



Book of Abstracts

5th European Turfgrass Society Conference

Turfgrass – Towards Sustainability and Perfection for
Aesthetic, Recreational and Sports

Salgados | Albufeira | Portugal

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5th European Turfgrass Society Conference
Turfgrass – Towards Sustainability and Perfection for Aesthetic, Recreational and Sports

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5th European Turfgrass Society Conference 2016

June 5-8th, Salgados, Algarve – Portugal

Turfgrass industry, which includes, among others, athletic field managers, golf course superintendents, architects, landscape designers, seed and sod producers, lawn homeowners, stakeholders, researchers, etc., is a sector facing great importance on society, since it brings societal, economical and environmental benefits. These three important pillars of turfgrass sustainability are the focus of the 5th European Turfgrass Society Conference, host in Salgados, between June 5th to 8th, under the organization of the University of Algarve.

As a growing industry, it is known that several important issues are normally addressed to turfgrass such as water use, pesticide use, fertilizer use and biodiversity, and those are significantly studied and research works are widely developed all around the world, under different climatic, edaphic and technological arrangements, enhancing and improving solutions for a better use of turfgrass.

The importance of turfgrass in Portugal and thus, the relevance of a conference concerning turfgrass subjects, result from the high number of golf courses, football pitches, private and public gardens and its relevance on the economy of the region. Portugal, and actually the Algarve region, is a privileged touristic destination for golf due to the excellent climate conditions and the high quality standards of most of its golf courses. Algarve holds nearly half of Portugal's golf courses, and they are all well regarded for their high quality standards, for the outstanding accommodations facilities, and the exceptional weather to play the game. Not surprisingly, the majority of our courses welcome more than 30,000 players across the year. With this high number of players, turfgrass stresses are usual and high standards of maintenance are necessary to establish and maintain the high quality of the golf courses.

Turfgrass also has a vital importance in several touristic and habitational areas such as Quinta do Lago, Vale do Lobo, Vilamoura and Albufeira, among others, where landscape architecture is often complemented with large areas of turf. Private gardens and public areas are covered with turfgrass from different kinds of species. With a soft and calm weather, stakeholders have a large number of varieties and species of turfgrass for use in recreational, aesthetical or for sports lawns.

The ETS Conferences are the *forum par excellence* for scientists, consultants, companies and practitioners to discuss technical issues related with the study of turfgrass.

Hosting the 5th ETS Conference 2016, it is our ambition to provide an optimum way to spread innovative applications for the benefit of the turf grass industry, national and local government, and the European public, promoting the exchange of information among turfgrass specialists from universities, official bodies and private companies.

On behalf of the Organizing Committee, I am pleased to welcome the turfgrass specialists to the 5th ETS Conference 2016 in Salgados, Albufeira, Portugal.

The convener,

Carlos Guerrero

WELCOME NOTE FROM ETS PRESIDENT

The European Turfgrass Society (ETS) was established in 2007 aiming to provide a common ground and forum for everybody that is involved in the turfgrass industry such as researchers, companies, greenkeepers and others.

ETS has achieved its goals through the organization of conferences and field days. So far ETS conferences were held in Pisa, Italy (2008); Angers, France (2010) and Kristiansand, Norway (2012), Osnabrück, Germany (2014) and the field days in Valencia (2009); Ghent, Belgium (2011); Copenhagen, Denmark (2015) and a regional field day in San Michele all'Adige - Trento, Italy (2012).

As a European Society ETS makes every effort to tackle turfgrass management and science in every possible aspect. Given that Europe constitutes a big puzzle of countries, mentalities, languages, stakeholders and customs, the main goal of ETS is to attract and bring together professionals and scientists from different European countries. This is the reason that ETS Conferences and Field Days have been covering a great range of European climatic zones, from the north to the south and from the east to the west. Management of cool and warm season turfgrasses has been presented to ETS membership from experts originating from different countries and subjected to various laws and regulations, budgets as well as techniques and equipment. Through all these years, the European turfgrass community has become richer in establishing an inventory of turfgrass professionals and scientists as well as opening channels of communication, and exchanging top of the edge technology and research among them. We are proud to say that all these have been feasible due to the works of ETS.

Following an extremely successful course, ETS is organizing the 5th European Turfgrass Society Conference 2016 in Salgados, Algarve – Portugal, from 5-8 June 2016. It is the time that ETS moves to its south and reaches the warmseason turfgrasses territory. The convener, Professor Carlos Guerrero, and the Organizing Committee, have created a Conference that provides multiple benefits to the participants. Knowledge through a high level and well structure scientific program that includes profound scientists and professionals as invited speakers and participants. Practical turfgrass experience through an exciting field trip to different turfgrass facilities. Aesthetics and civilization since it is located in one of the most beautiful southern European landscapes with several ancient historical locations. Luxury since Faro is the one of the most prestigious locations for vacation in Europe with impressive hotel resorts, golf courses, beaches, vibrant lifestyle and gourmet restaurants.

We are convinced that the 5th European Turfgrass Society Conference 2016 in Salgados, Algarve will be extremely successful and it is ETS Board pleasure to welcome all delegates from different parts of the world to participate at its works.

ETS President

Dr. P.A. Nektarios
Professor
Agricultural University of Athens

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TURFGRASS GENETICS AND BREEDING

ORAL PRESENTATIONS

GENETIC IMPROVEMENT OF BERMUDAGRASS FOR TURF QUALITY AND SUSTAINABLE TRAITS

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Introduction

Common bermudagrass [*Cynodon dactylon* (L.) Pers.] and interspecific hybrid bermudagrass between common bermudagrass and African bermudagrass (*C. transvaalensis* Burt-Davy) are arguably the most widely used warm-season turfgrasses in climates from tropical regions to transition environments in the US and other nations around the world. Turf bermudagrass is popularly used for residential lawns, sports fields, golf courses, road side vegetation, and other landscapes. Compared with other turfgrasses, bermudagrass forms dense sod, is more drought resistant and wear tolerant, and has relatively few insect and disease problems. But bermudagrass turf does not perform well in shade and has a risk of winterkill when its use is extended into transition climates. As water is becoming a limiting factor in the U.S. and many parts of the world, consumers need new cultivars with substantial improvement in drought resistance.

Common bermudagrass is widely distributed in geographic regions from 45° S. latitude to 53° N. latitude in the world, containing enormous genetic diversity for many important traits for turf performance and adaptation^{1,2}. The diverse germplasm provides rich genetic resources for breeding new turf cultivars for improved turf quality and sustainable traits. The turf bermudagrass breeding program at Oklahoma State University (OSU) was initiated by C.M. Taliaferro and colleagues in the 1980s with funding from the United States Golf Association (USGA). The initial goal of the OSU breeding program was to develop seed-propagated bermudagrass cultivars improved for textural fineness and cold hardiness for use in the U.S. transition zone. The OSU program was expanded to develop superior vegetatively-propagated turf cultivars in the 1990's. Currently, the major goal of the OSU breeding program is to develop new vegetatively- and seed-propagated cultivars combining high turf quality and enhanced resistance to winter freeze, summer drought, or shade. To increase sustainability of the turf production in the future, we also emphasize on improving resistance to spring dead spot (*Ophiosphaerella* spp.) and leaf spot (*Bipolaris* and *Exserohilum* spp.) diseases, and on salinity tolerance and reducing cultural inputs (*i.e.*, less mowing and fertilization) in breeding, selection, and evaluation protocols in addition to the major traits.

The OSU breeding program has one of the largest *Cynodon* germplasm working collections in the world. The OSU collection consists of over 1,000 plant introductions and preserved breeding lines. While many of the germplasm were collected by J.R. Harlan and colleagues from 1963 to 1967, over 350 new plant introductions have been added to the germplasm pool over the last two decades. Large genetic variabilities exist in a Chinese bermudagrass collection of more than 120 original accessions for adaptive, morphological, and fertility traits^{3,4,5}. Molecular markers and ploidy information further indicate substantial genotypic variation within the germplasm collection^{2,4,6}.

Bermudagrass is highly cross-pollinated with outcrossing enforced by strong self-incompatibility⁷. The major traits (seed production, textural fineness, stress resistance, etc.) requiring improvement in seed-propagated varieties are quantitatively inherited. Consequently, the development of seed-propagated varieties requires genetically modifying populations by increasing the frequency of desirable genes conditioning the traits under selection. Recurrent (cyclic) selection is used to effect incremental improvement in performance traits. Broad

¹ Harlan, J.R., and J.M.J. de Wet. 1969. Sources of variation in *Cynodon dactylon* (L.) Pers. *Crop Sci.* 9: 774-778.

² Wu, Y.Q., C.M. Taliaferro, G.H. Bai, and M.P. Anderson. 2004. AFLP analysis of *Cynodon dactylon* (L.) Pers. var. *dactylon* genetic variation. *Genome* 47: 689-696.

³ Wu, Y.Q., C.M. Taliaferro, G.H. Bai, D.L. Martin, J.A. Anderson, M.P. Anderson, and R.M. Edwards. 2006a. Genetic analyses of Chinese *Cynodon* accessions by flow cytometry and AFLP markers. *Crop Sci.* 46: 917-926.

⁴ Wu, Y.Q., C.M. Taliaferro, D.L. Martin, C.L. Goad, and J.A. Anderson. 2006b. Genetic variability and relationships for seed yield and its components in Chinese *Cynodon* accessions. *Field Crops Research* 98: 245-252.

