

Rhizobacterial Inoculants Increase Root and Shoot Growth in 'Tifway' Hybrid Bermudagrass¹

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Abstract

Plant growth-promoting rhizobacteria (PGPR) are non-pathogenic, beneficial bacteria that colonize seeds and roots of plants and enhance plant growth. Although there has been extensive PGPR research with agronomic crops, there has been little emphasis on development of PGPR for grasses in pastures or as turf. Accordingly, experiments were conducted to evaluate novel bacterial inoculants for growth promotion in 'Tifway' hybrid bermudagrass. Replicated laboratory and greenhouse experiments evaluated effects of various PGPR mixtures, each with 3 to 5 PGPR strains and applied as weekly root inoculations, in comparison to nontreated plants. Growth promotion was assessed by measuring foliar growth from 3 to 8 wk and root growth at 8 wk after the first treatment. In all experiments, at least one bacterial treatment of bermudagrass resulted in significantly increased top growth and greater root growth (length, surface area, volume, or dry weight). PGPR blends 20 and MC3 caused the greatest growth promotion of roots and shoots. These results suggest that the bacterial strains could be used in strategies to reduce nitrogen or water inputs to turf.

Index words: plant bacterial interactions, PGPR, turfgrass management, microbial treatments.

Species used in this study: 'Tifway' hybrid bermudagrass, *Cynodon dactylon* x *Cynodon transvaalensis* Burt-Davey.

Significance to the Horticulture Industry

There is growing concern in agronomic and amenity commodities about the potential overuse of fertilizers to sustain growth or productivity. Rhizobacterial inoculants are selected strains of root or root-zone colonizing bacteria that are beneficial to plants by reportedly enhancing nutrient uptake or pest resistance. We evaluated blends of rhizobacteria for growth promotion (root and shoot growth) in hybrid bermudagrass in growth chamber and greenhouse experiments. There was variability in the outcome depending on the blends of rhizobacteria used. However, two blends, Blend 20 and MC 3, provided consistent, positive growth impacts relative to nontreated plants. Although not yet commercially-available, these bacterial inoculants are relatively easy to apply and can potentially improve productivity of bermudagrass in pastures or as turfgrass.

Introduction

Plant growth-promoting rhizobacteria (PGPR) are non-pathogenic, beneficial, free-living, soil and root-inhabiting bacteria that are able to colonize seeds and roots (rhizosphere) (Kloepper and Schroth 1978, Kloepper 1993). Rhizobacteria, fungi, and associated microbes in the soil community influence the nitrogen cycle through nitrification, denitrification, fixation, and mineralization (Calvo 2013, Adesemoye et al. 2009). Enhancement of the soil microbe community can be accomplished by the use of specific microbial inoculants. For example, inoculation with *Azospirillum*, a N₂-fixing bacteria, increases root development in grains, leading to increased yield (Okon et al. 1998). PGPR inoculants interact with plant root exudates and metabolites to promote plant growth (Lutenberg and Kamilova 2009). Blends, or mixtures,

of PGPR strains have been used to improve consistency of results compared to single strains (Burkett-Cadena et al. 2008).

In order for inoculants to enhance plant growth, the bacteria must be able to survive inoculation, multiply in the rhizosphere in response to plant exudates, attach to the root surface, and colonize the developing root system (Kloepper 1993). The impacts of PGPR on plant growth and yield are accomplished through both direct and indirect mechanisms. However, the specific interactions are not fully understood (Whipps 2001, Nelson 2004). The most common direct impact of inoculants is growth promotion through biofertilization. Indirectly, PGPR are able to reduce the severity of pathogens and herbivore feeding through plant-acquired resistance, including induced systemic resistance (ISR) or systemically-acquired resistance (SAR). ISR and SAR utilize various plant defense pathways such as the salicylic acid, jasmonic acid, or ethylene pathways (Dimkpa et al. 2009, Lutenberg and Kamilova 2009, Howe 2004, Nelson 2004, Okon et al. 1998, Whipps 2001).

