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Effect of Symbiotic Microorganisms on Turfgrass under Two Irrigation Regimes

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Authors' contributions

This work was carried out in collaboration between all authors. Authors PB and ILT planned the experiment. Author PY performed the statistical analysis. Authors OK and DD managed the microbial protocols. Author GP had the overall management of the work. All authors contributed to the literature searches and the field work. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Efficient use of irrigation water is one of the main goal for turfgrass keepers. In this study, the ability of selected symbiotic microorganisms to improve turfgrass visual quality and growth under two different irrigation regimes was investigated.

Study Design: Three soil inoculants, *Bacillus amyloliquefaciens* FZB42 (B), *Glomus intraradices* (G) and *Trichoderma harzianum* (strain T-22) (T) were applied to a loamy sand soil in order to colonize *Festuca arundinacea*, 'Grande II'. Regarding irrigation differentiation, a stress cycle, during which soil moisture was kept at the level of 50% of available soil water, was applied.

Place and Duration of Study: The experiment was conducted under open field conditions in North West Greece during the summer of 2014.

Methodology: Twenty four rectangular compartments of 1x2m each hosted the plots. The experimental design was multi-factorial, involving two factors (the three above mentioned root symbiotic microorganisms and two soil moisture level (100% and 50% of available soil water) treatments) and completely randomized regarding the microorganisms, with 3 replications per treatment. A number of parameters like climatic conditions, soil moisture, colonization, dry weight of clippings and canopy spectral reflectance were measured.

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Results: The results showed that *T. harzianum* T-22 and *B. amyloliquefaciens* FZB42 promoted the aerial plant growth (total dry weight of clippings) of *F. arundinacea* under water stress conditions while *G. intraradices* had no effect on the growth. In qualitative terms, no statistically significant differences were found among treatments.
Conclusion: The results of this open field study provide promising evidence regarding the potential for agronomic application of the soil inoculants that were evaluated.

Keywords: Mycorrhiza; rhizobacteria; mediterranean environment; water stress; canopy reflectance indices.

ABBREVIATIONS

PGPR: Plant Growth Promoting Rhizobacteria; B: Bacillus amyloliquefaciens; G: Glomus intraradices; T: Trichoderma harzianum; R: Reference; W100%: Well-watered treatment, for which soil moisture was kept close to 100% of the available soil water content; W50%: Less-watered treatment, for which soil moisture was kept close to 50% of the available soil water content; DOY: Day of year; θ_t: soil moisture target.

1. INTRODUCTION

Water availability is one of the most ominous abiotic factors that limits the cultivation and preservation of landscape and groundcover plants. Fast-growing population, water scarcity or limited water availability and climatic alterations generate major problems regarding water resources management in the Mediterranean region [1] and are expected to considerably affect the grassland ecosystems [2].

Up to 80% of terrestrial plants have mycorrhizal associations during their life cycle and benefit from them. Arbuscular Mycorrhizal (AM) fungi inhabit the root cortical cells of most plants. They obtain photosynthates from the host plants while they transfer mineral nutrients from the host’s rhizosphere [3]. It is a beneficial symbiosis, helping the plant to grow, resist pathogens and survive under conditions of stress [4,5]. Other symbiotic micro-organisms like strains of *Trichoderma harzianum*, promote the plant growth via enhancement of root length, shoot height, number of roots, stem diameter, number of leaves etc. [6] while provide biocontrol [7] and activate plant resistance responses against abiotic stress conditions [8].

Plant growth promoting rhizobacteria (PGPR) grow around, on and/or in plant root tissues and enhance plant growth and yield. Among the mechanisms by which rhizobacteria exert beneficial effects on plants, are the facilitation of nutrients uptake such as phosphorus via phosphate solubilization, the synthesis of phytohormones such as auxins, cytokinins and gibberellins [9] and the aid regarding control of the deleterious effects of pathogens via synthesis of antibiotics or by inducing systemic resistance [10]. Some PGPRs act also beneficially by eliciting plant response reactions directed against abiotic stresses [11]. Very limited number of studies concerning the beneficial effects of PGPRs on turfgrass where found in the literature. The majority of them evaluated the effects of bacterial inoculants on diseases or pests [12,13].

The objective of the present work was to evaluate the effect of selected symbiotic microorganisms on turfgrass growth and quality characteristics under two different irrigation regimes (50% and 100% of available soil water content).