⁵ Wu, Y.Q., C.M. Taliaferro, D.L. Martin, J.A. Anderson, and M.P. Anderson. 2007. Genetic variability and relationships for adaptive, morphological, and biomass traits in Chinese bermudagrass accessions. *Crop Sci.* 47: 1985-1994.

⁶ Wu Y.Q., C.M. Taliaferro, G.H. Bai, and M.P. Anderson. 2005. Genetic diversity of *Cynodon transvaalensis* Burt-Davy and its relatedness to hexaploid *C. dactylon* (L.) Pers. as indicated by AFLP markers. *Crop Sci.* 45: 848-853.

⁷ Tan, C.C., Y.Q. Wu, C.M. Taliaferro, G.E. Bell, D.L. Martin, M.W. Smith, and J.Q. Moss. 2014. Selfing and outcrossing fertility in *Cynodon dactylon* (L.) Pers. var. *dactylon* under open pollinating conditions examined by SSR markers. *Crop Sci.* 54: 1832-1837.

genetic base populations serve as sources of elite clonal plants to serve as parents in narrower genetic-base synthetic seeded varieties. Currently, we have four common bermudagrass populations. Elite plants from the populations are selected to form experimental synthetics.

Numerous interspecific crosses between selected common and African parents have been made to produce superior vegetatively-propagated varieties. In determining their range of adaptation, interaction with environmental variables, and turf quality, rigorous and extensive field evaluations of experimental varieties are critical. Initial selection protocols include screening in the greenhouse and single-plot evaluation in the field. Advanced selections and commercially available standards are comprehensively evaluated in tests with appropriate management levels (mowing heights, fertility, water, etc.) and replications. Response variables include but not be limited to sod tensile strength, turf quality, winter survival, divot recovery rate, spring greenup, late-season color retention, spring dead spot, leaf spot and large patch disease incidence, shade resistance and water-use efficiency. Over the last five years (2010-2015) and next four years (2015-2019), as part of two large turfgrass breeding projects funded by the US Department of Agriculture, the OSU program, collaborating with turfgrass breeding programs at Texas A&M University (lead on the first project), University of Florida (lead on the second project), North Carolina State University, and University of Georgia, has generated and evaluated a large number of new hybrids for drought resistance and salinity tolerance at multiple locations. Elite experimental selections with excellent performance and wide adaptation have entered and will enter in the NTEP tests.

The success of the OSU program for seed-propagated bermudagrass improvement in cold tolerance, turf quality and seed yield is exemplified by the performance of Yukon⁸ and Riviera in the 1992, 1997 and 2002 NTEP bermudagrass trials, respectively. Riviera was selected as an official standard for the 2002, 2007, and 2013 NTEP bermudagrass tests⁹. The success of the program for vegetatively propagated cultivars improved in cold tolerance and turf quality is demonstrated by the performance of Patriot⁹, Latitude 36 and NorthBridge^{10,11}. New germplasm has been developed for improving sustainable traits. Seed- and vegetatively propagated experimental cultivars are currently tested in the 2013-18 NTEP bermudagrass test, local and regional trials. Performance and adaptation information of the OSU entries in the tests will be updated in the presentation.

⁸ Taliaferro, C.M., D.L. Martin, J.A. Anderson, M.P. Anderson, G.E. Bell, and A.C. Guenzi. 2003. Registration of 'Yukon' bermudagrass. *Crop Sci.* 43: 1131-1132.

⁹ Taliaferro, C.M., D.L. Martin, J.A. Anderson, M.P. Anderson, and A.C. Guenzi. 2004. Broadening the horizons of turf bermudagrass. *USGA Turfgrass and Environmental Online*. Vol. 3, No. 20: 1-9.

¹⁰ Wu, Y.Q., D.L. Martin, C.M. Taliaferro, J.Q. Moss, N. Walker, and G. Bell. 2010. Latitude 36 Turf Bermudagrass. Oklahoma Agricultural Experiment Station, OK. US Patent No. PP24271 P3. WO Patent No. 2,012,060,871.

¹¹ Wu, Y.Q., D.L. Martin, C.M. Taliaferro, J.Q. Moss, N. Walker, and G. Bell. 2010. NorthBridge Turf Bermudagrass. Oklahoma Agricultural Experiment Station, OK, USA. US Patent No: PP24116. WO Patent No.: 2,012,060,872.

A NON-GM HERBICIDE RESISTANT SYSTEM FOR SEASHORE PASPALUM TURFGRASS

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Introduction

A trait expressing enhanced herbicide resistance has long been desired by breeders and weed scientists as a means to improve weed control options in turfgrasses; however, attempts to commercialize genetically modified (GM) herbicide resistant turfgrasses have thus far been unsuccessful. Naturally occurring resistance to the grass-specific herbicide, sethoxydim, and also to other ACCase inhibiting herbicides has been reported in several weedy grass species as a result of various point mutations at the site of action. Seashore paspalum (*Paspalum vaginatum*, Swartz) is a warm-season turfgrass that has numerous morphological characteristics that make it desirable as a turfgrass. Increased interest in its use as a turf is most often related to its tolerance to salt and other abiotic stresses¹. Seashore paspalum is generally adapted to the same regions of the world as bermudagrass (*Cynodon dactylon* (L.) Pers.), and many existing golf courses around the world have replaced bermudagrass with paspalum². The most serious weed concern in paspalum plantings is bermudagrass, because it is highly competitive and difficult to eradicate once established. Invasion by bermudagrass and other weedy grasses can greatly reduce the aesthetic value and quality of paspalum turf. Currently there are no herbicides available that selectively control bermudagrass in seashore paspalum. Development of herbicide-resistant paspalum could provide an effective means of managing bermudagrass in paspalum and allow golf course and sporting venues to transition from bermudagrass to seashore paspalum.

Methods

Research was initiated to develop a novel source of resistance to sethoxydim in seashore paspalum using *in vitro* selection and regeneration protocols to select for naturally occurring mutations conferring herbicide resistance³. Callus was induced from immature inflorescences then plated on callus induction medium containing 10 μ M sethoxydim for selection. Green plants were regenerated from resistant callus, the Ile to Leu mutation at aa position 1781 documented, and expression of herbicide resistance confirmed using a series of greenhouse dose response studies. Levels of cross resistance to other ACCase inhibiting herbicides registered for use on turfgrass were determined for the Leu 1781 mutant. Initial herbicide resistant mutants generated from tissue culture contained many additional mutations that reduced fitness and rendered most plants completely sterile. The most vigorous and partially fertile herbicide resistant line, SR 31.15, was hand pollinated with pollen from 'Sealsle 1' and all resulting embryos rescued 10 to 14 days following pollination. Six vigorous and fertile herbicide resistant lines heterozygous for the Leu 1781 mutation were obtained. These were clonally increased and used in replicated field trials initiated in 2014 under high levels of bermudagrass pressure. Lines tested included the sethoxydim resistant lines SR 31.15-1, -4, -5, -7, -14, and -15; and susceptible controls UGA 1743 and Mauna Key. Each line was evaluated using a split plot design for tolerance to the ACCase inhibiting herbicides sethoxydim, clethodim, and fenoxypop at monthly X and 3X application rates. Data was collected on bermudagrass and paspalum coverage and injury following the monthly herbicide applications.

Results and Discussion

Results of a sethoxydim dose response experiment conducted on the tissue culture regenerated cell line, SR 11, are shown in Fig. 1. In repeated greenhouse experiments, the SR 11 mutant line heterozygous for the Leu 1781 mutation expressed high levels of resistance (less than 6% injury) to sethoxydim even at rates as high as 3200 grams a.i. ha⁻¹, a rate 15 times the labeled rate for centipedegrass. In contrast, the parental line, Mauna Kea had injury scores of >50% at rates of 200 g a.i. ha⁻¹ or higher.

¹ Duncan, R.R. and R.N. Carrow. 2000. *Seashore Paspalum-The Environmental Turfgrass*. John Wiley and Sons Inc., Hoboken, NJ, #p

² Raymer, P.L., S.K. Braman, L.L. Burpee, R.N. Carrow, Z. Chen, and T.R. Murphy. 2008. Seashore Paspalum: Breeding a turfgrass for the future. USGA Green Section Record. Jan-Feb:22-26.

³ Heckart, D.L., W.A. Parrott, and P.L. Raymer. 2010. Obtaining sethoxydim resistance in seashore paspalum. *Crop Sci.* 50:2632-2640.

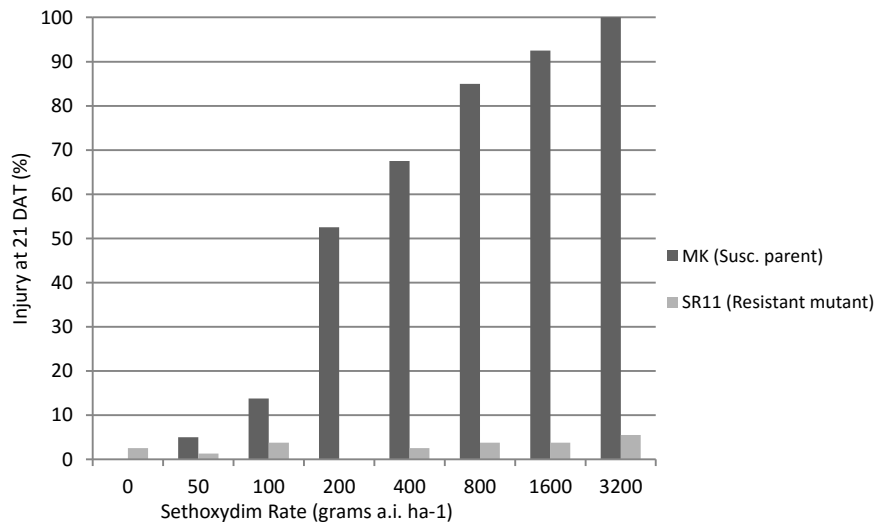


Figure 1. Whole-plant response of resistant SR11 mutant line and MK susceptible parent to a broad range of sethoxydim rates.