Despite extensive research on the use of microbial inoculants in agronomic crops, relatively few studies have considered the effect of the endemic microbial community or of inoculants on grass growth. Creeping bentgrass (*Agrostis stolonifera* Huds.) and hybrid bermudagrass (*Cynodon dactylon* x *Cynodon transvaalensis* Burt-Davey) putting greens support rich microbial communities in spite of manipulation of soil profiles and frequent use of pesticides (Bigelow et al. 2002, Elliot et al. 2004). Relative to food crops, there are few reports documenting positive growth promotion in grasses following inoculation with rhizobacteria. Baltensperger et al. (1978) evaluated nitrogen-fixing bacterial inoculants (*Azospirillum* and *Azotobacter*) with eight genotypes of bermudagrass in a greenhouse. Although there were no differences in responses among the different bermudagrass genotypes, bacterial inoculants increased foliar nitrogen and plant biomass under zero N fertility conditions compared to nontreated plants (Baltensperger et al. 1978). Bacterial inoculants and low fertilizer rates seem to be an option for lower input, more sustainable turfgrass management.

Yet studies evaluating this potential have not been conducted. Bacterial inoculants isolated by Auburn University's

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Department of Entomology and Plant Pathology have demonstrated growth promotion and biological control against plant diseases in different cropping systems. The objectives of these experiments were to evaluate bacterial blends for growth promotion in hybrid bermudagrass in growth chamber and greenhouse conditions similar to the research by Baltensperger et al. (1978).

Materials and Methods

Bacterial strains and inoculant preparation. The bacterial strains isolated by Auburn University's Department of Entomology and Plant Pathology, which are listed in Table 1, were transferred from cryovials maintained at -80°C (-112°F) for long-term storage to plates of tryptic soy agar (TSA; Hardy Diagnostics, Santa Maria, CA). After incubation at 28°C (82°F) for 48 hr, bacteria were scraped from TSA plates with inoculating loops, transferred to tubes (20 ml Glass Culturable, VWR, Radnor, PA) containing 10 ml of sterile water, and vigorously shaken to evenly distribute bacterial cells. Serial 10-fold dilutions were then made of each bacterial suspension into sterile water to a final dilution of 10:5.

Bacterial populations in the suspensions were determined by plating 50 μl of the serially-diluted bacterial suspensions onto TSA plates, incubating plates for 24 to 48 hr, and then counting the number of bacterial colonies that grew on each

plate. Once the concentrations in the prepared suspensions of each strain were determined, the populations of all strains were used to make a bacterial stock solution. Stock solutions for blends were prepared by the addition of equal portions by volume of each bacterium to achieve a blend with a final concentration of 1×10^7 colony forming units (cfu) $\cdot\text{ml}^{-1}$ of each strain.

Experiment 1 — growth chamber trials. Twelve PGPR blends (Table 1) as well as a distilled water treatment containing no added bacteria were used in the initial screening. Plugs of Tifway hybrid bermudagrass were collected from the Auburn University Turfgrass Research Unit. After harvesting, the plants were washed to remove field soil and then surface sterilized (1 minute in 10% sodium hypochlorite (bleach), 3 minutes in ethanol, and then rinsed 3 times in distilled water). Following methods developed for grasses by Mandyam et al. (2010), 4.331 g (0.15 oz) of Murashigie & Skoog media (MP Biomedicals, Santa Ana, CA) was added to 10 L (2.6 gal) distilled water, with 1.5% agar. This mixture was poured into plastic Petri dishes (160 by 15 mm; VWR, Radnor, PA). Once set, a single stolon was transplanted into each Petri dish (Fig. 1) and grown in a growth chamber at 28.6°C (83.5°F) $\pm 5^{\circ}\text{C}$, 14 hour light: 10 hour dark photoperiod, and $50 \pm 10\%$ relative humidity for 1 wk to adjust to the new conditions. After this period, the grass foliage was cut with

Table 1. Identity of rhizobacterial blends from Auburn University evaluated for growth promotion in hybrid bermudagrass.