2. MATERIALS AND METHODS

2.1 Experimental Set-up

The field experiment was conducted from May to September 2014 at TEIEP Kostakii Campus, Arta, Greece (latitude 39 70' N, longitude 20 56' E; WGS84). Arta's climate is of Mediterranean type with mild and rainy winters and hot and dry summers with occasional rain events.

Rectangular compartments of 1x2 m where used to host the plots. Their perimeter was framed by galvanized iron walls, down to a depth of 0.33 m. A high durability plastic sheet folded the content of each compartment and prevented mechanical and hydrological continuity between their content and its surroundings. The bottom surface had a slope of 3% and at its lowest point a collector guided any drainage to a separate for each compartment 120 L tank. Each plot was filled with a 0.08m depth layer of gravel at its bottom and above it, up to the surface, with a uniformly mixed locally available soil (texture LS: 86.00% sand; 11.64% clay and 2.36% silt). According to the characteristic water retention curve (LAB023V Soil Moisture Equipment Corp, USA), the levels of volumetric soil water content (θ) at field capacity (θ_{FC}) and at permanent wilt point (θ_{PWP}) were 15.30% and 7.10% respectively. Soil sterilization was not performed, as this is the common practice in commercial field projects. Irrigation was applied using a two stations multi-jet sprinkler system (MPR2000, Hunter Industries, San Marcos, California, USA), which was consisted of 6 sprinklers (4x90º and 2x180º) per station. The lower quarter distribution uniformity of the system was found to be around 71%.

The experimental design was multi-factorial, involving two factors (three root symbiotic microorganisms and two soil moisture level treatments) and completely randomized regarding the microorganisms, with 3 replications per treatment.

2.2 Plant Material and Its Maintenance

Tall fescue, *Festuca arundinacea*, 'Grande II' was seeded (40 g/m^2) in the plots on May 7. Soil moisture was kept close to θ_{FC} during the first 90 days. The granular fertilizers that were applied during the season were: 24-8-7, with a nitrogen content of 13.3% as NH_4^+ and 10.8% as NO_3 24.1N–5.2P–7.0K–2.0S; 15+5+20 (+2Mg+8S), with a nitrogen content of 8.0% as NH_4^+ and 7.0% as NO_3; 15N–3.5P–20.0K–2.0Mg–6.4S. During the evaluation period turfgrass was moved to a height of 4.5 cm every ten days.

2.3 Microorganisms Inoculation

Three symbiotic microorganisms: *B. amyloliquefaciens* FZB42 (*B*); *G. intraradices* (*G*) and *T. harzianum* T-22 (*T*) were evaluated against the reference treatment (R, no microbial inoculation). Regarding the plants inoculation with *G* strain, a commercial mycorrhizal inoculum ('Mycosin Tri-Ton', Vioryl Hellas, GREECE) containing fungal spores, was applied. Fifteen (15) g/m^2 of the inoculum were incorporated into the 2-3 cm top soil before seeding. Regarding plant growth promoting rhizobacterium, the inoculation was made using a commercial inoculum of *B* strain FZB42 (Greener, Intrachem Hellas, Greece) which contained industrially formulated bacterial endospores. Finally, for *T* inoculum, a commercial product ("Trianum–P" (KOPPERT B.V., Nederland) containing conidia was used. The spraying of soil with *B* (10^7 CFU/mL and 1 L/m^2) and *T* (0.45 g/L and 1L/m) inoculums was made four times per treatment. Specifically, one week after sowing (May 14) and again one two and three months later.

2.4 Application of Different Irrigation Regimes

Turfgrass water needs were estimated following the FAO paper 56 procedure [14]. The derived schedule was regularly adjusted using actual measurements of water balance factors. Two soil water regimes were established 90 days after turfgrass seeding: Well-watered (W100%), for which soil moisture was kept close to 100% of the available soil water content (ASW=θ_{FC}-θ_{PWP}) and water stressed (W50%), for which soil moisture was kept close to 50% of AWC. After 20 days, a recovery schedule during which soil moisture was kept close to 100% of ASW was applied to W50%. This phase lasted for 30 days. To sum up, the following treatments were available for evaluation: a) group of W100%: R_W100%, *G*_W100%, *B*_W100%, *T*_W100%: DOY 126-267, θ_t at 100% AWC and b) group of W50%: R_W50%, *G*_W50%, *B*_W50%, *T*_W50%: DOY 126-215 (establishment), θ_t at 100% AWC; DOY 216-236 (soil water status differentiation), θ_t at 50% AWC; DOY 237-267 (recovery), θ_t at 100% AWC. The R_W100% treatment was treated as the universal reference one.

