

Plant Growth–promoting Rhizobacteria Have Varied Effects on Quality and Yield of Drought-stressed Creeping Bentgrass (*Agrostis stolonifera*)

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KEYWORDS. *Agrostis stolonifera*, creeping bentgrass, drought, plant growth–promoting rhizobacteria, turfgrass

ABSTRACT. Recently, there has been renewed interest in reducing excess inputs into turf, with a special emphasis on reducing water use. One potential mechanism to achieve this goal is the use of plant growth–promoting rhizobacteria (PGPR) applications. Plant growth–promoting rhizobacteria research in turfgrass is limited, but the few studies that have been conducted show that PGPR can reduce biotic and abiotic stress in turfgrass. Two creeping bentgrass (*Agrostis stolonifera*) cultivars (Pennncross and 007) were treated with either PGPR Blend 20, PGPR DH44, water, or nitrogen fertilizer before being subjected to a dry-down period during which half of each treatment was either irrigated or drought stressed. Results indicate that PGPR DH44 may help maintain greater quality in drought-stressed ‘007’ creeping bentgrass compared with nitrogen-treated plots; however, quality did not differ between DH44- and water-treated plots. When drought stressed, PGPR did not help creeping bentgrass maintain clipping yield compared with nitrogen-fertilized or water-treated plots, nor did PGPR affect root biomass. More research is needed before recommendations can be made regarding PGPR applications to turfgrass.

Golf course superintendents are facing increased pressure to reduce the quantity or quality of irrigation water used on their courses (Unruh 2017). Water scarcity and persistent drought conditions in the arid west have led to reductions in water use and increased interest in the establishment of and transition to low-water-use landscapes and plantings. Many western states have programs aimed at reducing irrigation use, and

several programs specifically target turfgrass landscapes (della Cava 2015).

The increased scrutiny golf turfgrass managers face has led to a demand for grasses that require less water and the implementation of best management practices to reduce water use and maintain turfgrass under reduced irrigation. Golf courses have adopted agronomic conservation practices including the use of wetting agents and soil amendments, hand watering, increasing no-mow acreage, and reducing irrigated acres (among others) to help reduce irrigation use (Gelernter et al. 2015). Of the golf courses that reduced turfgrass acreage between 2005 and 2013, 75% and 32% cited water conservation and drought, respectively, as reasons driving the decision to reduce turfgrass acreage (Gelernter et al. 2015). Between 2005 and 2013, US golf course water use decreased by 21.8% nationally (Gelernter et al. 2015).

Regulation and public scrutiny of golf irrigation methods, frequency, and rates have increased (Gelernter et al. 2015); however, drought and reductions in irrigation can have a lasting impact on turfgrass health. Drought can be a major limiting factor for turfgrass health and can lead to reductions

in turfgrass growth and vigor, wilting, desiccation, and overall reduced turfgrass quality. When drought stress is severe, turfgrass can be permanently damaged. Superintendents must balance the call for reduced inputs with the high visual quality and playability requirements expected of golf course turfgrass and turfgrass health in general.

Plant growth–promoting rhizobacteria (PGPR) are one potential tool for reducing irrigation inputs on golf courses. Plant growth–promoting rhizobacteria act upon plants in various ways, including metabolic adjustments, direct biostimulation, and production of growth-promoting compounds such as phytohormones and volatile compounds that may increase rooting and water and mineral absorption (Errickson and Huang 2023; Ortiz-Castro et al. 2009). Other mechanisms include antagonism of plant pathogens or stimulation of plant immune responses (Calvo et al. 2014; Pieterse et al. 2014). Although many bacteria may act as PGPR, the most bioactive strains identified so far across various crops include various species of *Pseudomonas*, *Bacillus*, *Rhizobium*, and *Actinobacteria* (Calvo et al. 2014; Pieterse et al. 2014).