Trial ^a	Name	Bacterial strains in formulation ^b	Identification of strains
Lab, 1,2	Blend 8	AP188, AP209, AP217, AP218	<i>Paenibacillus macerans</i> , <i>Bacillus atrophaeus</i> , <i>Brevibacillus brevis</i> , <i>Bacillus subtilis</i>
Lab, 1,2	Blend 9	AP136, AP188, AP219, AP295	<i>Bacillus subtilis</i> , <i>Paenibacillus macerans</i> , <i>Bacillus subtilis</i> , <i>Bacillus vallismortis</i>
Lab	Blend 11	AP3, AP279, AP280, AP282	<i>Bacillus pumilus</i> , <i>Bacillus subtilis</i> , <i>Bacillus pumilus</i> , <i>Bacillus sphaericus</i>
Lab	Blend 12	AP272, AP282, AP283	<i>Bacillus mycoides</i> , <i>Bacillus sphaericus</i> , <i>Bacillus pumilus</i>
Lab	Blend 13	AP3, AP278, AP279, AP282	<i>Bacillus pumilus</i> , <i>Bacillus amyloliquefaciens</i> , <i>Bacillus subtilis</i> , <i>Bacillus sphaericus</i>
Lab	Blend 14	AP7, AP271, AP282	<i>Bacillus pumilus</i> , <i>Bacillus megaterium</i> , <i>Bacillus sphaericus</i>
Lab	Blend 15	AP32, AP33, AP34, AP40, AP50	<i>Bacillus circulans</i> , <i>Bacillus pumilus</i> , <i>Bacillus megaterium</i>
Lab	Blend 16	AP188, AP204, AP209, AP217, AP218	<i>Paenibacillus macerans</i> , <i>Bacillus amyloliquefaciens</i> , <i>Bacillus atrophaeus</i> , <i>Brevibacillus brevis</i> , <i>Bacillus subtilis</i>
Lab	Blend 17	AP136, AP153, AP188, AP204, AP219	<i>Bacillus subtilis</i> , <i>Brevibacillus laterosporus</i> , <i>Paenibacillus macerans</i> , <i>Bacillus amyloliquefaciens</i> , <i>Bacillus subtilis</i>
Lab, 1,2	Blend 18	AP143, AP153, AP204, AP217, AP218	<i>Paenibacillus macerans</i> , <i>Brevibacillus laterosporus</i> , <i>Bacillus amyloliquefaciens</i> , <i>Brevibacillus brevis</i> , <i>Bacillus subtilis</i>
Lab, 1,2	Blend 19	AP 223, AP 279, AP 282, AP 283	<i>Bacillus circulans</i> , <i>Bacillus subtilis</i> , <i>Bacillus sphaericus</i> , <i>Bacillus pumilus</i>
Lab, 1,2	Blend 20	AP 7, AP 18, AP 282	<i>Bacillus pumilus</i> , <i>Bacillus pumilus</i> , <i>Bacillus sphaericus</i>
2	MC 1	AP 188, AP 209, AP 282	<i>Paenibacillus macerans</i> , <i>Bacillus atrophaeus</i> , <i>Bacillus sphaericus</i>
2	MC 2	AP 7, AP 188, AP 209, AP 282	<i>Bacillus pumilus</i> , <i>Paenibacillus macerans</i> , <i>Bacillus atrophaeus</i> , <i>Bacillus sphaericus</i>
2	MC 3	AP 18, AP 188, AP 209, AP 282	<i>Bacillus pumilus</i> , <i>Paenibacillus macerans</i> , <i>Bacillus atrophaeus</i> , <i>Bacillus sphaericus</i>
2	MC 4	AP 188, AP 204, AP 209, AP 282	<i>Paenibacillus macerans</i> , <i>Bacillus amyloliquefaciens</i> , <i>Bacillus atrophaeus</i> , <i>Bacillus sphaericus</i>

^aLab = initial laboratory screening of Blends using M-S media in Petri dishes. Trials 1 and 2 were separate 5 wk experiments using potted hybrid bermudagrass in a greenhouse.

^bAP strains contain rhizobacteria unique to the Auburn University PGPR collection.