2.5 Measurements and Statistical Analysis

Meteorological data, namely air temperature and relative humidity (T_{air}, ºC and RH_{air}, %), solar radiation (RS, W/m^2), wind speed (v, m/s) and rain (R, mm), were recorded every 30 min by means of a nearby station (HOBO Weather Station, ONSET instruments, USA). These data were used for evapotranspiration (ET) calculations. Soil moisture (θ) at a depth of 0.15 m was recorded every 30 min using a relevant network of sensors (ten 10HS sensors installed at characteristic for each plot locations and two Em50 dataloggers, Decagon Devices Inc., USA). Additionally manual measurements of soil moisture using a Thetaprobe sensor (Delta-T Devices Ltd, UK) and of drainage water volume were made every two days. In order to perform growth evaluation, turfgrass cuttings from each replication were collected. The sampling of cuttings was made by using a hedge trimmer and a 0.3x0.3x0.045 m metal frame for delimitation of the sample. Cuttings were collected by using a vacuum, dried at 70ºC and then weighed. The sampling was made before each mowing event.

While turfgrass quality is typically evaluated by integrating factors of canopy color, density and texture, canopy spectral reflectance (SR) can also provide an objective means to evaluate turfgrass quality status [15,16]. In the framework of the present work, SR was measured using a hand-held multispectral radiometer (model MSR87, Crop Scan, USA) which provides reflectance data at 8 wavelengths centered on 460, 510, 560, 610, 660, 710, 760 and 810 nm (band width: 10 nm). The sensor was placed at 1 m above ground level which according to the manufacturer corresponds to a circular measuring area of Ø0.5 m. Reflectance was measured around solar noon and a precaution for a 3 hours time period from the last irrigation event was also applied. All plots were fully vegetated and thus, soil background effects were considered negligible. A Photochemical Reflectance Index (PRI) for estimating light use efficiency [17] and a Normalized Difference Vegetation Index (NDVI) for estimating canopy phenology [18] were used for quality evaluation of each treatment in comparison to the reference one. The indices were calculated as: PRI $(R_X - R_{Ref}) / (R_X + R_{Ref})$ equals to $(R_{560} – R_{510}) / (R_{560} + R_{510})$ while NDVI $(R_{NIR} – R_{Red}) / (R_{NIR} + R_{Red})$ equals to $(R_{810} – R_{660}) / (R_{810} + R_{660})$, where R denotes reflectance at the specified wavelength, X is a wavelength in the absorbance of xanthophyll pigments spectral region; Ref is the reference wavelength for PRI measurements, NIR is the near infra red and Red is the red region of the spectrum.

Statistical processing was performed by using the SPSS 20 work package (IBM, Armonk, New York, USA). Analysis of variance (one way ANOVA) was applied and when significant treatment effects were indicated by it, means were separated by applying the Fisher's least significant difference test at P = .05. Turfgrass rhizosphere colonization by *B* was analyzed by t-test. Correlation analysis has been performed using Pearson's coefficient (r).

2.6 Estimation of Bacterial Population in Plant's Rhizosphere

Rhizosphere soil samples were collected from *B*_W100% and *B*_W50% treatments at the experimentαl period. Samples were obtained using a 5.5 cm diameter soil probe to a depth of 10 cm. Three cores were collected per plot and after mechanical processing; serial dilutions were prepared from the resulting rhizosphere soil suspensions. 0.1 ml from 10-3 and 10-4 dilutions was spread on Nutrient Agar (NA) and sheep blood agar plates (Biolife, GREECE) supplemented with cycloheximide (100μg/ml) to prevent fungal growth. The agar plates were incubated at 25°C for 3 days before counting *B. amyloliquenfaciens* colonies. The counts were expressed as CFUs (Colony Forming Units) per g of dry soil. *B. amyloliquefaciens* colonies were detected among the other rhizosphere microorganisms on the basis of: a) their typical morphology on NA medium (white–creamy colonies with an undulate margin and flat elevation) and b) hemolytic halo formed around colonies after plating dilution series on sheep blood agar.