Cross resistance dose response studies indicated that the mutant line SR11 showed significantly less injury than the susceptible checks at all fenoxaprop and fluazifop rates above 50 g a.i. ha⁻¹. SR11 was also more tolerant to clethodim than the susceptible checks but only at rates up to 200 g a.i. ha⁻¹. In the field study conducted under high levels of bermudagrass pressure, paspalum lines heterozygous for the Leu 1781 mutation were tolerant to 3x recommended rates of both fenoxypop and sethoxydim. Tolerance to clethodim in the heterozygous Leu 1781 mutation was not sufficient to allow for its use as an herbicide resistant system. Repeated applications of fenoxypop at X and 3X and sethoxydim at 3X the recommended rate were highly effective in controlling bermudagrass. In summary, seashore paspalum genotypes with excellent vigor and tolerance to both sethoxydim and fenoxypop were identified and successfully field tested. This novel germplasm offers great hope for the development of a non-genetically modified herbicide resistance system with the ability to manage bermudagrass and other problematic grassy weeds in seashore paspalum.

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INTRODUCED BROWN PATCH DISEASE RESISTANCE IN TRANSGENIC TALL FESCUE

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Introduction

Tall fescue (*Festuca arundinacea* Schreb.) is an important turfgrass widely used for home lawns and on golf courses. The most serious and frequently occurring disease of tall fescue is brown patch, caused by a basidiomycete fungus, *Rhizoctonia solani*. To breed brown patch resistant tall fescue cultivars is a challenge to the breeders. This research used two transgenic approaches to introduce transgenes into tall fescue and obtained resistance to brown patch disease: expression of a shrimp *Penaeidin 4-1* (*Pen4-1*) gene, or by Host Induced Gene Silencing (HIGS).

Penaeidin 4-1 is a peptide of 47 amino acid residues and belongs to a large group of antimicrobial peptides (AMPs) produced by animals and plants as part of their innate immunity to defend themselves against a wide range of microbes. Expression of the *Pen4-1* gene in creeping bentgrass confers resistance to brown patch caused by *R. solani* subgroup AG2-2(IIIB) and dollar spot caused by *Sclerotinia homoeocarpa*¹. However, AG2-2(IIIB) does not infect tall fescue. The subgroup that causes brown patch in tall fescue is AG1-IA.

Host Induced Gene Silencing, or HIGS, is a relatively new technology for resistance to pest, including fungi, in plants. Successful resistance to several plant fungal diseases by HIGS has been reported². HIGS expresses, in host plants, RNAi constructs of the “essential” genes of a pathogen. The siRNAs somehow (mechanisms remains to be elucidated) could penetrate into fungal cells, confer post-transcriptional silencing to those essential genes and thus suppress fungal growth.

Methods

Both *Pen4-1* gene and the HIGS RNAi constructs were under control of the rice *rub13* promoter and introduced into tall fescue cv. “Coronado” by Agrobacterium-mediated transformation, and the transgenic plants were selected by hyg B. Fungal inoculation was performed in polyethylene box with high humidity. Lesion data were collected four days later. At least three replicates were performed and the data were statistically analyzed.

Results and Discussion

Four out of five *Pen4-1* transgenic plants had significantly smaller lesions ($p < 0.05$) with two being highly resistant ($p < 0.001$) after inoculation with AG1-IA (Fig. 1). Results from our experiments, together with Zhou et al. report¹, indicate that *Pen4-1* has good efficacy and is resistant to various subgroups of *R. solani*, and likely could confer resistance to a wide spectrum of fungal diseases in transgenic plants.

Table 1. Brown patch resistance by HIGS

E	Plant	Lesion%	Small RNA	Resistance significance P value
	CK	56.8±9.4	/	/
	NR1	10.7±9.2	yes	0.0008
	NR3	35.2±4.9	yes	0.0661
	NR12	15.0±5.7	yes	0.0017
	NR14	17.3±13.0	n.t.	0.0026
	MC12	19.35±9.8	yes	0.0431
	MC15	11.0±4.43	yes	0.0188

¹ Zhou, M., Q. Hu, Z. Li, D. Li, C.F. Chen and H. Luo. 2011. Expression of a novel antimicrobial peptide Penaeidin4-1 in creeping bentgrass (*Agrostis stolonifera* L.) enhances plant fungal disease resistance. *PLoS One*, 6(9), e24677.

² Koch A. and K.-H. Kogel. 2014. New winds in the sails: improving the agronomic value of crop plants through RNAi-mediated gene silencing. *Plant Biotechnol. J.* 12: 821-831.

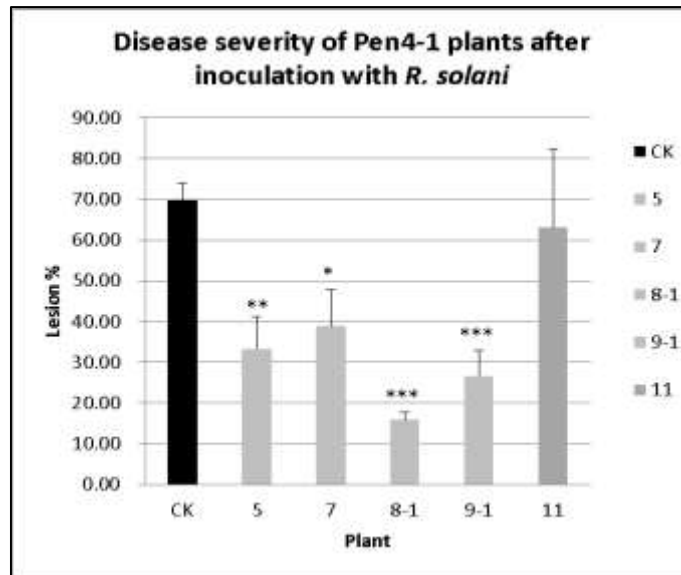


Figure 1. *Pen4-1* resistance in tall fescue

In HIGS approach, four essential genes of *R. solani* were identified by *in vitro* fungal growth assay when incubated with dsRNAs of the genes. Four RNAi constructs were made, each being a fusion gene of the original or modified DNA fragments of two essential genes. Among 12 plants showing accumulation of siRNAs, six displayed significantly improved resistance against *R. solani* (Table 1) whereas plants without siRNA accumulation did not show any resistance, indicating that the observed resistance was caused by the siRNA produced using HIGS approach. *R. solani* is a tough pathogen to cope with. However, our results suggest that brown patch disease could be controlled by biotechnology approaches. In addition, to our knowledge, this is the first case that RNAi constructs of pathogen genes introduced into a host plant confers resistance against a necrotrophic fungus. Possible mechanisms on how HIGS works will be discussed. The manuscripts have been accepted for publication^{3,4}.

³ Zhou B. A. Bailey, C.L. Niblett, and R. Qu. 2016a. Control of brown patch (*Rhizoctonia solani*) in tall fescue (*Festuca arundinacea* Schreb.) by host induced gene silencing. Plant Cell Rep. DOI 10.1007/s00299-015-1921-7

⁴ Zhou, B., H. Luo and R. Qu. 2016b. Expression of the shrimp antimicrobial peptide Penaeidin 4-1 confers resistance against brown patch disease in tall fescue. Plant Cell Tiss. Org. Cult. 2016. DOI 10.1007/s11240-016-0963-z

ASSOCIATIONS BETWEEN PLOIDY LEVEL AND GENETIC DIVERSITY OF BERMUDAGRASS AS ASSESSED BY IPBS RETROTRANSPOSON MARKERS

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Introduction

A large genetic variation is present for common bermudagrass [*Cynodon dactylon* (L.) Pers.] in the eastern Mediterranean, reported to be within the center of diversity for the species¹. *Cynodon*, considered to be an autoploid², includes all the ploidy series from diploids to hexaploids¹ with base chromosome number of (x) 9³. The region from Turkey to West Pakistan is recognized as the area of evolutionary activity for this species⁴. Bermudagrass is widely used in tropical and subtropical regions as a warm season turfgrass. Although number of protein coding genes do not vary much among plant species, their genome sizes differ greatly. Plant genomes commonly expand due to the copy-and-paste proliferation of a few long terminal repeat retrotransposons (LTRs)⁵. LTRs retrotranspose via an mRNA intermediate in a “copy-and-paste” process leading to extremely high copy numbers in the genome. Kalendar et al⁶ described the PCR-based iPBS marker system. The method is based on the virtually universal presence of a tRNA complement as a reverse transcriptase primer binding site (PBS) in LTR retrotransposons. Maize genome comprises of 85% repeat sequence, most of which constitute transposable elements⁷. In sorghum and rice, with around 500 Mbp genomes, transposable element fraction is about 30%⁸. As dispersed and ubiquitous transposable elements, LTRs are well suited as molecular markers.

