 20 mixed with 2,4-D at 0 DAT had a greater weed composition ($P = 0.0099$). At 14 DAT, Blend 20 and 2,4-D Amine had a lower weed composition than 2,4-D Amine control at 0 DAT ($P = 0.0219$). Blend 20 with 2,4-D Amine at 28 DAT, had a lower weed composition than Blend 20 with Sterling Blue at 0 DAT ($P = 0.0457$). At 0, 14, and 28 DAT, the Blend 20 control had a lower weed composition than Blend 20 with Sterling Blue at 0 DAT ($P = 0.0456$, $P = 0.00339$, $P = 0.0131$, respectively). Within the 14 and 28 DAT ratings, Blend 20 control had a greater weed composition than the DH 44 control (P = 0.0087, P = 0.0287, respectively). Blend 20 and Prowl® H_2O at 0, 14, and 28 DAT had a lower weed composition than Blend 20 with Sterling Blue at the 0 DAT (P = 0.0111, P = 0.0012, P = 0.0012, respectively). At 28 DAT, Blend 20 with Sterling Blue had a lower weed composition than the Sterling Blue control at 0 DAT (P =0.0125). The 2,4-D Amine control at 0 DAT had a greater weed composition than DH 44 with 2,4-D Amine at 28 DAT (P = 0.0457). However, the 2,4-D Amine control at 14 and 28 DAT had a lower weed composition than DH 44 with 2,4-D Amine at 0 DAT (P = 0.0336, P = 0.0025, respectively). The Sterling Blue control at 0 DAT had a greater weed composition DH 44 with Sterling Blue at 28 DAT (P = 0.0336). However, at 14 and 28 DAT, the Sterling Blue control was less than DH 44 with Sterling Blue at 0 DAT (P = 0.0099, P = 0.0054). DH 44 with 2,4-D Amine at 0 DAT, had a greater weed presence than DH 44 control at 0, 14, and 28 DAT (P = 0.0002, P = 0.0001, P = 0.0001, respectively). At 28 DAT, DH 44 control had less of a weed composition than DH 44 with Prowl® H_2O at 0 DAT (P = 0.0382) as well as DH 44 with Sterling Blue at 0 and 14 DAT (P = 0.0001, P = 0.0382, respectively). At 14 and 28 DAT, DH 44 with Prowl® H_2O had a lower weed composition than DH 44 with Sterling Blue at 0 DAT (P = 0.0175, P = 0.0413, respectively). Lastly, DH 44 and Sterling Blue at 0 DAT had a greater weed composition than the same treatment at 28 DAT (P = 0.0054).

There was a PGPR × herbicide interaction effect on the weed composition (P < 0.0002, Table 4). DH 44 combined with 2,4-D Amine resulted in the greatest weed composition of the plots averaged across a 28 d period (79%); however, it was not different than DH 44 and Sterling Blue (P = 0.7933) and DH 44 with Prowl® H_2O (P = 0.02315). DH 44 mixed with 2,4-D Amine had 11% greater weed composition compared with Prowl® H_2O. The DH 44 control resulted in the least weed composition (54%) among treatments but was not significantly different than

Blend 20 mixed with Prowl® H_2O (56%). The Blend 20 control and Blend 20 mixed with 2,4-D Amine had a greater weed composition than Blend 20 mixed with Prowl® H_2O ($P = 0.0182$, $P = 0.0054$, respectively). However, Blend 20 mixed with Prowl® H_2O had a lower weed composition than Blend 20 mixed with Sterling Blue ($P = 0.0066$).

There was an herbicide effect on the weed composition of the field plots ($P < 0.0020$). 2,4-D Amine had a greater weed composition averaged across a 28 d period than Prowl® H_2O ($P = 0.0101$) but was not different than Sterling Blue ($P = 0.7081$). 2,4-D Amine resulted in 20% greater weed presence over Prowl® H_2O (76 vs. 64%, respectively). Prowl® H_2O had a lower weed composition than Sterling Blue ($P = 0.0264$).

Table 4: Average ground cover composition (%) of each PGPR treatment and the associated *P* value.

Treatment		Weeds	Bermudagrass	*P* value
PGPR†	Herbicide‡	%		
Blend 20		69^{ab}	31^{ab}	0.0001
	2,4-D	74^{ab}	26^{ab}	0.0001
	S. Blue	74^{ab}	26^{ab}	0.0001
	Prowl	56^{c}	44^{c}	0.0001
DH 44		54^{c}	46^{c}	0.0001
	2,4-D	79^{a}	21^{a}	0.0001
	S. Blue	77^{a}	23^{a}	0.0001
	Prowl	71^{ab}	29^{ab}	0.0001

a,b,c Means within the column followed by a common letter are not different ($P > 0.05$).
†AP 18, AP 7, AP 282, DH 44
‡Sterling Blue (0.002oz sq. m^{-1}), Prowl® H_2O (0.020oz sq. m^{-1}), 2,4-D Amine (0.004oz sq. m^{-1})