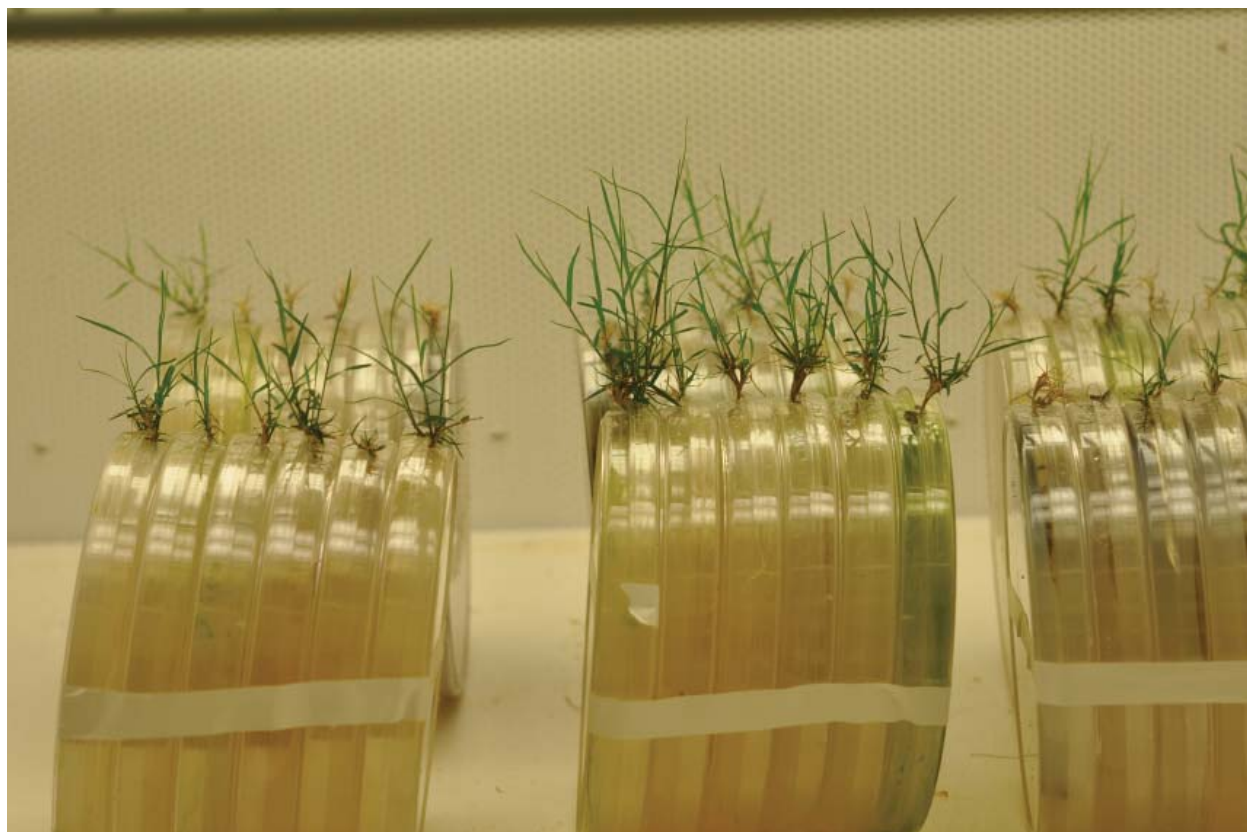


Fig. 1. Hybrid bermudagrass growing in petri dishes containing M-S media in a bioassay to initially screen rhizobacteria for growth promotion.

scissors to a height of 5 cm (2 in). Each week for 5 wk, 2 ml (0.068 fl oz) of an aqueous bacterial suspension of 10^7 cfu·ml⁻¹ or distilled water were applied to the growing bermudagrass plants. Weekly, the grass was re-cut to 5 cm, and the foliage was collected and weighed (Braman et al. 2002). After the 6th wk (5 applications), the bermudagrass plugs were destructively sampled to determine root growth impacts. This experiment was replicated 12 times, six replicates in each of two separate trials.