Phylogenetic analysis can help our understanding of evolution, history of polyploidization, and endemism, leading to current distributions. Understanding of associations among ploidy level and genetic diversity of *Cynodon* accessions may enhance our understanding of the evolutionary history of this warm season grass species. The objective of this study was to investigate associations between ploidy level and genetic diversity as assessed by iPBS retrotransposon marker, (2) determine whether retrotransposon gene family followed a similar evolution, and (3) correlate between retrotransposon marker and four nuclear molecular marker systems (SRAP, ISSR, POGP and RAPD) for *Cynodon* accessions' genetic analyses.

Materials, Methods and Results

Of 44 *Cynodon* accessions, 40 represent diploids, triploids, tetraploids, pentaploids and hexaploids collected in Turkey and four were triploids obtained from controlled crosses. They were all genotyped in this study using iPBS retrotransposon markers. The 14 iPBS primers generated 117 polymorphic markers. ISSR, RAPD and POGP marker data were kindly provided by Gulsen et al. (1). The similarity matrix based on simple matching was used to construct a dendrogram using the unweighted pair-group method arithmetic average (UPGMA) to determine genetic relationships among the accessions studied. This clustered the 44 genotypes into two subclusters. The first group included 3 of 6 diploids, 2 of 4 triploids, 7 of 13 tetraploids, 9 of 10 pentaploids and 2 of 7 hexaploids, which failed to indicate ploidy-based clustering except for pentaploids and hexaploids. However, iPBS type retrotransposon markers very closely clustered the four hybrids (T6-D2, T4-A35, T4G10, T1-A1). from the same parents, pointing the efficiency of the analysis. Neighbor-joining (NJ) analysis was also performed for estimating phylogenetic trees and based on the idea of parsimony yielding relatively short

¹ Gulsen, O., S. Sever Mutlu, N. Mutlu, M. Tuna, O. Karaguzel, R.C. Shearman, T.P. Riordan and T.M. Heng-Moss. 2009: Polyploidy creates higher diversity among *Cynodon* accessions as assessed by molecular markers. *Theor. Appl. Genet.* 118, 1309–1319.

² Zeven, A.C. 1979: Polyploidy and domestication: The origin and survival of polyploids in cytotype mixtures. In: LEWIS, W.H. (ed): Polyploidy, biological relevance. Plenum Press, New York, pp. 385–408.

³ Wu, Y.Q., C.M. Taliaferro, G.H. Bai, D.L. Martin, J.A. Anderson, M.P. Anderson and R.M. Edwards. 2006: Genetic analyses of Chinese *Cynodon* accessions by flow cytometry and AFLP markers. *Crop Sci.* 46, 917–926.

⁴ Wu, Y., 2011. *Cynodon*. In: Kole, C. (Ed.), *Wild Crop Relatives: Genomic and Breeding Resources. Millets and Grasses*, Springer-Verlag, Berlin, pp. 53–71.

⁵ Lee, S.I., and Kim, N.S. 2014. Transposable elements and genome size variations in Plants. *Genomics Inform* 12(3):87-97.

⁶ Kalendar, R., K. Antonius, P. Smykal, A.H. Schulman. 2010 iPBS: a universal method for DNA fingerprinting and retrotransposon isolation *Theor. Appl. Genet.*, 121 (2010), pp. 1419–1430

⁷ Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, Pasternak S, et al. The B73 maize genome: complexity, diversity, and dynamics. *Science* 2009;326:1112-1115.

⁸ Michael TP. Plant genome size variation: bloating and purging DNA. *Brief Funct Genomics* 2014;13:308-317

estimated evolutionary trees. Similarly, this failed to discriminate ploidy series. However, unlike the UPGMA analysis, the NJ tree scattered four hybrids little more distantly from each other and indicated no grouping pattern in general. In addition, a two-way Mantel test⁹ was used to estimate Mantel correlation coefficients between each pair of similarity matrices of iPBS and four previously evaluated markers (SRAP, ISSR, RAPD and POGP markers) by using MXCOMP option and 10,000 permutations. This computed the product-moment correlation, r , and the Mantel test statistic, Z , to measure the degree of relationship between the two matrices. This revealed that the similarity matrix of iPBS markers poorly associated ($r < 0.35$) with that of the four marker systems. This implied that iPBS markers significantly evolved in different ways from the four marker systems listed above. In our previous study, chloroplast (cp) RFLP markers efficiently distinguished diploids from the rest of ploidy levels, but failing for the other ploidy series. Overall, evaluated five nuclear genome based marker systems (iPBS, SRAP, ISSR, RAPD and POGP) failed to make ploidy based grouping among the 44 bermudagrasses with ploidy series from diploid to hexaploid, whereas chloroplast genome based cpDNA RFLP marker distinguished diploids from the rest. In conclusion, this study indicated monophyletic origin and continued diversification after polyploidization among the bermudagrasses, and iPBS markers evolved in different path from the other marker systems.

⁹ Mantel NA (1967) The detection of disease clustering and a generalized regression approach. *Cancer Res* 27:209–220.

GAMMA-RAY IRRADIATION IMPROVES TURFGRASS CHARACTERISTICS OF ST. AUGUSTINEGRASS

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Introduction

St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze, is a warm-season turfgrass with medium to superior shade tolerance, a valuable trait for use in lawns, particularly in smaller residential landscapes and other green spaces where trees are dominant. It is well adapted to a wider range of soil conditions than other warm-season turfgrasses¹ (1). However, long internodes, very coarse leaf and stolon texture are undesirable in home lawns and public spaces. Gamma-ray (γ) irradiation has successfully been used to induce useful variations for various morphological traits in turfgrass breeding^{2,3,4,5,6}. Each of these mutant turf cultivars possessed finer texture and superior turfgrass quality than their parental genotypes. Gamma-rays irradiation has also been used to develop superior St. Augustinegrass cultivars⁷. The most frequent mutant character from the irradiated plants is dwarfism^{8,4,9,6}. Many semi-dwarf type mutant lines with reduced internode and leaf blade length were developed by gamma irradiation of 'Raleigh' St. Augustinegrass⁷.

The objective of the present study was to induce dwarf/semi-dwarf phenotypes from two St. Augustine genotypes. The genotype 'S1' was irradiated with 50, 100, 150 and 300 Gy; and 'S2' with 20, 40, 80 and 120 Gy using a ⁶⁰Co source.

Methods

Stolons of genotypes were collected from the mature field plots at Akdeniz University, Antalya, Turkey and attached soil was washed off with tap water. The stolons were cut into single node cuttings, sealed in plastic bags. A total of 4301 cuttings irradiated with a dose rate of approximately 35 Gy/h at 100 cm source-to-sample distance. The control cuttings were not irradiated, but placed in petri dishes and kept in 5°C to maintain moisture until planting. Each treatment consisted of 260 and 780 single node cuttings for S1 and S2 genotypes, respectively. After irradiation, the cuttings were immediately planted in the mixture of peat, vermiculite and perlite (3:1:1, v/v/v) for regeneration in the greenhouse at 25 ± 5°C under natural light conditions. Survival of the cuttings was scored 8 and 12 weeks after irradiation treatment. Then, 18 weeks after irradiation treatment, preselection based on morphological differences was made where 180 plants were transplanted into the plastic pots for further observation of growth and turf characteristics. Among them, 15 mutant lines exhibiting distinct semi-dwarf characteristics with finer leaf texture, shorter canopy height than parental plants were chosen eight weeks after transplantation, and clonally propagated for detailed evaluation. Experimental design was a randomized complete block with 4 replications. Plants were allowed to grow for 3 months in a greenhouse under natural light and at temperature of 25-35°C. During the establishment period, plants were fertilized with 2.5 g N m⁻² with 15N-6.6P-12.5K, a complex fertilizer, biweekly and irrigated daily to prevent visual wilt symptoms.

Survival rate was calculated as a percentage of cuttings with re-growth divided by the total number. The data-points obtained in this way were fitted to the probit function: $probit(p) = a + b\sqrt{2}erf^{-1}(2p - 1)$ where a is the y -intercept, b is slope and p is the normalized survival probability. The fit was performed by using the ROOT

¹ Busey, P., M.D. Casler, and R.R. Duncan. 2003. St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze. In: Turfgrass biology, genetics, and breeding (M.D. Casler, R.R. Duncan eds), p 309-330. John Wiley and Sons, Inc, New Jersey.

² Busey, P. 1980. Gamma ray dosage and mutation breeding in St. Augustinegrass. *Crop Science*, 20: 181-184.

³ Dickens, R., W.J. Johnston, and R.L. Haaland. 1981. Variability observed in centipede grass grown from ⁶⁰Co irradiated seed. *Agronomy Journal*, 73:674-676.

⁴ Burton, G.W. 1985. Registration of Tifway II bermudagrass. *Crop Science*, 25:364.

⁵ van Harten, A.M. 1998. Mutation Breeding: theory and practical applications. Cambridge Univ. Press, Cambridge, UK.

⁶ Hanna, W.W. and J.E. Elsner. 1999. Registration of TifEagle bermudagrass. *Crop Science*, 39:1258.

⁷ Li, R., A.H. Bruneau, and R. Qu. 2010. Morphological mutants of St. Augustinegrass induced by gamma ray irradiation. *Plant Breeding*, 129:412-416.

⁸ Powell, J.B., G.W. Burton, and J.R. Young. 1974. Mutation induced in vegetatively propagated turf bermudagrass by gamma radiation. *Crop Science*, 14(2):327-333.