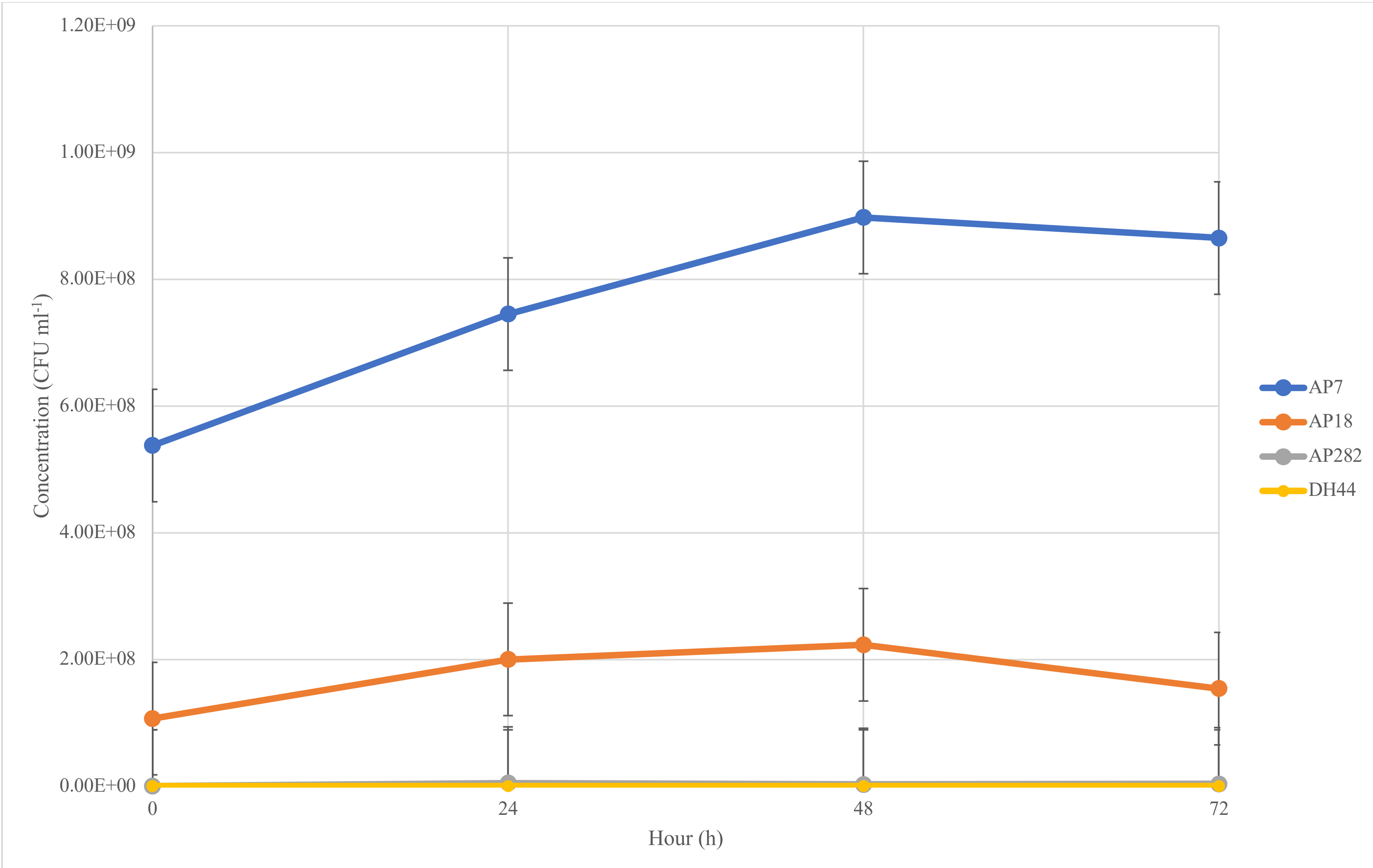

Figure 13: Concentrations, in CFU ml^{-1}, of four PGPR strains (AP7, AP18, AP282, and DH44) inoculated with Sterling Blue after 0, 24, 48, and 72 h incubation period. Active ingredient: Diglycolamine at a rate of 1.4µl 0.25 sq. ft^{-1}.