Experiment 2 — greenhouse trials. After identifying potential candidate blends of PGPR in Experiment 1, we tested those blends using plugs with soil in a greenhouse. Four PGPR blends as well as a nontreated control were evaluated in Greenhouse Trial 1, and 8 blends (Table 1) were evaluated in the Greenhouse Trial 2. The MC PGPR blends were designed by reviewing data from Experiment 1, Greenhouse Trial 1, and previous published (Calvo Velez 2013, Yellaredi 2013) and unpublished studies conducted at Auburn University with the PGPR collection.

For these trials, Tifway hybrid bermudagrass plugs (3.8 cm diameter) from the Auburn University Turfgrass Research Unit were harvested on April 1 and July 24, 2013, respectively, for each trial. After harvesting, plugs were washed free of field soil and transplanted. Bermudagrass plugs were grown in SC7 Stubby cone-tainers (3.8 by 14 cm, Stuewe & Sons, Tangent, OR) in a greenhouse facility set at 28.6 ± 5 C (83.5F), 14 hour light: 10 hour dark natural photoperiod, and $50 \pm 10\%$ relative humidity. Bermudagrass plugs were planted in clean sand. The plants were given 3

wk to acclimate to the new conditions. During acclimation, fertilizer (305 ppm Nitrogen, Peterson's 20N-20P-20K; Alix, Alberta, Canada) was mixed weekly at a rate of 5 mg per 3.78 L (0.0002 oz per gal), and 50 ml (1.7 oz) were applied to each plant. After acclimation (4th week), the plants were cut to a height of 5 cm, and supplemental fertilization was stopped. Each week, 2 ml of a freshly-prepared aqueous bacterial suspension of 10^7 colony forming units (cfu·ml⁻¹) from PGPR stock solutions or distilled water were applied to the growing media of each pot followed by 30 ml of tap water. Plants were watered daily to saturation, except when PGPR applications were made. At the end of the 8th week (5 applications), the bermudagrass plugs were destructively sampled. Each trial had 12 replicates for each treatment in a randomized complete block design.

Evaluations of growth promotion. The bermudagrass foliage was cut weekly to a height of 5 cm, and the top growth was collected. Foliage was dried in an oven at 70C (158F) for 40 min, and dry mass was recorded. For the experiment conducted in Petri dishes it was not possible to extract roots completely, so a rating of root coverage was used to make comparisons between bacterial inoculants and with the nontreated plants. Coverage was rated visually by dividing each dish into eight equal sections. If the root structure was present in 50% or more of a section, then it was counted. Each replicate was rated (0 to 8) based on the sections containing roots.

For experiments with plants in cone-tainers, the root system of each plant was washed in the lab. After washing,

analysis of the root structure was conducted using a root scanning system (Regent Instruments, Inc., Sainte-Foy, Quebec), which consisted of a scanner (LA 1600+) and WinRhizo software (version 2004a). Based on image analysis, the software computed the following parameters: root length, root surface area, root volume, root tips. Total dry root weight was calculated using a PB303-S scale (Mettler-Toledo Inc., Columbus, OH). The data collected were used to compare root growth and shoot growth to determine if strains and blends caused growth promotion in bermudagrass relative to the nontreated plants.

Statistical analysis. Top growth for all trials was analyzed using repeated measures of multivariate analysis of variance (MANOVA) ($P < 0.05$, JMP Version 10, SAS Institute Inc., Cary, NC, 1989–2007). Root ratings for the growth chamber trials were transformed ($\log + 0.5$) before the analysis of variance, and means were subject to Student's *t*-test ($P < 0.05$, JMP) for comparisons. Treatment means for root parameters in the greenhouse trials were subjected to analysis of variance and compared using a Student's *t*-test to determine if there were differences between bacterial treatments ($P < 0.05$, JMP).