⁹ Hanna, W.W., R.N. Carrow, and A.J. Powell. 1997. Registration of 'Tift 94' bermudagrass. *Crop Science*, 37:1012.

package¹⁰. In addition an inverse fit was performed as a consistency check and for easy display of data. Measurements for morphological characterization included canopy heights, leaf length and width, seed head density, spike length, number of spikelets per inflorescence, peduncle length, stolon length, stolon internode length and diameter, stolon leaf length and width, number of stolons per pot, relative chlorophyll content (Field Scout CM 1000; Spectrum Technologies, Inc., Plainfield, IL), and turfgrass color by CR-400 chroma meter (Konica Minolta Sensing, Inc., Osaka, Japan). Treatment differences were tested using analysis of variance procedures with PROC GLM (SAS release 8.0; SAS Institute, Cary, NC). Means were separated using Fisher's protected least significant difference ($P < 0.05$) procedure.

Results and Discussion

The LD₅₀ for 'S1' and 'S2' genotypes were 95 and 103Gy, respectively (Fig 1). The linear reduction of survival rate with increasing gamma-rays was highly correlated ($r^2 = 0.96$ and 0.78). Similar results were reported with other species of Poaceae family^{2,11,12,13}. The 15 morphological mutants (0.35 % of the irradiated plants) with a semi-dwarfed growth habit were identified among 4301 node cuttings. The useful mutant rate was 0.3% in bermudagrass and St. Augustinegrass^{14,7}. The mutant lines exhibited up to 25% shorter canopy height, finer leaf (up to 29% reduction in length and 16% in width) texture than parental genotypes. The internode length and diameter were reduced by 33% and 19% in selected lines. The stolon length was up to 36% shorter than parental genotypes. The reduction in number of stolon ranged from 1% to 65%. In conclusion, improved mutants with finer texture were identified. Mutant lines developed in this study offer a great potential to develop improved St. Augustinegrass cultivars for landscaping.

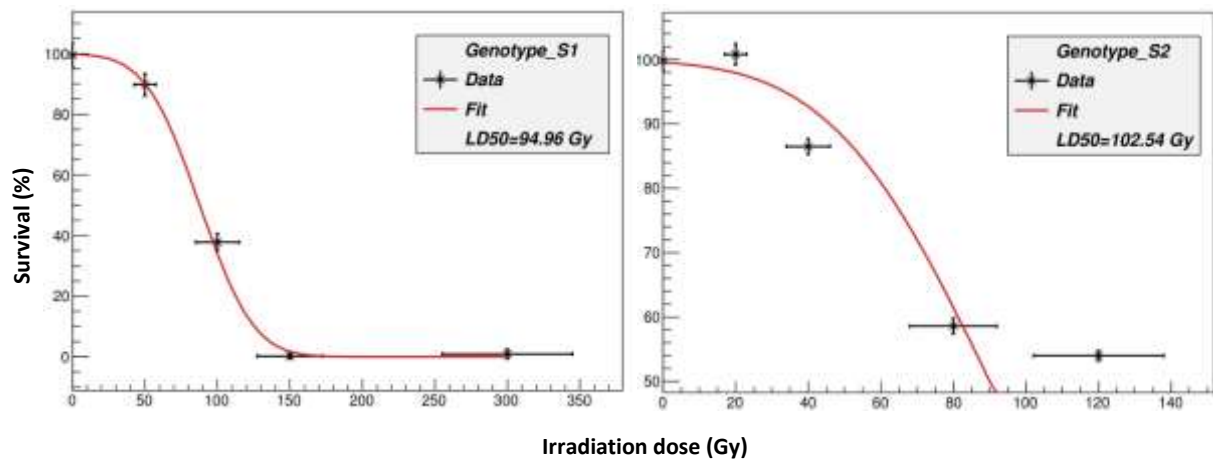


Fig. 1. Gamma ray dosage effects on survival of two St. Augustine genotypes

¹⁰ Brun R. and F. Rademakers. 1997. ROOT - An object oriented data analysis framework. Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment, 389:81-86.

¹¹ Krishna, G., G. Shivashankar, and J. Nath. 1984. Mutagenic response of rhodes grass (*Chloris gayana* Kunth.) to gamma rays. Environmental and Experimental Botany, 24(2):197-205.

¹² Hase, Y., K. Shimono, M. Inoue, A. Tanaka, and H. Watanabe 1999. Biological effects of ion beams in *Nicotiana tabacum* L. Radiation and Environmental Biophysics, 38:111-115.

¹³ Zaka, R., C.M. Vandecasteele, and M.T. Misset. 2002. Effect of low chronic doses of ionizing radiation on antioxidant enzymes and G6PDH activities in *Stipa capillata* (Poaceae). Journal of Experimental Botany, 53(376):1979-1987.

¹⁴ Sever Mutlu, S., H. Djapo, S.F. Ozmen, C. Selim, and N. Tuncel. 2015. Gamma-ray Irradiation Induces Useful Morphological Variation in Bermudagrass. Not Bot Horti Agrobi, 43(2):515-520.

OVERCOMING APOMIXIS TO DEVELOP TURF-TYPE BAHIAGRASS

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Introduction

The development of a turf-type bahiagrass (*Paspalum notatum* Flügge) with enhanced aesthetic value could have a remarkable impact in the billion-dollar turf industry in the southeastern US. Two grass species dominate the sod industry in Florida, St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] and bahiagrass¹. Bahiagrass requires less frequent irrigation due to its capacity to avoid water stress² and the current recommended nitrogen (N) rates in Florida are twice for St. Augustinegrass compared to bahiagrass³. While there are economic and ecological advantages for using bahiagrass in turf applications, currently available cultivars lack improved turf traits. Breeding strategies differ for diploid and tetraploid bahiagrass due to their associated reproductive modes. At the diploid level, traditional breeding methods can be used because diploids reproduce sexually⁴. However, apomixis⁵ constitutes a barrier for traditional plant breeding in tetraploids. Mutagenic breeding approaches have been employed in bahiagrass with limited success^{6,7}. Improvements in leaf texture, color, plant height, flowering behavior and seed production are warranted for the adoption of bahiagrass for use in higher value landscapes. The goals of this study were: i) identify genotypes with improved turf attributes in the University of Florida bahiagrass breeding population, and ii) determine the reproductive mode and seed production of these selected genotypes.

Methods

The population under study was composed of: i) commercial cultivars: Pensacola, Argentine and Wilmington, ii) 40 tetraploid mutant plants obtained after treating Argentine and Wilmington with different mutagenic compounds, iii) 23 wild-type accessions, and iv) four tetraploid hybrids. Each genotype was vegetatively propagated and planted using 24 plugs on 30 cm centers at the Plant Science Research and Education Unit, Citra, FL and at the West Florida Research & Education Center, Jay, FL. The plots were planted in the Spring 2012 in a randomized complete block design with three replications. Plants were irrigated at establishment to promote adequate root growth and throughout the study fertilization was applied at the recommended rate for bahiagrass³. Monthly ratings were taken from August 2012 until July 2014 at Citra, and in the spring and summer 2013 at Jay. Visual ratings were collected for turf color and turf quality using a one to nine scale as described by the National Turfgrass Evaluation Program (NTEP) guidelines; seed head density was recorded using a 1 to 5 scale (1=no flowers; 2= 1-5; 3= 5-10; 4=10-30; 5= more than 30 seed heads per plot) and flowering period was documented as the number of months that each genotype flowered. Foliage and seed head height were measured with a meter stick placed in the center of each plot (average of three leaves and seed heads), as well as leaf texture and length were measured (three leaf blades per plot) in July 2013 and 2014. Thirty genotypes were selected for evaluation of their mode of reproduction and seed production. Reproductive modes were determined by cytoembryological observations of ovaries collected at anthesis in July of 2012 at Citra and in late August 2013 in Jay. Seed heads were fixed in FAA (18 Ethanol 70%: 1 Formaldehyde 37%: 1 glacial acetic acid), at least 20 pistils per genotype were dissected out of the spikelets, cleared with alcohol and methyl salicylate solutions and then examined under a differential interference contrast microscope. Ovules bearing a single *Polygonum* type embryo sac were classified as sexual (SES) (Figure

¹ Satterthwaite, L.N., A.W. Hodges, J.J. Haydu, and J.L. Cisar. 2009. An agronomic and economic profile of Florida's sod industry in 2007. Univ. of Florida, IFAS, Florida Agric. Exp. Stn., Florida Coop. Ext. Serv., Gainesville, FL.

² McCarty, L. B., and J. L. Cisar. 1995. Bahiagrass for Florida lawns. In L. B. McCarty, K. C. Ruppert, and R. J. Black, eds. Florida Lawn Handbook. Gainesville, FL: Florida Cooperative Extension Service.

³ Trenholm, L.E. 2013. Urban Turf Fertilizer Rule for Home Lawn Fertilization. [Online]. Available at <https://edis.ifas.ufl.edu/ep353>

⁴ Burton, G.W. 1955. Breeding Pensacola bahiagrass, *Paspalum notatum*: I. method of reproduction. *Agron. J.* 47:311-314.

⁵ Burton, G.W. 1948. The method of reproduction in common Bahiagrass, *Paspalum notatum*. *J. Am. Soc. Agron.* 40:443-452.

⁶ Burton, G.W. 1974. Radiation breeding of warm season forage and turf grasses. In: Polyploidy and Induced Mutations in Plant Breeding; Bari, 1972. IAEA, Vienna, pp. 35-41.

⁷ Kannan, B., N.H. Davila-Olivas, P. Lomba, and F. Altpeter. 2015. In vitro chemical mutagenesis improves the turf quality of bahiagrass. *Plant Cell, Tissue and Organ Culture* 120:551-561.