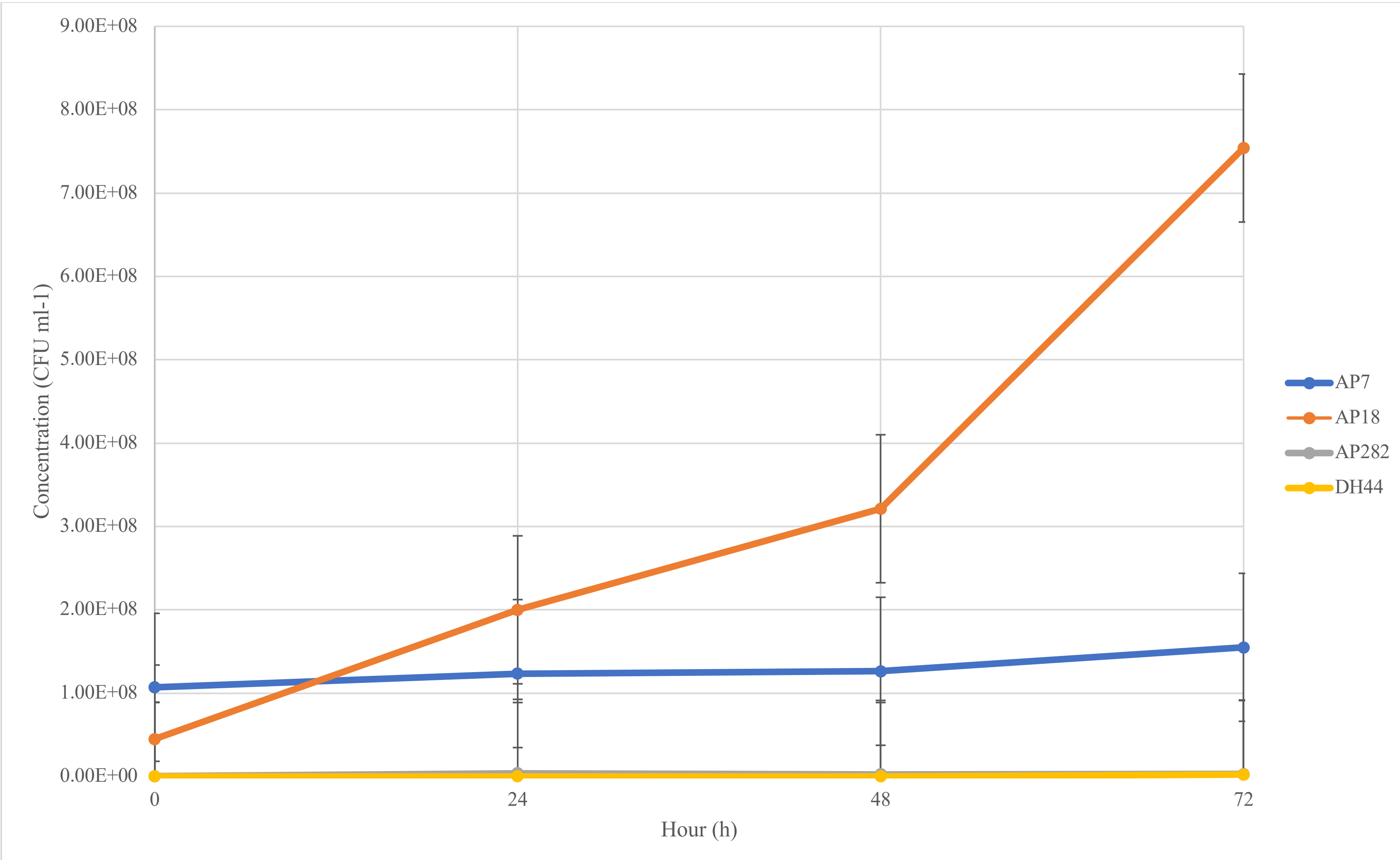

Figure 14: Concentrations, in CFU ml^{-1}, of four PGPR strains (AP7, AP18, AP282, and DH44) inoculated with Prowl® H_2O after 0, 24, 48, and 72 h incubation period. Active ingredient: pendimethaline at a rate of 1.3µl 0.25 sq. ft^{-1}.