Results and Discussion

Experiment 1 — growth chamber trials. Shoot weight was significantly enhanced by treatment with 6 blends (Blends 8, 14, 16, 18, 19, 20) by 236 to 345% relative to the nontreated control ($P < 0.01$; Fig. 2). Treatment of bermudagrass with Blends 9, 11, and 17 resulted in less top growth (37 to 98%; $P \leq 0.95$) relative to nontreated plants. In 6 wk, roots of bermudagrass treated with Blends 8, 18, 19, and 20 covered more than half the Petri dish on average (ratings > 4) compared to nontreated plants ($P < 0.05$), which had a median rating of 3.67 (Table 2). Twelve blends were initially screened for growth promotion using the M-S media bioassay. Negative shoot and root impacts may be a result of the production of certain phytohormones and gibberellins produced directly by the bacteria or bacteria-induced changes within the plant. This impact on signaling pathways represents plant-bacterial interactions that may result in growth production or antagonism depending on the bacterial species present in the inoculant (Bottini et al. 2004). The M-S media bioassay was beneficial for a high throughput, rapid evaluation, which disqualified over half of the initial bacterial candidates before moving to greenhouse trials. However, M-S media bioassays limit the amount of data that can be collected from root systems. For example, it was impossible to extract the roots from the media for analysis using the scanning system, which resulted in the use of a more rudimentary visual rating system.

Experiment 2 — greenhouse trials. Top weight was significantly enhanced by treatment with Blends 8, 18, 19, and 20 in Trial 1 (158–197%; $P < 0.003$) relative to nontreated plants over the 8 wk study (Table 3). This confirms the top growth results obtained by using the M-S media bioassay. Among these treatments, however, only bermudagrass treated with Blend 8 had significantly greater (11%; $P < 0.01$) root mass relative to nontreated plants and all other treatments. Only Blend 20-treated plants had a greater (157%; $P < 0.001$) root length relative to nontreated plants. Bermudagrass treated with Blends 19 and 20 had significantly greater ($\geq 173\%$; P

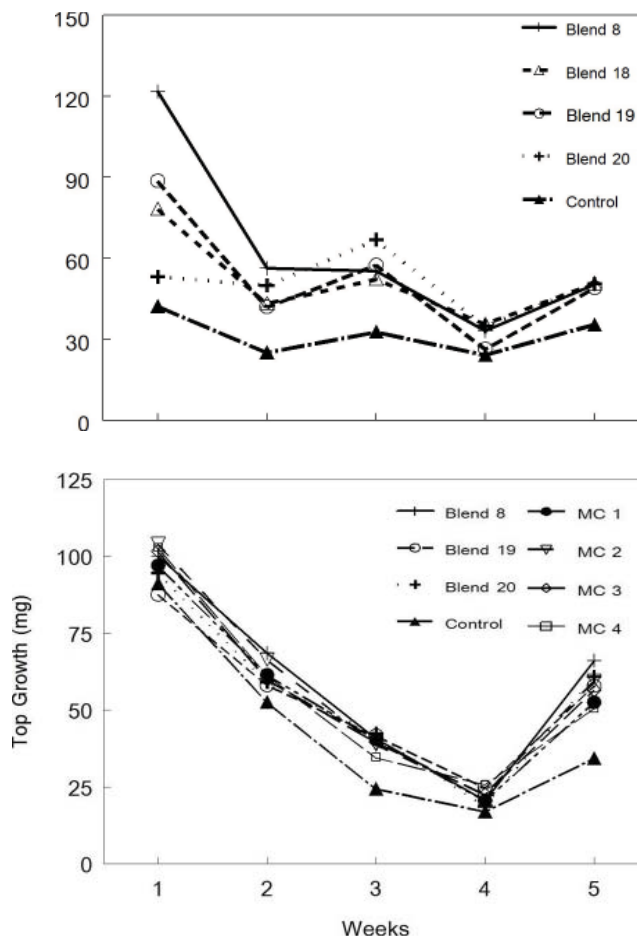


Fig. 2. Dry mass (mg) of hybrid bermudagrass foliage produced weekly from Greenhouse Trials 1 (top) and 2 (bottom) as affected by applications of bacterial inoculants.