Plant growth–promoting rhizobacteria have been shown to reduce fertilizer requirements, increase drought tolerance, and mitigate pest pressure in various crops such as tomato (*Solanum lycopersicum*, formerly *Lycopersicon esculentum*) and rice (*Oryza sativa*) (Adesemoye et al. 2009; Burkett-Cadena et al. 2008; Ramamoorthy et al. 2001). Cereal grains of the Poaceae family, including barley (*Hordeum vulgare*) and rice, have also shown positive responses from PGPR applications (Kloepper 1993). Results from turfgrass trials indicate that PGPR can increase root and shoot growth in hybrid bermudagrass (*Cynodon dactylon* × *C. transvaalensis* Burt-Davey) (Coy et al. 2014) and increase color rating and clipping yield in perennial ryegrass (*Lolium perenne* L.) and tall fescue [*Schedonorus arundinaceus* (Schreb.) Durmort, formerly *Festuca arundinacea* L. Schreb.], although not in Kentucky bluegrass (*Poa pratensis* L.) (Acikgoz et al. 2016). There is research suggesting PGPR may alter drought stress response in bermudagrass (Coy et al. 2017) and in tall fescue (Mahdavi et al. 2020). In creeping bentgrass (*Agrostis stolonifera* L.), PGPR inoculation can result in improved

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tillering number and rate, root viability, and root growth during drought stress and drought recovery (Errickson et al. 2023).

There are an estimated 163,800 km² ($\pm 35,850$ km²) of residential and commercial lawns, parks, golf, and sports turfgrass in the United States (Milesi et al. 2005); an estimated 6087 km² of that area is maintained golf course turfgrass (Golf Course Superintendents Association of America 2007). Creeping bentgrass is the most commonly used cool-season turfgrass species on US tees, greens, and fairways (Golf Course Superintendents Association of America 2007). This economically important turfgrass species may provide an opportunity to reduce water inputs on golf courses if PGPR can help mitigate drought stress in creeping bentgrass. Therefore, the goal of this study was to determine whether PGPR enhanced the drought tolerance of creeping bentgrass.

Materials and methods

The trial was conducted at the Utah State University Research Greenhouses in Logan, UT, USA, using PGPR strains from the David Held collections at Auburn University. Creeping bentgrass was seeded at 5.37 g·m⁻² on 28 Feb 2021 into 10.16-cm tree-pots filled with a sand/peat mixture at 2% organic matter by weight. The pots were maintained on a day temperature of 21 °C and a night temperature of 15 °C with a 14-h photoperiod. The pots were irrigated three to four times per week and fertilized weekly during establishment (28 Feb to 30 Aug 2021) to ensure a healthy stand. Stands were maintained at a 1-cm height of cut.

This study was conducted on pots of two bentgrass cultivars, Pennncross or 007, generally considered to be relatively drought susceptible and drought tolerant, respectively (Brilman L, personal communication). Treatments included 1) PGPR Blend 20 (equal parts *Bacillus pumilus* strain AP7, *B. pumilus* strain AP18, and *Bacillus sphaericus* strain AP282); 2) PGPR DH44 (*Paenibacillus riograndensis*); 3) a nitrogen-fertilized control applied as 500 mL·m⁻² dissolved urea solution (46-0-0; Frontier Fertilizer, Johnstown, CO, USA) at a rate of 0.49 g·m⁻² N; and 4) an unfertilized control irrigated with 500 mL·m⁻². The PGPR blends were applied as 500 mL·m⁻² of the aqueous PGPR suspension of 1×10^7 cfu/mL.

Treatments were applied weekly for 4 weeks, starting 6 months after seeding. All turfgrass was irrigated with 131 mL of water after treatments were applied to move the treatments into the root zone. All turfgrass was irrigated daily with fertilizer-free irrigation water on non-treatment application days. After 4 weeks of treatment applications, irrigation was discontinued on half of each treatment's pots for a 9-week dry-down period, while the remaining pots continued to be irrigated. The experiment was arranged as a completely randomized design, with three replications of each application $\times$ irrigation combination.

During the 9-week dry-down period, clippings were collected weekly at a 1-cm height of cut, dried at 60 °C for 72 h, and weighed to determine aboveground biomass throughout the stress period. The pots were then destructively sampled to determine the root biomass. Aboveground tissue and thatch were removed, and roots were washed in water to remove sand and peat. Roots were then dried at 60 °C for 72 h and weighed to determine dry root biomass.

Photos of each turfgrass plug surrounded by a purple frame (flat paint, color 4001-10C, "Cosmic Berry"; Val-spar, Minneapolis, MN, USA) were taken daily during the dry-down period using a light box (Karcher and Richardson 2013). The images were digitally analyzed to determine visual quality on a 1 to 9 scale (Skogley and Sawyer 1992), with 9 being dark green, dense, uniform turf; 1 being dead or dormant brown turfgrass; and 6 being minimum acceptable quality (Turf Analyzer Green Research Services, LLC, Fayetteville, AR, USA). The data were averaged by week for analysis.