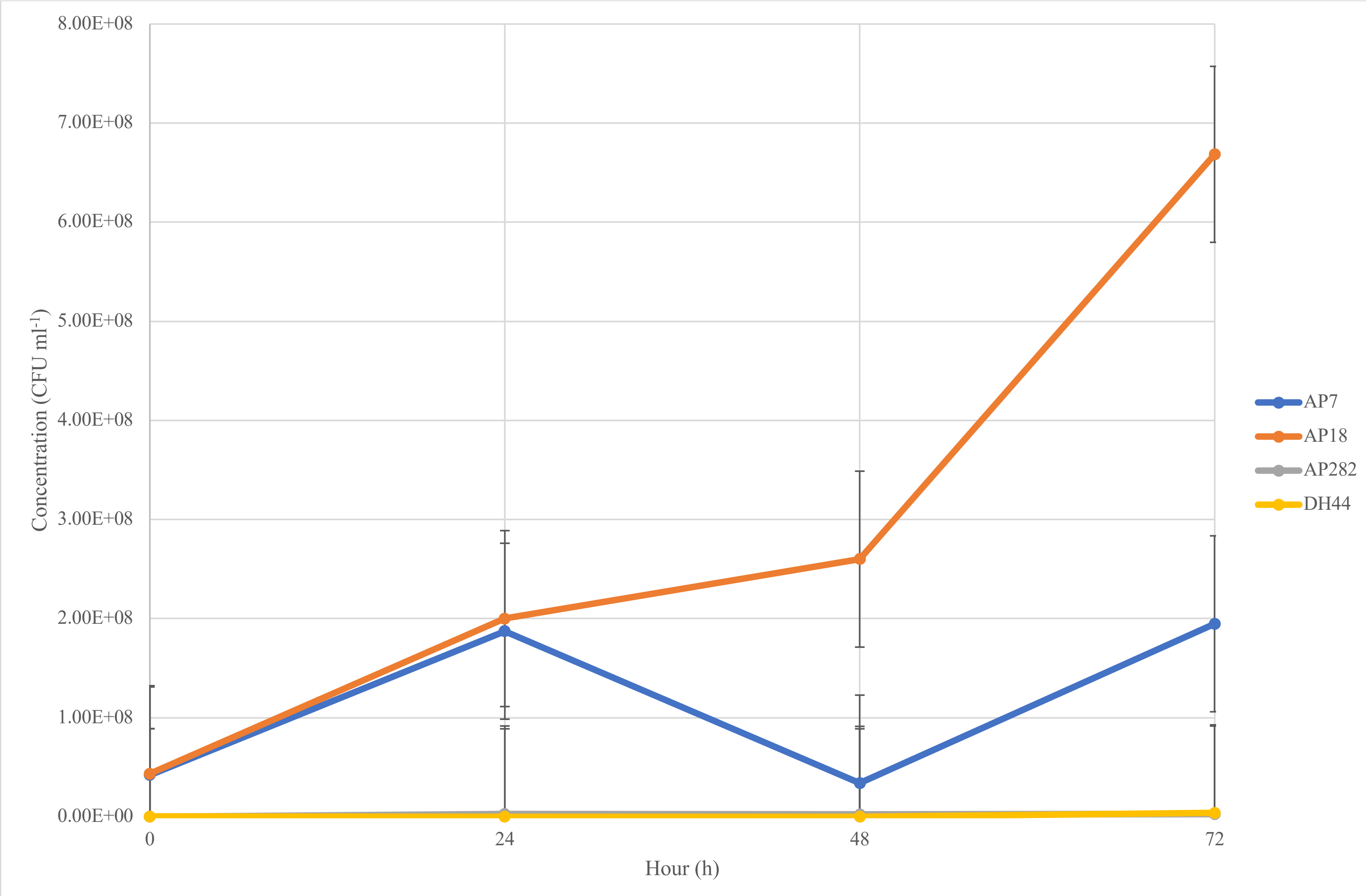

Figure 15: Concentrations, in CFU ml^{-1}, of four PGPR strains (AP7, AP18, AP282, and DH44) inoculated with 2,4-D Amine after 0, 24, 48, and 72 h incubation period. Active ingredient: 2,4-dicholoro-phenoxyacetic acid at a rate of 2.8µl 0.25 sq. ft^{-1}.