< 0.03) root surface areas than nontreated plants. Similarly, bermudagrass treated with Blends 19 and 20 resulted in 150 to 186% greater root volume relative to the nontreated plants ($P < 0.05$). Root volume and root length for plants treated with Blends 8 and 18 were numerically greater than nontreated plants. However, these pairwise comparisons were not significant at $\alpha = 0.05$ but were significant at $P = 0.062$.

In Greenhouse Trial 2, blends unique to Trial 2 (MC 1, MC 2, MC 3) as well as Blends 8, 18, and 20 produced significantly greater top growth than the nontreated plants (Fig. 2; $P < 0.03$), but plants treated with MC 4 and Blend 19 did not differ significantly from nontreated plants. Treatment with MC2 resulted in root dry weight that was significantly greater than that found in the nontreated plants and all other treatments ($P \leq 0.05$). In general, root length and root surface area were generally lower in Trial 2. Plants treated with MC 1, MC 2, MC 3, MC 4, Blend 18, Blend 19, and Blend 20 resulted in root systems that had significantly greater root length relative to the nontreated plants and Blend 8 ($P \leq 0.05$). Additionally, plants treated with MC 3 had a root length that was significantly greater than those treated with MC 1, MC 2, MC 4, and Blend 20 ($P < 0.024$). Blend 19 produced a greater root length in plants than Blend 20 did ($P < 0.021$). Blend 8-treated plants resulted in the lowest root surface area, which was significantly different from MC 2, MC 3, MC 4, Blend 18, Blend 19, Blend 20, and nontreated

Table 2. Mean root ratings of hybrid bermudagrass plants as affected by rhizobacterial blends in a growth chamber trial using Petri dishes.

Name	Rating ^z
Nontreated	3.7cd
8	5.9a
9	1.8e
11	2.3de
12	2.3de
13	1.7e
14	4.3bc
15	2.8de
16	4.6abc
17	4.6abc
18	5.6ab
19	5.3ab
20	5.8a

^zVisual rating of roots occupying 8 sections of the dish. A 0 = no root presence, a 4 represents half of the sections containing roots and an 8 all sections. Means presented are actual means. Within a column, means followed by the same letter are not significantly different from each other ($P < 0.05$; JMP; ANOVA Student's t-Test; [SAS Institute Inc., Cary, NC, 1989–2007]).

plants ($P \leq 0.02$). Blends 19 and MC 3-treated plants resulted in the greatest root length and surface area, and both blends were significantly greater than the nontreated plants. Plants treated with Blend 19 also had a root surface area that was significantly greater than those receiving MC 1, MC 2, and Blend 20 ($P < 0.006$); whereas plants treated with MC 3 had root surface areas significantly greater than those treated with MC 1 ($P < 0.001$). None of the blends in Trial 2 resulted in a root volume that was significantly greater than the nontreated plants ($P \leq 0.05$).

The treatment of bermudagrass with PGPR blends 8, 18, 19, and 20 resulted in significantly greater top growth than the nontreated plants, and all blends except for Blend 18 had a significant impact on one or more root parameters (Table 3). The key first steps in the plant-bacterial interactions involve the ability of bacterial blends to survive inoculation, attach to the root surface, and colonize the developing root system (Kloepper 1993). There is little information on the success of rhizobacteria in these first steps for grasses. Additionally, a few studies have characterized the persistence of bacteria after inoculation for crop plants (Durham 2013). All PGPR-treated plants averaged about 150% greater root length than the control. These results may have important implications for turf and pasture grasses with low fertility inputs. In pastures, an increase in top growth makes the pasture more productive as forage.

In our experiments, inoculants were applied weekly; however, Durham (2013) found that a single application to cotton persisted and was detectable up to 12 weeks post inoculation. Perhaps these bacteria could be applied 1 to 2 times per season, possibly in a granular or wettable powder form similar to Nortica (*Bacillus firmus* (strain I-1582), a PGPR product in turfgrass aimed at providing season-long growth promotion and nematode protection (Bayer Environmental Science, 2013). In turf settings, applications are easier to make than in pasture and could be tank mixed with fertilizer applications, possibly allowing for nitrogen rate reductions and enhanced nutrient uptake (Baltensperger 1978). This hypothesis is currently under further investigation. The increase in root growth when inoculants are present could

Table 3. Bermudagrass root measurements after 5 wk exposure to bacterial inoculants in a greenhouse trial.