All of the data were analyzed using the PROC GLIMMIX procedure in SAS 9.4 (SAS Institute, Cary, NC, USA), with a model in which cultivar, application, irrigation, and week were fixed effects, and the correlation of repeated measures over weeks was modeled with first-order autoregressive structure. Pairwise comparisons among the treatments were made using Tukey-Cramer's method adjusting for multiplicity with a significance level of 0.05. The results were visualized using RStudio (RStudio, PBC, Boston, MA, USA).

Results and discussion

QUALITY. There was a large amount of variation in quality, which was

affected by the significant interaction between week, cultivar, drought stress status, and treatment (Table 1). However, there were no significant differences between treatments in 'Pennncross' bentgrass quality during any of the 9 weeks of the dry-down period (data not shown). At no point was there a significant difference in '007' quality between irrigated or drought-stressed treatments within any of the four applications (Fig. 1). From week 4 through week 9, drought-stressed '007' treated with DH44 showed a trend of greater quality than drought-stressed, nitrogen-treated '007' bentgrass, although neither treatment varied significantly from the other two drought-stressed treatments (Fig. 1). Although the irrigated water-treated '007' bentgrass was the only '007' treatment of acceptable quality at the end of the dry-down period, the quality was only significantly greater than the drought-stressed nitrogen treatment (Fig. 1).

Overall, the results indicate that PGPR applications before the onset of drought may help maintain greater quality in drought-stressed '007' creeping bentgrass compared with nitrogen-treated plots but do not necessarily provide better quality than simply applying water before the onset of drought. The results from the present study do not align with Mahdavi et al. (2020), who determined that the application of *Pseudomonas fluorescens* PGPR improved the turfgrass quality of tall fescue subjected to drought compared with uninoculated control treatments. Differences in results could be due to the turfgrass or PGPR species used, maintenance practices, or the timing of treatment applications. In the present study, treatments were applied before the onset of drought. More research is needed to determine whether application during drought may help maintain turfgrass quality or whether application after a drought period can help turfgrass recover more quickly from drought stress or damage. Similarly, the results were cultivar-specific, and no impact on quality was measured in 'Pennncross' creeping bentgrass, indicating that PGPR applications may need to be tested on a wide selection of cultivars to determine general efficacy.

CLIPPING YIELD. Clipping yield was significantly affected by the two-way interactions of week with drought stress and week with treatment (Table 1).

Table 1. Summary of modeled effects on creeping bentgrass (cultivars Penn-cross and 007) quality and yield in response to four experimental treatments (water, nitrogen fertilizer, plant growth-promoting rhizobacteria Blend 20, and plant growth-promoting rhizobacteria DH44) and two drought levels (drought-stressed or irrigated).

Variable	df	Quality	Yield
C	1	**	NS
T	3	NS	*
C × T	3	NS	NS
D	1	*	**
C × D	1	NS	NS
T × D	3	NS	NS
C × T × D	3	NS	NS
W	1	***	***
W × C	1	*	NS
W × T	3	**	*
W × C × T	3	NS	NS
W × D	1	***	**
W × C × D	1	NS	NS
W × T × D	3	NS	NS
W × C × T × D	3	*	NS

C = Cultivar; D = drought; NS = nonsignificant; T = Treatment; W = week; * = significant at the 0.05 *P* level; ** = significant at the 0.01 *P* level; *** = significant at the 0.001 *P* level.

At week 5 of the 9-week dry-down period, clipping collection was halted due to insufficient volume of clippings. Clipping yield did not differ significantly between cultivars, nor was

‘007’ creeping bentgrass clipping yield significantly different between any treatments at any week during the trial (data not shown).

Generally, between the pretreatment period and week 1 of the dry-down period, ‘Penn-cross’ clipping yield decreased across all treatments and continued to decrease throughout the 5 weeks of the dry-down period during which clippings were collected (Fig. 2). At week 1 of the dry-down period, irrigated fertilized ‘Penn-cross’ plots had greater dry clipping yield than drought-stressed fertilized plots, irrigated DH44 plots, irrigated water-treated plots, and drought-stressed DH44 plots (Fig. 2). Between weeks 2 and 5 of the dry-down period, ‘Penn-cross’ clipping yield did not differ significantly between any of the drought-stressed treatments (Fig. 2), indicating that when drought stressed, PGPR applications did not help creeping bentgrass maintain top growth compared with fertilized or water-treated plots. This is again counter to findings by Mahdavi et al. (2020), who determined that application of *P. fluorescens* PGPR increased fresh weight (combined root, clipping, and verdure) of tall fescue subjected to drought, compared with uninoculated control treatments. These differences may be due to the nature of collected

tissue (clippings compared with root, clipping, and verdure), species of the turfgrass and PGPR, method or length of drought stress, and general management practices.