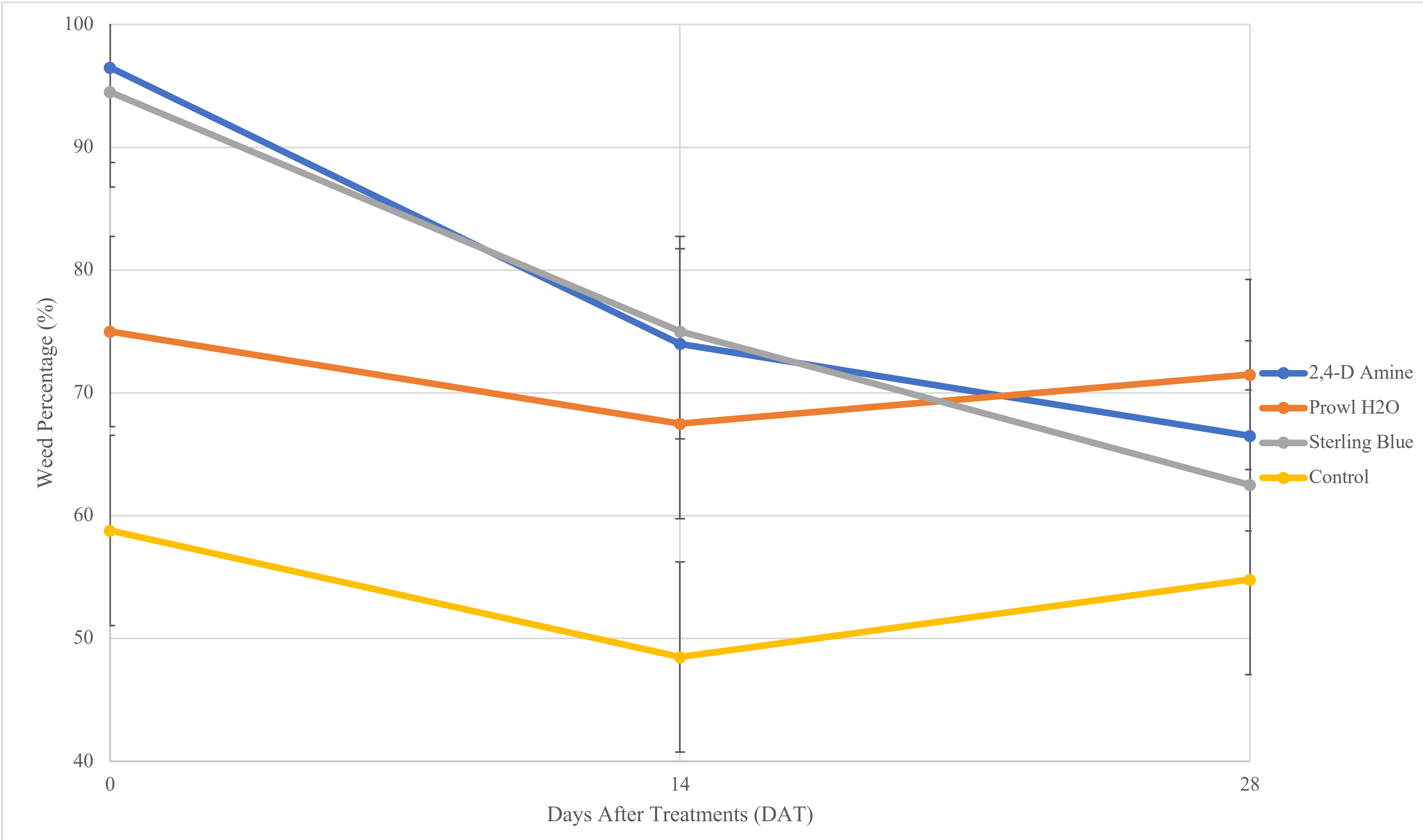

Figure 16: Percentage of weeds in plots treated with DH 44, PGPR strain, 2,4-D Amine, Prowl® H_2O, and Sterling Blue, and DH 44 control at 0, 14, and 28 days after treatment (DAT). Active ingredients: 2,4-D Amine = 2, ,4-dicholoro-phenoxyacetic acid at 0.004 oz sq. m^{-1}, Prowl® H_2O = pendimethalin at 0.020 oz sq. m^{-1}, Sterling Blue = Diglycolamine at 0.002 oz sq. m^{-1}.