1-A); spikelets with ovules bearing both sexual and aposporous embryo sacs were classified as facultative (FES) (Figure 1-B) and ovules having multiple embryo sacs were classified as aposporous (AES) (Figure 1-C). Genotypes were classified as sexual, facultative and obligate apomictic based on the embryo sacs observed. The same 30 genotypes were tested for seed production. Mature seed heads were harvested and percent seed set, germination and reproductive efficiency were calculated. Data were analyzed using linear mixed models using the statistical software ASReml v.3. Genotypes were ranked based on genotypic values and clonal repeatability (broad sense heritability) was estimated using $H^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_m^2 + \sigma_e^2)$.

Results and Discussion

The identification of wild-type bahiagrass and the use of mutagenic treatments produced large morphological variation and clonal repeatability (cf. broad sense heritability) (H^2) ranged from 0.27 to 0.90 (Table 1). Flowering period and seed head density were extremely reduced in some mutants, which also exhibited dark green color, finer texture and reduced height, significantly improving the aesthetic value of bahiagrass. Because foliage and seed head height were also highly reduced, the dwarf genotypes may not need to be mowed as frequently, reducing the cost of maintenance. Some wild-types had finer texture and low canopy height, but prolific flowering. The high H^2 observed for some traits (Table 1) indicated that large genetic effects contributed to the observed phenotypes, thus selection for improved turf attributes could be successfully performed in this population.

Table 1. Clonal repeatability (H^2) for eight turf traits for data taken at Citra during two years, and H^2 and type B correlation ($rg^2_{B(g)}$) for a multi-location analysis in seventy bahiagrass genotypes.

Trait	Citra - 2 years	Citra and Jay combined	
	H^2 (SE ¹)	H^2 (SE)	$rg^2_{B(g)}$ (SE)
Turf color	0.27 (0.04)	0.33 (0.05)	0.89 (0.09)
Turf quality	0.31 (0.04)	0.37 (0.06)	0.87 (0.08)
Foliage height	0.71 (0.04)	-	-
Leaf length	0.76 (0.04)	-	-
Seed head density	0.79 (0.03)	0.63 (0.05)	0.91 (0.05)
Seed head height	0.83 (0.03)	-	-
Leaf width	0.85 (0.02)	-	-
Flowering period	0.90 (0.02)	-	-

¹SE=standard error

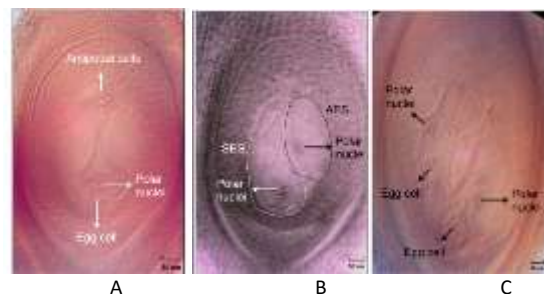


Figure 1. Embryo sac observations in bahiagrass: (A) pistil from diploid bahiagrass; (B) ovule from a diploid plant bearing a single *Polygonum* type meiotic embryo sac (SES); (C) tetraploid ovule bearing a meiotic (white) (SES) and an aposporous (black) embryo sacs (AES); (D) ovule from a tetraploid plant bearing multiple aposporous embryo sacs (AES).

Diploid bahiagrass reproduced sexually and tetraploids by obligate or facultative apomixis (Figure 1). Some wild-types showed higher potential for sexual reproduction towards the end of the flowering season, exhibiting the same behavior as previous reports⁸. This dynamic reproductive mode provides an opportunity to incorporate traditional breeding methods into our breeding program by crossing the improved facultative apomicts with the outstanding obligate apomicts. In addition, the higher apomictic potential during peak flowering can be exploited during seed production to produce clonal seed for cultivar propagation. While mutant plants exhibited large morphological variation, the random mutations did not affect their reproductive mode and seed production. The improved turf attributes exhibited by some mutant genotypes are novel traits in bahiagrass and they represent very important characteristics to increase the use of this species in higher value landscapes. Mutagenesis proved to be a useful technique to create variation for essential turfgrass traits in bahiagrass.

⁸ Rios, E., A. Blount, K. Kenworthy, C. Acuña and K. Quesenberry. 2013. Seasonal expression of apospory in bahiagrass. *Tropical Grasslands-Forrajes Tropicales*: Vol. 1 No. 1

GENETIC, ADAPTIVE, MORPHOLOGICAL AND DROUGHT RESISTANCE VARIABILITIES IN EASTERN MEDITERRANEAN ACCESSIONS

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Introduction

Bermudagrass [*C. dactylon* (L.) Pers.] is a long-lived perennial sod-forming warm-season grass species used for turf, pasture, forage, soil stabilization, and remediation of contaminated soils^{1,2}. It is the most widely distributed and genetically variable species in the genus. The genus *Cynodon* (L.) Rich. contains nine species and ten varieties³ within the family Gramineae (Poaceae)⁴. Bermudagrass is widely distributed in the warmer temperate and tropical regions of the world. It has a widespread distribution and is referred to as “common” bermudagrass⁵. The base chromosome number of the species is $X = 9$, including all ploidy series from diploids to hexaploids⁶. *Cynodon* is considered an autopolyploid⁷. Intra- and inter-ploidy level crosses can produce viable seeds². Variety *dactylon* is one of the taxa of predominant turf importance and contains enormously variable plant types ranging from the small, fine-textured plants used as turf to the large robust plants with high biomass production capability, suitable for cultivated pasture². The center of origin may extend from West Pakistan to Turkey^{8,6}. Eastern Mediterranean region inhabits all known ploidy levels of *Cynodon dactylon* (L.) Pers⁶. The large genetic variation of *Cynodon* accessions identified in the region may have significant contribution to bermudagrass improvements. However, information is lacking on genetic variation on drought resistance, adaptive and morphological characters in native Turkish accessions. Thus, the objectives of the study were to quantify genetic variability for morphological, adaptive, and drought resistance related traits; and characterize relationship among the traits.

Material and Methods

The 95 clonal accessions originated from Mediterranean coastal region of Turkey and belonged to five ploidy levels were used in the study. The genotypes were selected from 182 *Cynodon* accessions collected from the Mediterranean coastal region of Turkey in 2006 and described by Gulsen et al.⁶. Selection was made based on general morphological turfgrass characteristics (leaf texture, color, growth habit, shoot density etc.) and ploidy levels. The selected accessions comprised 66 tetraploids ($2n = 4x = 36$), 17 pentaploids ($2n = 5x = 45$), six hexaploids ($2n = 6x = 54$), three triploids ($2n = 3x = 27$) and three diploids ($2n = 2x = 18$). The accessions along with five commercial bermudagrass cultivars as controls (Tifway, Blackjack, NuMex Sahara, Riviera, Contessa), were evaluated for drought resistance, morphological and adaptive traits in two independent field experiments in Mersin, Turkey during 2007-2009. Greenhouse grown clonal plants of each accession and bermudagrass cultivars were transplanted to field plots on the Alata Agricultural Research Station, Erdemli, Mersin (lat. 36°37'N, long. 34°42'E). The experimental designs were randomized complete block with three replications. Plot size was 1.0 x 1.0 m with 0.5 m alleys between plots. Each plot was established by planting five 5.0-cm-diameter evenly distributed plugs. Drought resistance was evaluated by ceasing irrigation for 45 d from June 15th to 1st of August in 2008-2009. At the beginning of drought stress treatment, grasses were at 100% green cover and plots were watered to saturation. Percentage of leaf firing and quality were recorded weekly

¹ Burton G.W. 1947. Breeding bermudagrass for the southeastern United States. *Agron J*, 39:551–569.

² Taliaferro, C.M. 2003. Bermudagrass (*Cynodon* (L.) Rich). In: *Turfgrass biology, genetics, and breeding* (M.D. Casler, R.R. Duncan eds), p 235–257. John Wiley and Sons, Inc, New Jersey.

³ Harlan, J.R., J.M.J. De Wet, W.W. Huffine, and J.R. Deakin. 1970. A guide to the species of *Cynodon* (*Gramineae*). Bull B-673. Oklahoma Agric Exp Stat, Stillwater.

⁴ Renvoize, S.A., and W.D. Clayton. 1992. Classification and evolution of the grasses. In: *Grass evolution and domestication*, p 3–37. Cambridge University Press, New York.

⁵ Kenworthy, K.E., D.L. Martin, and C.M. Taliaferro 2007. Growth habit determination of genotypes of African bermudagrass. *HortScience*, 42:1513–1516.

⁶ Gulsen, O., S. Sever Mutlu, N. Mutlu, M. Tuna, O. Karaguzel, R.C. Shearman, T.P. Riordan, and T.M. Heng-Moss 2009. Polyploidy creates higher diversity among *Cynodon* accessions as assessed by molecular markers. *Theor. Appl. Genet.*, 118: 1309–1319.

⁷ Zeven, A.C. 1979. Polyploidy and domestication: The origin and survival of polyploids in cytotype mixtures. In: *Polyploidy, biological relevance* (W.H. Lewis ed.), p 385–408. Plenum Press, New York.