2.7 Detection of Root Colonization by Fungi

Ten root samples per *G* and *T* treatments were randomly selected at the end of the study and were stained according to the protocol of [19]. After staining, a histological examination of all root tissues was performed using a compound microscope.

3. RESULTS AND DISCUSSION

3.1 Climatic and Meteorological Information

In Table 1, the estimated daily ET and the monthly precipitation during the experimental period are presented against the relevant climatic values (20 years averages [20]). Although in general the summer of 2014 was characterized by high temperatures for the period June-August, the conditions for a number of European areas, among which was NW Greece, deviated from the general trend [21]. In Arta, ET and precipitation were lower during July and August when compared to the climatic conditions of the area.

Table 1. Climatic (20 years) and meteorological (5 months) information

Month	Climatic (20 years avg)		Experiment period	
	ET_o [a]	R [b]	ET_o [a]	R [b]
	mm/day	mm/month	mm/day	mm/month
May	3.99	58.50	4.33 (±0.04)	77.82
June	4.78	21.80	5.38 (±0.19)	53.14
July	5.03	12.60	4.19 (±0.17)	9.00
August	4.48	17.20	4.26 (±0.20)	2.60
September	3.39	43.50	3.28 (±0.14)	122.10

[a] ET_o is the monthly reference evapotranspiration as calculated according to FAO paper 56; [b] R is the cumulative precipitation; The standard error of the values -when applicable- are provided in the parentheses

As shown in Fig. 1 Fig.before the application of the stress irrigation regime, θ was at a similar level in all treatments. After the initiation of deficit irrigation, θ in the W50% treatments started to decrease gradually down to the level of 50% of AWC and was kept around that level during the whole stress period. At the same period, for W100% treatments, θ values remained around the θ_{FC} level.

3.2 Plant's Rhizosphere Colonization by *B. amyloliquefaciens* FZB42

It was estimated the population density of *B. amyloliquefaciens* in turfgrass rhizosphere under the two irrigation regimes. It was revealed that *B. amyloliquefaciens* was successfully established in the plant's rhizosphere of both irrigation treatments (mean and standard error: 6.33±0.29 x 10^5 and 5.86±0.38 x 10^5 CFU/g dry soil for *B*_W100% and *B*_W50% treatments respectively; means are not significantly different as determined by t-test (P < .05). Furthermore, the microorganism's development was performed in significant populations. Finally, it was observed that *B. amyloliquefaciens* cell density was not influenced by limited soil water conditions.

3.3 Plant's Root Colonization by *G. intraradices* and *T. harzianum*

All *T* treatments root samples were found colonized to a high extent. In *G* treatments, all samples were found to be colonized regardless the applied irrigation regime. The mycorrhizal fungus formed arbuscules, vesicles, spores and intra- as well as inter-cellular hyphae in the root cells. No root colonization by both fungi was found in the reference samples.