On weeks 1, 3, and 4, the clipping yield of irrigated, Blend 20-treated ‘Penn-cross’ creeping bentgrass was statistically similar to that of irrigated, fertilized ‘Penn-cross’ creeping bentgrass; however, at no point was clipping yield of Blend 20-treated creeping bentgrass statistically different from the irrigated, water-treated plots (Fig. 2). Similarly, clipping yield in weeks 4 and 5 was not significantly different between irrigated, DH44-treated plots and irrigated, fertilized plots, but neither were they different from irrigated, water-treated plots. By week 5, there was no significant difference in dry clipping yield between any of the irrigated treatments (Fig. 2). These results indicate that PGPR applications do not affect the dry clipping yield of irrigated creeping bentgrass turf.

ROOT BIOMASS. Treatment and drought status did not significantly affect root biomass. Bentgrass cultivar was the only significant factor affecting root biomass, with ‘Penn-cross’ root biomass exceeding ‘007’ root biomass (6.243 ± 0.296 g and 3.096 ± 0.147 g, respectively). This is counter to prior studies, which found that Blend 20 stimulated root growth to help enhance tolerance of bermudagrass and tall fescue to root herbivores such as white grubs, without the use of additional fertilizer (Coy et al. 2019). Another study by Groover et al. (2020) found increased root weights of Blend 20-inoculated ‘Princess 77’ hybrid bermudagrass and DH44-inoculated ‘Tifway’ hybrid bermudagrass in glasshouse and microplot studies, respectively. Differences in results between these Blend 20 applications could be due to differences in turfgrass species, method of turfgrass establishment and stand age, growing media and management conditions, application timing and duration, and manner of stress, all of which could in turn affect PGPR colonization success as well. Also counter to the results in the present study, Errickson et al. (2023) recorded increased root viability, length, surface area, volume, and dry weight of PGPR-inoculated ‘Penn-cross’ creeping bentgrass subjected to drought stress; however, the PGPR used in that study was

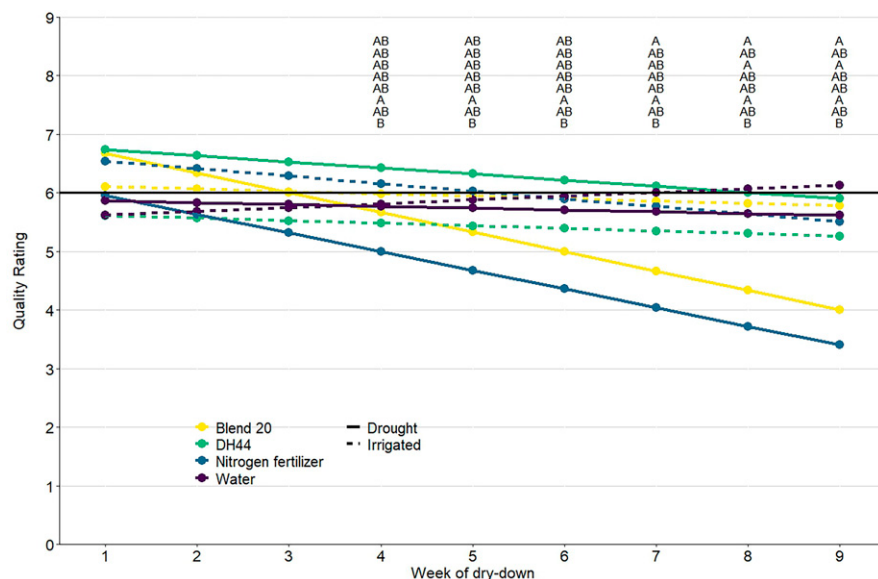


Fig. 1. Quality rating of ‘007’ creeping bentgrass treated before the onset of the dry-down period with plant growth-promoting rhizobacteria (PGPR) Blend 20, PGPR DH44, nitrogen fertilizer ($0.49 \text{ g} \cdot \text{m}^{-2} \text{ N}$), or water. The solid line at the quality rating of 6 indicates the minimum acceptable turfgrass quality. Letters above each week indicate significant differences in quality ratings during that week. From top to bottom, the letters represent irrigated water, drought-stressed water, irrigated Blend 20, drought-stressed Blend 20, irrigated DH44, drought-stressed DH44, irrigated nitrogen, and drought-stressed nitrogen treatments, respectively.