⁸ Harlan, J.R., and J.M.J. De Wet 1969. Sources of variation in *Cynodon dactylon* (L.) Pers. *Crop Sci.*, 9:774–778.

throughout drought stress. Subsequently, to assess post drought response of the turfgrasses, the plots were saturated with water and normal watering frequency was maintained thereafter for the turf recovery phase until dormancy in the late fall. Establishment rate, turfgrass quality, spring greenup, fall/winter color retention, genetic color, growth habit were assessed by visual ratings of field plots. Number of stolon per plot, stolon length, internode length and diameter, leaf blade length and width, shoot density, leaf pubescence, the number racemes per inflorescence, raceme length, the number of inflorescence, plant height with seed head), and turfgrass color by CR-400 Chroma meter (Konica Minolta Sensing, Inc., Osaka, Japan) were measured in the field. Plot means were used for statistical analyses for traits with multiple measurements. Treatment differences were tested using analysis of variance procedures with PROC GLM (SAS release 8.0; SAS Institute, Cary, NC). A test was conducted for differences within and among the ploidy levels. Means were separated using Fisher's protected least significant difference ($P < 0.05$) procedure. The PROC CORR procedure was used to perform correlation analyses among morphological, adaptive and drought resistance trait descriptors.

Results and Discussion

Significant differences were detected among the accessions for most of the traits. The variation among the accessions was the greatest within tetraploids and the lowest within diploids. As compared to the controls, superior tetraploid accessions exist for quality, genetic color, fall color retention and drought resistance. Variability for drought resistance was greater among Turkish accessions compared to control cultivars. The superior drought resistance was detected as evidenced by low leaf firing under prolonged drought stress and fast post-drought stress recovery. The accessions existed with very high drought resistance belonging to tetraploid, pentaploid and hexaploid.

The accessions exhibited large morphological variations. The leaf and stolon texture got coarser as ploidy increased, pentaploids having the coarsest morphology. The diploids and triploids had the highest shoot density. Of all the accessions, some of the tetraploids established faster than the controls. Variability for summer, fall and winter quality were greater among the accessions compared to control cultivars. The triploids on average had the highest quality while the tetraploids had the largest variations. The variation for spring greenup (GU) ratings were found to be larger than the cultivars, indicating some of the accessions initiated growth earlier and provided green cover faster. Among the five ploidy levels, the hexaploids and diploids had the highest and lowest GU averages, respectively, while the tetraploids had the largest ranges. Turkish accessions have comparable to and/or superior fall color retention than the cultivars. Variability for genetic color was greater among Turkish accessions compared to control cultivars. Among the five ploidy levels, the pentaploids and hexaploids had darkest green color on average while tetraploids had larger ranges. The variability in the growth habit was higher in Turkish accessions and ranged from prostrate (1.0) to upright (5.0) while the cultivars were 2.0 to 4.0. Significant correlations were detected among some of the traits. Ploidy level and turfgrass density was negatively correlated ($r=-0.32$; $p\leq 0.0001$). Drought resistance was positively correlated ($r=0.30-0.40$; $p\leq 0.0001$) with verdure, density and fall color retention.

Substantial genetic variation was found among the 95 *C. dactylon* accessions from Eastern Mediterranean for drought resistance as well as some selected morphological traits. Such variation among *C. dactylon* accessions from geographic areas other than Turkey was also reported^{1,3,8}. Variation was the greatest among tetraploid accessions, similar to Chinese accessions⁹. Existing variation among the accessions could be used to enhance turfgrass characteristics and drought resistance of bermudagrass cultivars. Genetic variation among bermudagrasses for turf performance traits has been reported to be heritable and useful in breeding improvement^{1,10}. The variation present in the Turkish *Cynodon* accessions may have significant contribution to *C. dactylon* breeding programs for various uses such as turf, forage, soil stabilization, and remediation, as well as our understanding evolution of this warm-season grass.

⁹ WU, Y.Q., C.M. Taliaferro, D.L. Martin, J.A. Anderson, and M.P. Anderson 2007. Genetic variability and relationships for adaptive, morphological, and biomass traits in Chinese bermudagrass. *Crop Sci.*, 46: 917–926.

¹⁰ Wofford, D.S., and A.A. Baltensperger 1985. Heritability estimates for turfgrass characteristics in bermudagrass. *Crop Sci.*, 25:133-136.

TURFGRASS GENETICS AND BREEDING

POSTER PRESENTATIONS

USE OF GENOTYPING-BY-SEQUENCING TO DEVELOP A HIGH DENSITY SNP-BASED LINKAGE MAP IN ZOYSIAGRASS

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Introduction

Zoysiagrasses (*Zoysia* spp.) are warm season turfgrasses with great potential as lower input grasses because of their low growth habit, reduced fertilizer demands, and general tolerance to abiotic stresses like drought, shade and salinity. However, one factor limiting the widespread use of zoysiagrasses is a relative lack of freezing tolerance, especially when compared to cool-season grasses. Limited progress has been made in the development of new cold-tolerant cultivars since 'Meyer' was released in 1951. The identification of markers linked to genomic regions controlling cold tolerance in zoysiagrass would improve the accuracy and effectiveness of selection, which would ultimately lead to an increase in the availability of cold-tolerant cultivars. Genome mapping is a pre-requisite for identifying such associations. Genotyping by sequencing (GBS) is a high throughput, cost-effective sequencing technology that can be used for the development of genome-wide single nucleotide polymorphisms (SNPs) even in species without a reference genome. GBS was implemented in a zoysiagrass mapping population and used for the rapid discovery of SNPs using the GBS-SNP Calling Reference Optional Pipeline (GBS-SNP-CROP)¹. These SNPs will in turn be used in conjunction with phenotypic data to locate quantitative trait loci (QTL) associated with freezing tolerance in zoysiagrass.

Methods

Plant Materials and Field Testing: A pseudo-F₂ mapping population consisting of 175 individuals was developed from the cross of freeze-tolerant 'Meyer' with freeze-susceptible 'Victoria'. For phenotypic evaluations of winter survival this population and nine controls were planted in the spring of 2014 in a randomized complete block design (RCBD) with three replications at the Upper Mountain Research Station (Laurel Springs, NC) and the William H. Daniel Turfgrass Research and Diagnostic Center (West Lafayette, IN) based on the range of winter temperatures at these locations. These plants were evaluated for percent green cover and winterkill in the springs of 2015 and 2016.

Library Preparation: DNA from mapping population samples was extracted using the Cetyltrimethyl ammonium bromide (CTAB) extraction method detailed in Afanador et al.². A library was prepared according to the genotyping-by-sequencing protocol described by Poland et al.^{3,4}. DNA at a concentration of 20 ng/ul was double-digested using the restriction enzymes *Pst*I and *Msp*I before being ligated to adapters designed for use with these enzymes and containing unique barcodes 5-8 bases long. Multiplexing with these unique barcodes allowed for the sequencing of all 177 individuals in a single lane. This library was cleaned and brought to a concentration of 30ng/ul for a MiSeq 150 single read (SR) run.

Data Analysis: Sequence reads were processed with GBS-SNP-CROP¹, a reference-optional pipeline. This pipeline runs seven Perl scripts that filter and demultiplex raw single-ends reads, assemble mock reference by clustering reads, align and parse reads, then filter SNPs. Only SNPs that are polymorphic between Meyer and Victoria will be used in mapping. **Map Construction:** A total of 239 simple sequence repeat (SSR) primers were selected based on previous successful amplification and mapping in zoysiagrass as described in Guo et al.⁵, Ma



Figure 1. Progeny exhibits variation for aggressiveness, genetic color, density and texture.

¹ Melo et al. (2015) GBS-SNP-CROP: A reference-optional pipeline for SNP discovery and plant germplasm characterization using genotyping-by-sequencing data. BMC Bioinformatics. 17:29. DOI 10.1186/s12859-016-0879-y.

² Afanador L, Haley SD, Kelly JD. 1993. Adoption of a "mini-prep" DNA extraction method for RAPD marker analysis in common bean (*Phaseolus vulgaris* L.). Annual Report of the Bean Improvement Cooperative 36: 10-11.

³ Poland, J, P. Brown. 2011. *Pst*I-*Msp*I GBS: Genotyping-by-sequencing Protocol.

⁴ Poland JA, Brown PJ, Sorrells ME, Jannink JL (2012) Development of High-Density Genetic Maps for Barley and Wheat Using a Novel Two-Enzyme Genotyping-by-Sequencing Approach. PLoS ONE 7(2): e32253. doi: 10.1371/journal.pone.0032253

⁵ Guo, H.L., J.P. Xuan, J.X. Liu, Y.M. Zhang, Y.Q. Zheng. 2012. Association of molecular markers with cold tolerance and green period in zoysiagrass (*Zoysia Willd.*) Bred. Sci. 62: 320-327.

et al.⁶, and Li et al.⁷. From these, 125 polymorphic markers were used for genotyping in the mapping population on LICOR sequencers⁸. These markers in conjunction with the identified SNPs will be used to construct a linkage map in JoinMap¹⁰⁹. This map will be used along winter survival data to locate quantitative trait loci (QTL) associated with cold tolerance.

Results and Discussion

Field Evaluation: Winter survival data was taken in spring 2015 with 59% survival in North Carolina and 21% in Indiana (Figure 2). Thirty-six lines suffered no winter injury in Laurel Springs, NC, and seven lines suffered no winter injury in West Lafayette, IN.

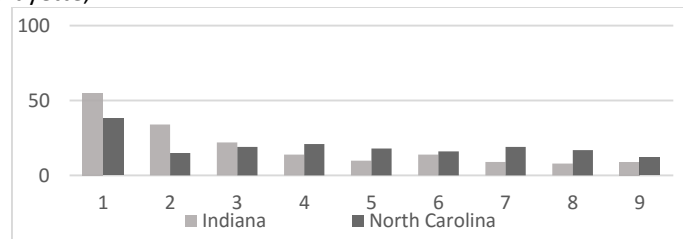


Figure 2: Distribution of winterkill for 175 progeny, Meyer, and Victoria at Laurel Springs, NC and West Lafayette, IN in 2014. Winterkill is a measure of winter injury on a scale of 1 (completely dead) to 9 (no winterkill).