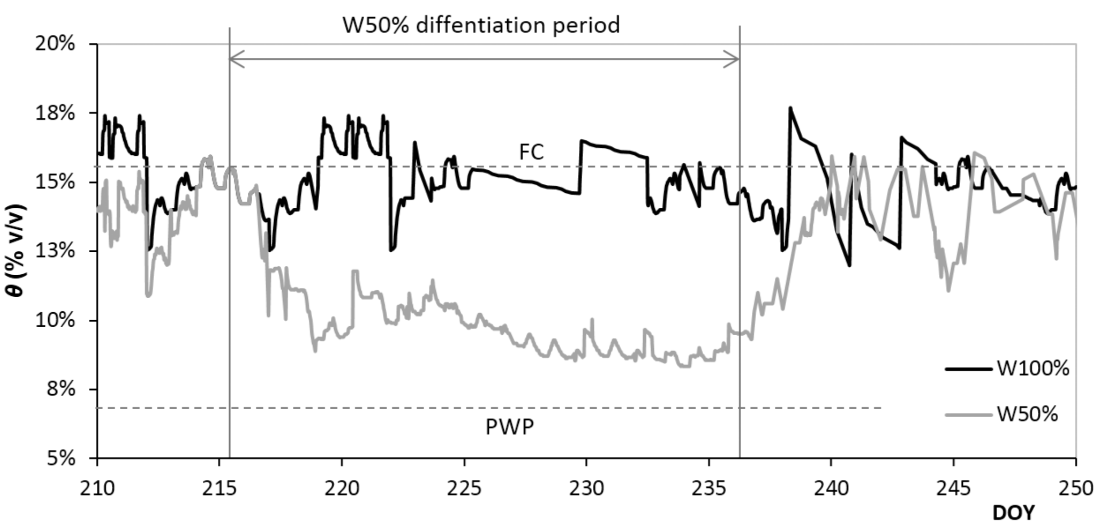

Fig. 1. The variance of average soil moisture for all W50% and W100% treatments during the limited and recovery water period for W5

3.4 Turfgrass Growth

The effects of the inoculated symbiotic microorganisms on the growth of the aerial part of the turfgrass (total dry weight of clippings) for all W50% and W100% irrigation treatments are presented in Fig. 2.

Statistically significant differences were found only for W50% treatments (ANOVA *P* = .03). More specifically: a) the dry weight of clippings in *B*_W50% (LSD, *P* = .015, *P* = .01) and *T*_W50% (LSD, *P* = .05, *P* = .03) treatments showed better development when compared to *G*_W50% and R_W50% treatments; b) *B*_W50% and *T*_W50% treatments, produced similar dry weight of clippings (LSD, *P* = .433) and no statistically significant difference when compared to the R_W100% treatment (LSD, *P* = .889, *P* = .516); c) *G*_W50% and R_W50% treatments produced similar dry weight of clippings (LSD, *P* = .095) and d) the plants that showed the largest total growth under water deficit conditions were those of *B*_W50% and *T*_W50% treatments, which presented no statistically significant difference when compared to each other (LSD, *P* = .433) or when compared to the R_W100% treatment.

Macroscopic observation revealed that the only plots which showed signs of partial wilt were those of R_W50% treatment. Regarding the effects of symbiotic microorganisms at W100% treatment, after the 3rd mowing the *B*_W100% showed statistically significant better development when compared to all other treatments. The *G*_W100% (LSD, *P* = .355) and *T*_W100% (LSD, *P* = .606) showed no difference to the R_W100%.

3.5 Turfgrass Quality

Concerning turfgrass quality, PRI values were found to be between 0.14 and 0.19 while those of NDVI were found to be between 0.67 and 0.91, a result similar to those of other researchers [16]. During the evaluation period, the majority of PRI and NDVI values showed no statistically significant differences between treatments. Fig. 3 presents the course of PRI for the W50% treatments along with this of the relevant θ level. Statistical analysis revealed a positive correlation between θ and PRI (r = 0.60, 0.5, 0.72 and 0.76 for R_W50%, *G*_W50%, *T*_W50% and *B*_W50% treatments respectively). This finding is in accordance with those of [22-24] which reported a decrease in PRI with time after application of water withhold.

Among the studied symbiotic microorganisms, *T. harzianum* (*T*) and *B. amyloliquefaciens* (*B*) performed better than *G. intraradices* (*G*). Both *T* and *B* assisted *F. arundinacea* to compensate for limited soil water conditions in both quantitive and qualitive terms. Specifically, the inoculated plants of *T*_W50% and *B*_W50% treatments presented similar aerial part yield in comparison to the R_W100%. This behavior is probably