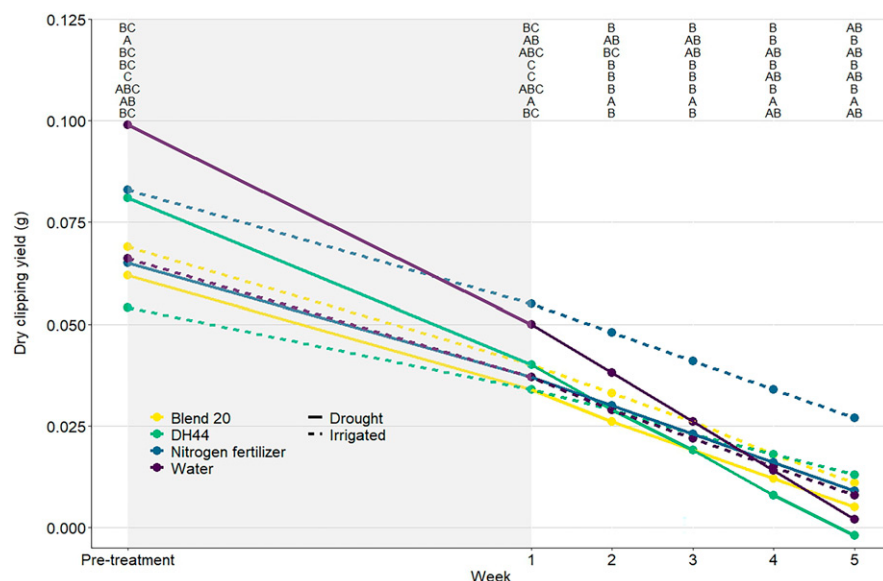


Fig. 2. Clipping dry weight of ‘Penncross’ creeping bentgrass treated with plant growth-promoting rhizobacteria (PGPR) Blend 20, PGPR DH44, nitrogen fertilizer ($0.49 \text{ g}\cdot\text{m}^{-2} \text{ N}$), or water before the onset of the dry-down period. The gray box indicates the treatment period, during which clippings were not collected. Letters above each week indicate significant differences in quality ratings during that week. From top to bottom, the letters represent irrigated water, drought-stressed water, irrigated Blend 20, drought-stressed Blend 20, irrigated DH44, drought-stressed DH44, irrigated nitrogen, and drought-stressed nitrogen treatments, respectively.

Paraburholderia aspalathi WSF23, and root measurements were recorded after a 15-d recovery period following 35 d of drought stress, which may account for differences between study results.

Prior study results suggest that PGPR Blend 20 used in the current study may affect various biotic and abiotic stress responses of turf. For example, applications of Blend 20 have increased root and shoot growth in ‘Tifway’ bermudagrass (Coy et al. 2014). Additionally, although Blend 20 helped improve tolerance to root-feeding herbivores by stimulating root growth (Coy et al. 2019), and fall armyworms laid fewer eggs on bermudagrass treated with Blend 20, larvae reared on Blend 20-treated bermudagrass clippings pupated faster and produced heavier pupae than those reared on untreated bermudagrass clippings (Coy et al. 2017). Additionally, Blend 20 was identified as one of several promising PGPR biological control agents for root-knot nematode (*Meloidogyne incognita*) management in turfgrass (Groover et al. 2020). These prior studies suggest there may be benefits of Blend 20 applications to turfgrass, despite the lack of drought stress-specific response in the current study.

CONCLUSIONS. Plant growth-promoting rhizobacteria strains and

blends are unique in their ability to promote beneficial responses in turfgrasses but did not do so in the present study. Response can vary by bacterial strain or blend of strains, turfgrass species, environmental conditions, turfgrass management, and the specific nature of the abiotic and biotic stressors. Broad generalizations cannot be made regarding the effects of PGPR blends on different responses of various grass species or cultivars. More research is needed to evaluate specific PGPR, including Blend 20 and DH44, applied to commonly used turfgrass cultivars in field settings, in various climates, and against various turfgrass biotic and abiotic stressors.

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