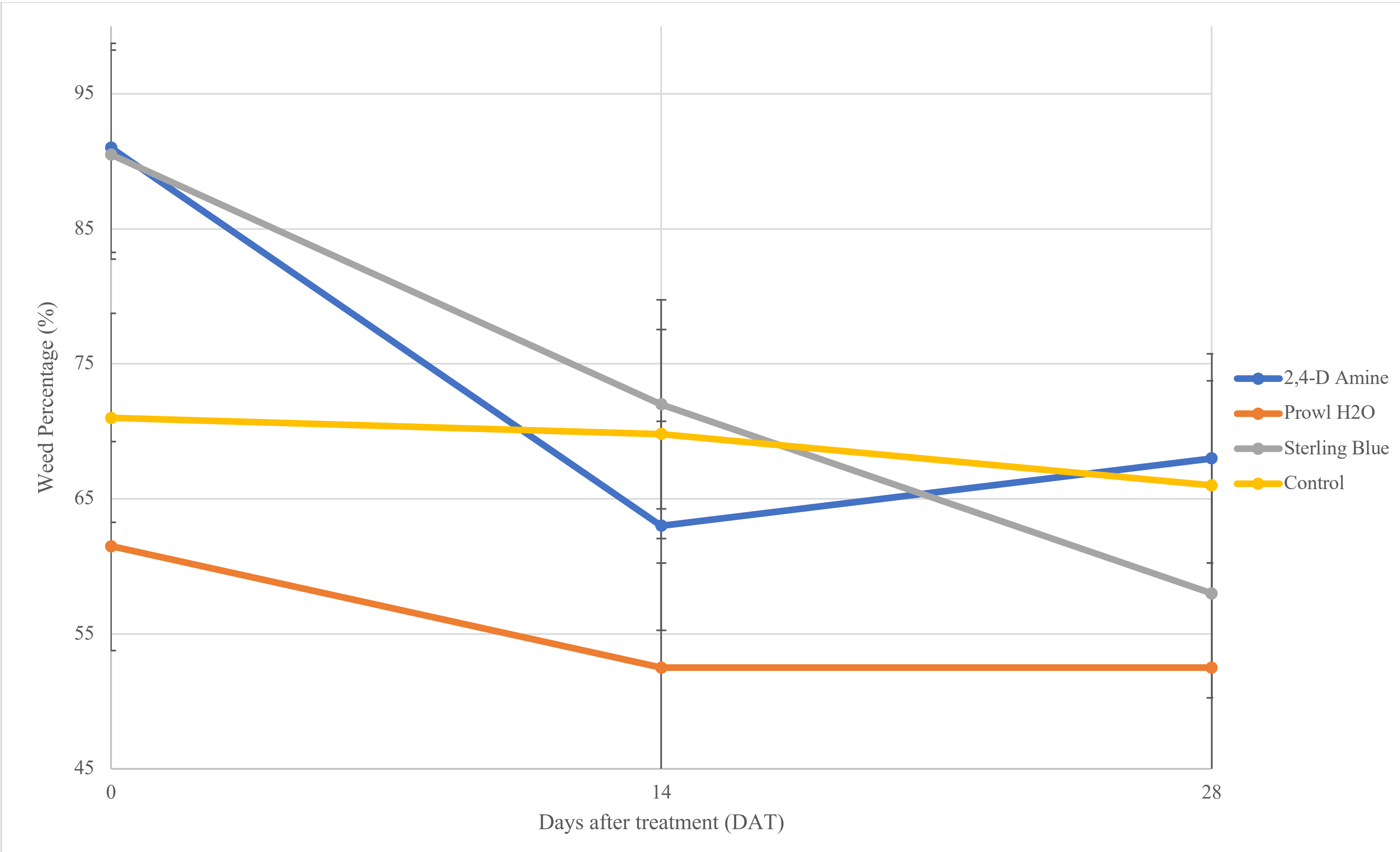

Figure 17: Percentage of weeds in plots treated with Blend 20, PGPR blend, 2,4-D Amine, Prowl® H_2O, and Sterling Blue, and Blend 20 control at 0, 14, and 28 days after treatment (DAT). Active ingredients: 2,4-D Amine = 2, ,4-dicholoro-phenoxyacetic acid at 0.004 oz sq. m^{-1}, Prowl® H_2O = pendimethalin at 0.020 oz sq. m^{-1}, Sterling Blue = Diglycolamine at 0.002 oz sq. m^{-1}.

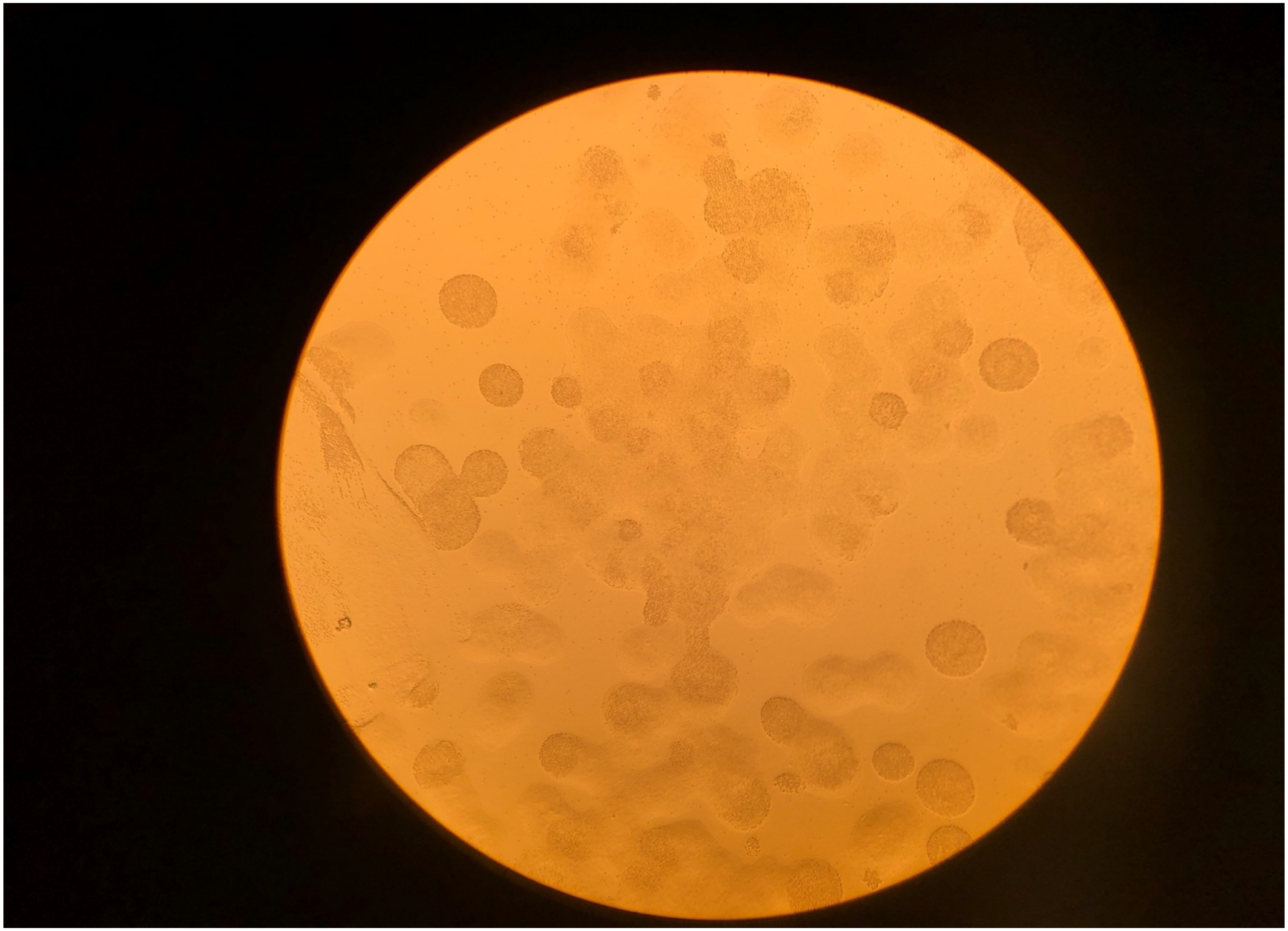

Figure 18: Microscopic view of the PGPR strain, DH 44, grown on tryptic soy agar plates under 40x magnification.