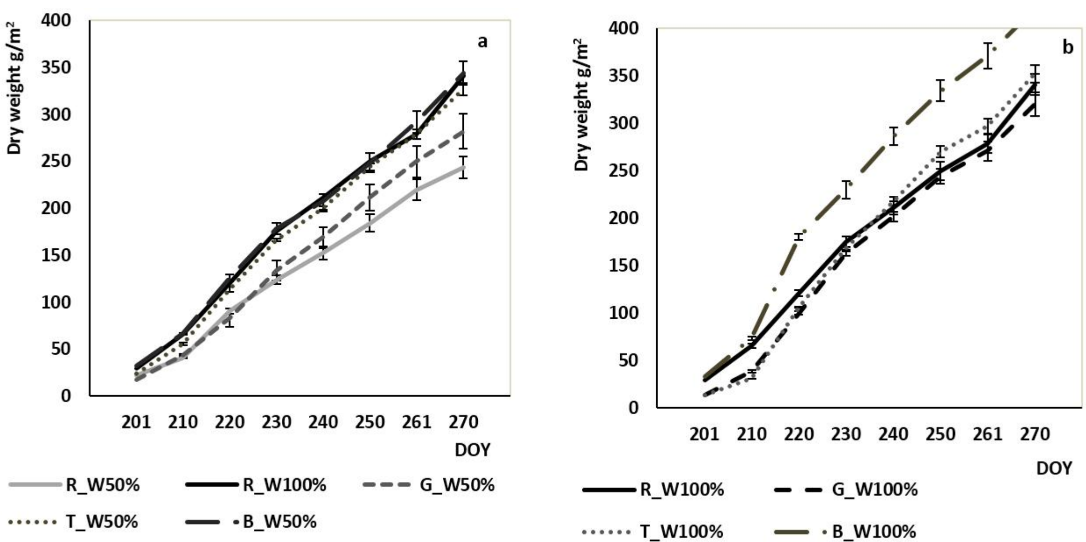

Fig. 2. Effects of symbiotic microorganisms on aerial part growth (dry weight) a) in W50% treatments, in comparison to the W50% and W100% reference treatments, b) in W100% treatments. Error bars represent ± standard error

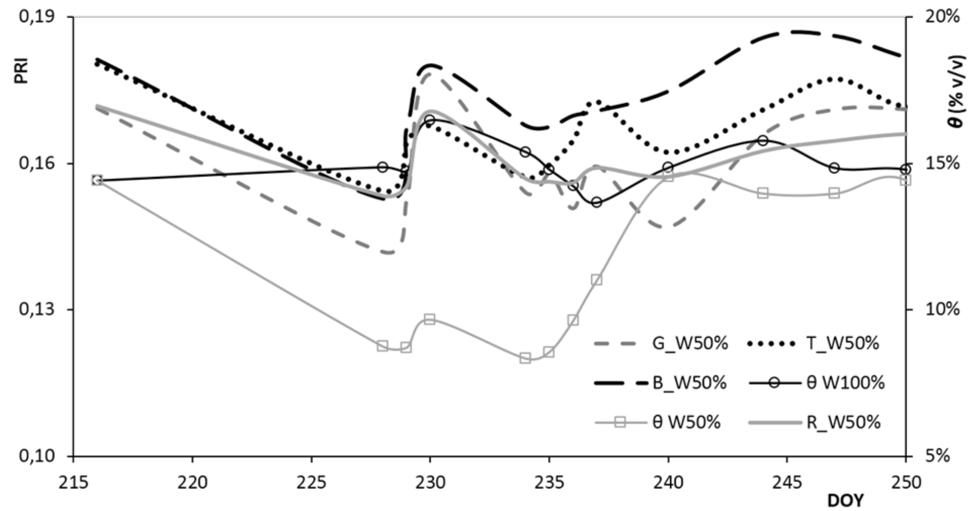

Fig. 3. Correlation of PRI for the W50% treatments with soil moisture level

based on the enhancement of root length and branching (lateral root formation) that these symbiotic microorganisms induce [6,9,25]. These changes in root growth and morphology result in greater root surface area which enhances water uptake capacity of plant.

Under sufficient soil moisture conditions *B* promoted turfgrass growth while *T* and *G* showed no effect on it. The positive effect of the *B* to turfgrass growth agrees with the findings of an introductory experiment that was done by our team, using pots in greenhouse [26].

It is noticeable that the limited soil water conditions did not affect the root's high extent colonization by *T*. On the other hand under sufficient soil moisture conditions, *B* promoted turfgrass growth, while *T* and *G* showed no effect on it. This may suggest that water stress stimulates the beneficial effect of root colonization by both symbiotic fungi and shows the necessity of the specific inoculation under more intense water stress. In contrary, the high infectivity of *G* in all treatments did not enhanced plant's growth as much as expected.

4. CONCLUSION

The study showed that the inoculation of *F. arundinacea* by *T* and *B* presents great potentials regarding its performance under limited soil water conditions. Also the extended period of stress (20 days) showed that it would be possible to maintain inoculated turfgrass using less water, without lowering the expectations regarding quality and/or gain surviving time in case of damage in the irrigation system.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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