Treatment	Trial	Root measurements ^z			
		Length (cm)	Surface area (cm ²)	Volume (cm ³)	Dry weight (g)
Nontreated	1	849.1b	138.9b	1.8b	2.3b
Blend 8	1	1,213.6ab	204.1ab	2.8ab	2.6a
Blend 18	1	1,207.4ab	203.9ab	2.8ab	2.4b
Blend 19	1	1,199.5ab	214.0a	3.01a	2.4b
Blend 20	1	1,341.2a	239.4a	3.4a	2.5b
Nontreated	2	365.0d	126.3c	3.7a	2.3bc
Blend 8	2	192.0e	88.8d	3.3ab	2.1c
Blend 19	2	575.3ab	169.3a	3.9a	2.3c
Blend 20	2	469.4c	138.5c	3.2abc	2.3bc
MC 1	2	455.8bc	129.0c	2.5c	2.3bc
MC 2	2	543.5bc	137.4c	2.9bc	2.5a
MC 3	2	655.1a	162.0ab	3.3abc	2.4b
MC 4	2	573.8bc	145.4bc	3.2abc	2.3bc

^zNumbers presented are actual means ($N = 12$), although transformed data was used for statistical analysis. For each Trial, means in the same column followed by the same letter are not significantly different from each other (Trial 1 and 2: $P < 0.05$; JMP; Student's t-Test).

increase nutrient and water acquisition from soils, which could enhance drought tolerance (Coy, unpublished data) or decrease water inputs for turfgrass managers.

In general, Greenhouse Trial 2 had less growth than Trial 1. One limitation to these experiments is that root parameters may vary among the test plants after the 3 wk of fertilization and before beginning the bacterial inoculation. The plants in each trial were harvested and grown in the greenhouse at different times of the year. To compensate for this variation, a larger number of replicates were used. Each trial, for example, typically had 12 replicates, and many treatments were repeated across multiple trials. Interestingly, Blend 8 resulted in marginally to significantly greater root parameters than nontreated plants in Greenhouse Trial 1, yet plants treated with Blend 8 in Trial 2 were typically less vigorous than nontreated plants. In Trial 2, only Blend 19 and MC 3 increased root length and surface area, and MC 2 increased root dry weight. However, a shade cloth was placed over the greenhouse between weeks 1 and 2 of Trial 2, which resulted in a reduction in growth compared to Trial 1, as Tifway bermudagrass is not a shade tolerant grass (Fig. 2).

Blends (MC1–4) were designed by combining PGPR strains that had previously demonstrated the capacity to promote growth of bermudagrass (growth chamber trial) or of similar monocot crops (corn and rice) (data not shown). Different blends from the Auburn University PGPR collection may offer growth promotion in one crop, but the same benefits may not be seen in another crop (Kloepper, personal communication). Performance of bacterial inoculants may be crop dependent and influenced by not only the blend's strains but their interactions with plant physiology (C_3 vs. C_4) or root morphology. For example, Blend 9 has been credited with increased root growth in cotton (Ngumbi 2011) but failed to demonstrate this benefit in turfgrass in regards to top and root growth parameters. Also, the more fibrous monocot root system may be more easily colonized by some strains than the tap roots of dicots.

Bacterial inoculants in the Auburn University collection were selected based on success in root colonization (endophytically or ectophytically), and some are being

commercially developed for use in different plant production systems. Certain companies already have bacterial inoculants available for use in turfgrass, like Nortica (Bayer Environmental Sciences 2013). However, the aim of this study was to evaluate novel bacterial blends rather than to test existing commercial PGPR products. In this study we present data indicating that Blends 19, 20, MC 2, and MC 3 should be further evaluated for use in pasture and amenity grass systems. Further research should consider the performance of individual strains compared to blends as well as field performance of these bacterial inoculants in relation to fertility and pest management.

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