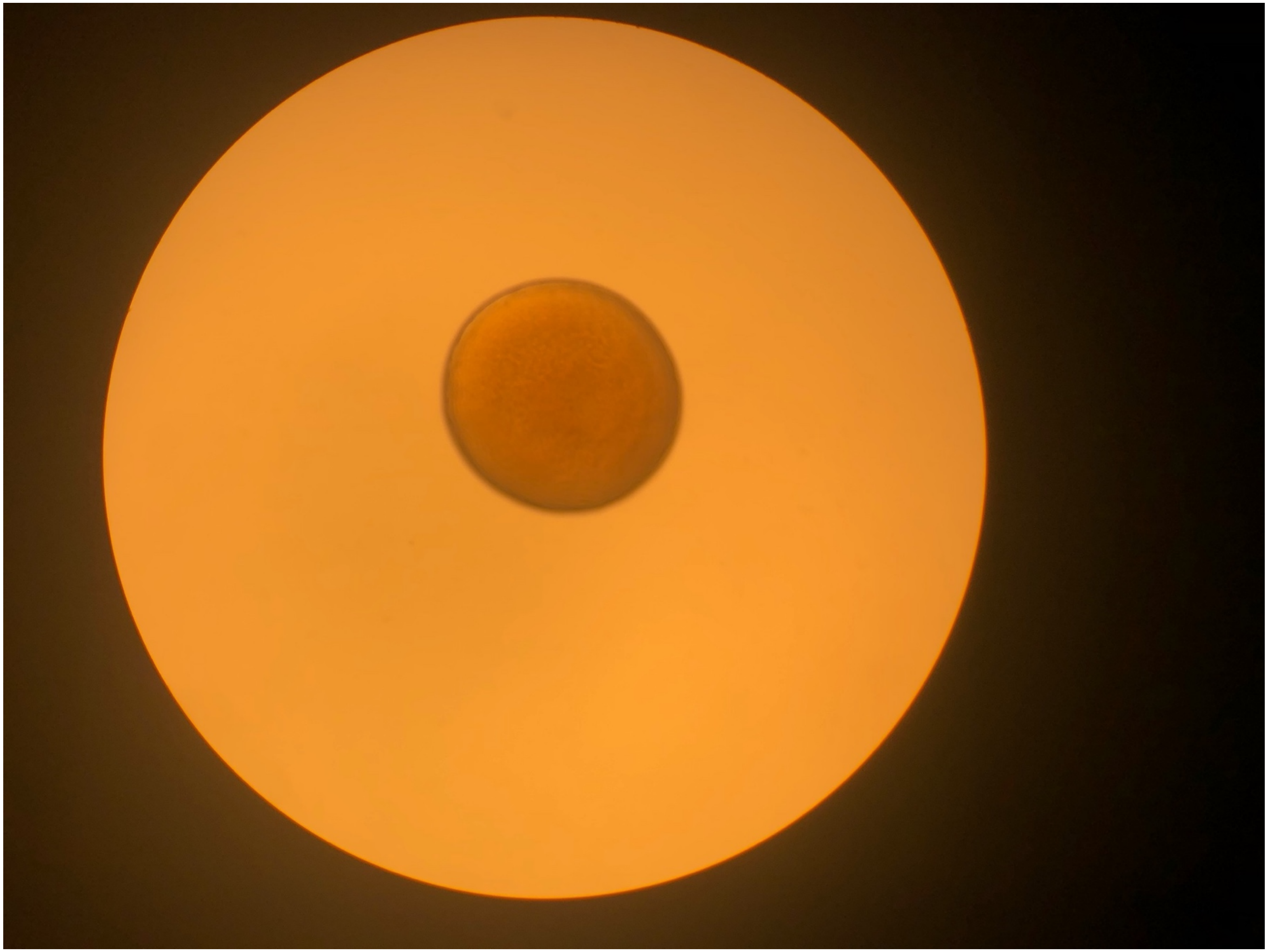

Figure 19: Microscopic view of the PGPR strain, AP 7, grown on tryptic soy agar plates under 40x magnification.

Figure 20: Microscopic view of the PGPR strain, AP 18, grown on tryptic soy agar plates under 40x magnification.

Discussion

Experiment 1: Laboratory Inoculation of PGPR and Herbicides

In this experiment, each strain in Blend 20 (*B. pumilus* AP 7*, B. Pumulis* AP 18*, B. sphaericus* AP 282) and DH 44 (*Paenabacillus* spp.) was analyzed independently and mixed with three herbicides commonly used in bermudagrass forage systems to control grassy and broadleaf weeds for a 72 h period. DH 44 and AP 282 lost some of the initial population when inoculated with all three herbicides. There were small variations noted which could be attributed to the bacterial distribution in the flasks, serial dilutions, or even the difference in growth rates of the bacterial colonies. Among the three herbicides, flasks with Sterling Blue were significantly greater in bacterial population among all incubation periods than Prowl® H_2O and 2,4-D Amine. The combination of AP 7 and Sterling Blue was had a significantly greater bacterial population among incubation periods (7.6125×10^8 CFU ml^{-1}) compared with all other mixtures. All three herbicides, when mixed with DH 44, had the least bacterial populations among all incubation periods. Coy (2017) reported that the strains comprising Blend 20 were compatible and stable once mixed with three common insecticides used to control mole crickets. The population stability of PGPR when mixed with pesticides and measured by survival, is likely dependent on PGPR strain, method of application, and PGPR formulation (Shuvakumar et al., 2000; Kloepper et al., 1981). Myresiotis et al. (2012) reported bacterial populations of 0.80 to 2.12×10^7 CFU ml^{-1} immediately after PGPR were inoculated with different pesticides. The author reported the maximum population between 24 and 30 h of incubation ranging from 1.06 to 2.20×10^9 CFU ml^{-1}. After the initial growth phase increase, the bacterial populations decreased to 0.94 – 1.74 and 0.74 – 1.36×10^9 CFU ml^{-1} at the 48 and 72 h incubation, respectively. The current study showed maximum growth at 48 h for the AP 7 and Sterling Blue mixture (8.975×10^8) and 72 h period for the rest of the mixtures, excluding AP 282 and DH 44. DH 44 and AP 282 consistently grew less than the other bacterial strains throughout all incubation periods. In a study performed by Wijekoon et al. (2018), two PGPR species *Psedomonas* and *Bacilllus* sp. were mixed with varying concentrations of glyphosate to determine the PGPR population and efficiency of pesticide degradation. The author reported both species having significant growth at the lesser concentrations (1.5×10^8 CFU ml^{-1}). However, when concentrations of glyphosate were increased, inhibition of microbial population growth for both species was observed (Wijekoon et al., 2018). Even though the current study did not evaluate the efficacy of the PGPR or herbicides, the populations of AP 7 and AP 18 were similar to those in the previous study.

The *Bacillus* strains are fast growing with larger colonies compared with DH 44, which takes at least 72 h for much smaller colonies to form. This could be a reason DH 44 did not do as well in the lab portion of this experiment than the other *Bacillus* strains. DH 44 was also discovered during a droughty summer in Alabama, potentially meaning it can tolerate higher temperatures. If this is true, increasing the incubation temperature for DH 44 may reduce its growth time. Ash et al. (2009) suggested grinding air-dried plants and adding to the media and autoclaving at 121°Cfor 15 minutes then straining to remove the ground matter and re-autoclaving for 30 minutes. This could potentially give the *Paenibacillus* species more nutrients during incubation and the growth phase.

There was a discernable amount of bacterial growth on the TSA plates that was not the PGPR strains in question, which could be due to the herbicides not being sterilized properly and/or the unidentified bacteria being associated with the herbicide suspension itself. Future research studies are encouraged to determine what species of bacteria are associated with the herbicides and if there is a possible way to sterilize the herbicides without denaturing the chemical compounds.

Experiment 2: Field Application of PGPR and Herbicides

The location at which the field study was performed had less bermudagrass than rye grass present. The PGPR was made into suspension prior to the day of application; however, it was not introduced to the herbicide until they were tank-mixed on the day of application. Results from the laboratory study showed that the bacterial populations increased over time after being exposed to the herbicide. If this remains true, and the efficacy of the PGPR remain stable, then populations of the PGPR in the soil should increase after 48 h of exposure. This could be detrimental to the efficacy of the herbicide as there is work showing PGPR degrading many different pesticide compounds. The PGPR could be using the carbon sources in the pesticides as nutrients and ultimately breaking apart the pesticide compounds.

Conclusions

The results of this study indicate that PGPR can be used as a biostimulant to supports forage biomass production in Russell bermudagrass hay while maintain or improving the nutritive value relative to non-treated or synthetic fertilizers. The nutritive value in the current study is within the ranges reported in literature. Griffin et al. (2020) reported that Blend 20 out-produced DH 44 but not the synthetic fertilizer.

AP 282 and DH 44 appear to level or have slight declines when incubated in solution with Sterling Blue, Prowl® H_2O, and 2,4-D Amine. Interestingly, populations of AP 18 and AP 7 increased in most herbicide solutions. Sterling Blue inoculated with AP 7 and AP 18 showed great promise to maintain PGPR populations. This may indicate that AP 18 is using the carbon in these herbicides for growth. This has implications for tank mixing PGPR with certain herbicides as well as for potential phytoremediation.

Further research is necessary to investigate the effects of PGPR inoculants with greater synthetic fertilizer rates. It is also necessary to further investigate the ISR response induced by PGPR on the host plant. Evaluating PGPR's influence on the animal performance and forage palatability in grazing studies should be included in future research. Studies evaluating the performance of the PGPR when tank-mixed with a pesticide for longer periods of time without a growing media could be helpful in determining the shelf life of mixed products.

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