Field Evaluation: Winter survival data was taken in spring 2015 with 59% survival in North Carolina and 21% in Indiana (Figure 2). Thirty-six lines suffered no winter injury in Laurel Springs, NC, and seven lines suffered no winter injury in West Lafayette, IN.

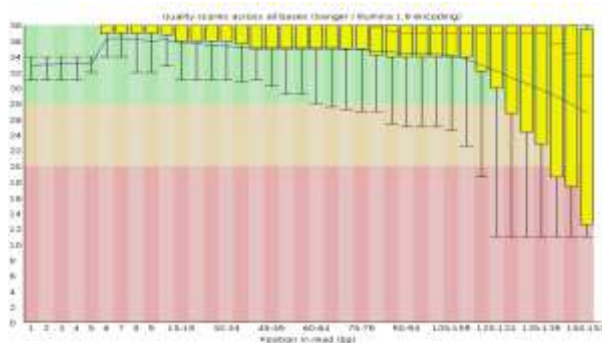


Figure 3: FastQC base per sequence quality scores

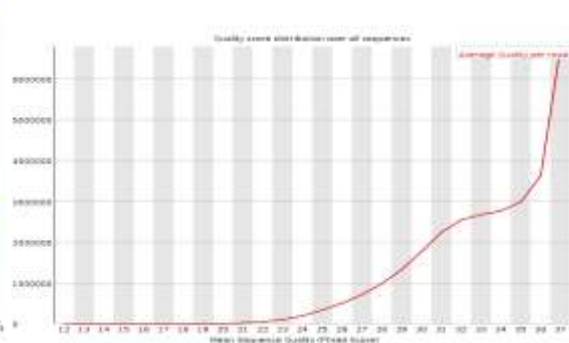


Figure 4: FastQC per sequence quality scores.

GBS reads quantity and quality: A total of 29,794,721 reads were obtained from the MiSeq 150 SR run. Of this total number of reads, 27,387,481 (91%) were determined to be usable for SNP calling based on identifiable restriction sites and barcodes. FastQC was used to further verify the quality of these reads. The base per sequence quality readings (Figure 3) indicated that the first 125 bases of each sequence were consistently high quality and adequate for SNP identification. In addition, the per sequence quality scores (Figure 4) displayed Phred scores satisfactory for SNP calling. Reads per sequence length were normally distributed. GC content was also normally distributed and N content was zero across all bases in all sequences. Reads will be trimmed down according to frequent and infrequent cut sites for alignment and SNP calling. GBS-SNP-CROP was able to produce a mock reference sequence based on the Meyer and Victoria parent sequences which will be helpful in alignment as there is currently no reference sequence for zoysiagrass. As SNPs are identified, the pipeline will produce additional mock reference sequences using sequence information from the progeny to ensure the maximum number of SNPs is called. The GBS-SNP-CROP pipeline is currently being used to filter and call SNPs in these reads. Identified SNPs will allow for the construction of a high density linkage map with adequate genome coverage for QTL identification.

⁶ Li, M., Y. Nana, H. Mariko, J. Chen, Y. Wang, and H.W. Cai. 2009. Construction of a high-density SSR marker-based linkage map of zoysiagrass (*Zoysia japonica* Steud). *Euphytica* 170:327–338.

⁷ Ma, K.H., D.H. Jang, A. Dixit, J.W. Chung, S.Y. Lee, J.R. Lee, H.K. Kang, S.M. Kim and Y.J. Park. 2007. Characterization of 30 new microsatellite markers, developed from enriched genomic DNA library of zoysiagrass *Zoysia japonica* Steud. *Mol. Ecology Notes*. 7: 1323-1325.

⁸ Kimball, J.A., M.C. Zuleta, K.E. Kenworthy, V.G. Lehman, K.R. Harris-Shultz and S. Milla-Lewis. 2012. Genetic Relationships in *Zoysia* Species and the Identification of Putative Interspecific Hybrids Using Simple Sequence Repeat Markers and Inflorescence Traits. *Crop Sci.* 53: 285-295.

⁹ JoinMap 4.1, Kyazma B.V., Netherlands.

TURFGRASS LANDSCAPE

ORAL PRESENTATIONS

TURF MANKIND AND LANDSCAPE

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Turf is an important component of the urban and rural landscape. It is hard to imagine our urban gardens and parks without turf, the same way as the countryside without pastures and meadows. In geobotany herbaceous plants make most of the pioneer stages in ecological succession¹. In natural landscapes, grass also appears in the clearings in the middle of the woods or as the lower strata in sparse woods. Plant formations/biomes turf mimics are the tropical savanna, the temperate grasslands (steppe and the prairies) and the tundra. In tundra, trees and tall plants are excluded because of low temperature or short growing season. In the tropical savanna it is the long dry season that benefits grasses leaving trees small and spaced out. The temperate grasslands appear in temperate, seasonal, dry climates and have a lack of trees (deep organic soils develop and organic matter accumulates)². In many of these biomes, at least the above ground part of the plants, if not the entire plant, dies in the unfavorable season (due either to drought or cold) and re-sprout or germinate from seed when climate becomes favorable.

Gardens and parks, in which turf is included, provide many functionalities/benefits, being them of environmental, aesthetic, recreational, economic, sociologic and psychological/physiological nature, as have been reviewed^{3,4}. Among these benefits/functionalities some are only fully achieved if turf is present, which, most probably, is the reason for turf immense popularity in private, as well as in public gardens or parks.

Aesthetics are the paramount factor in landscaping decisions, environmental and other interests come after⁵. Signs that the landscape has been taken care of, like orderly plantings and well-kept lawns, are highly valued by residents although these signs may depend on culture⁵. Turf has an important aesthetic value, as the simple background for all the other items of the landscape, it becomes the frame that enhances/highlights other features (like the house). It sets the ground level for the height. It reveals the land shape as a tight garment. It gives the tidy look which is important in many cultures. Recent inquiries (as of 2013) in the U.S.⁶ revealed that for the lawn owners the main turf benefits were, first the enhanced property aesthetics, second the increment in property value and third the provision of a recreation area. Also, the people's perception is that "healthy, green landscaping" improves their quality of life, enhances public safety, and reduces crime. Recreationally, turf allows for the permeable, non-heatable, injury protecting, impact cushioning, cheap, open space surface, needed for many sports/games. Sociologically/culturally turf can become a symbol of wealth and power, and/or the mirror of the lawn-owner character: a badly kept turf meaning a careless owner. This popularity of turf, and the competition among lawn-owners, has probably lead to some exaggeration on the way turf is maintained and on what one expects from a lawn.

As a consequence, turf has come under criticism. The main points being high water consumption, decreased biodiversity, high fossil energy use (a bad carbon footprint) and, incorrect use of fertilizers, herbicides and pesticides, leading to important environmental problems⁷.

In some European areas, the use of drinking water for garden irrigation increased more than 400% between the mid-1970s and the mid-1990s⁸. The importance of adequate turf irrigation has been frequently emphasized and water conservation is a complete set of strategies, not a single factor decision. Nevertheless if irrigation is

¹ Wang, K., Shao, R., Shangguan, Z. 2010. Changes in species richness and community productivity during succession on the Loess plateau (China). *Pol. J. Ecol.* 58: 549–558.

² Colinvaux, P. 1986. *Ecology*. John Wiley and Sons, Inc., NY, USA. 725 pp

³ Brethour, C., Watson, G., Sparling, B., Bucknell, D., Moore, T. 2007. Literature review of documented health and environmental benefits derived from ornamental horticulture products. George Morris Center. Available online at https://www.agrireseau.net/horticulture-arbresdenoel/documents/Reports_Ornamentals_Health_Benefits.pdf Viewed January 2010.

⁴ Cameron, R.W.F., Blanusa, T., Taylor, J.E., Salisbury, A., Halstead, A.J., Henricot, B., Thompson, K. 2012. The domestic garden – Its contribution to urban green infrastructure. *Urban Forestry & Urban Greening* 11: 129–137.

⁵ Hayden, L., Cadenasso, M.L., Haver, D., Oki, L.R. 2015. Residential landscape aesthetics and water conservation best management practices: Homeowner perceptions and preferences. *Landscape and Urban Planning* 144: 1–9.

⁶ Khachatryan, H., Rihn, A., Dukes, M. 2014. Consumer lawn care and fertilizer use in the united states. IFAS, Univ. of Florida. Available online at http://gardeningsolutions.ifas.ufl.edu/clce/faculty/pdf/pubs/clce_fertilizer_report_dec1214.pdf Viewed March 2016.

⁷ Helfand, G.E., Park, J.S., Nassauer, J.I., Kosek, S. 2006. The economics of native plants in residential landscape designs. *Landscape and Urban Planning* 78: 229–240.

⁸ Krinner, W., Lallana, C., Estrela, T., Nixon, S., Zabel, T., Laffon, L., Rees, G., Cole, G. 1999. Sustainable water use in Europe. Part 1: Sectoral use of Water, Environmental assessment report No 1. European Environment Agency.

significantly reduced, the benefits of turf may be reduced or invalidated. Also, instead of yes or no options some compromises can be taken and intermediate solutions can be achieved. Mimicking nature may offer some solutions. In some climates, like the Mediterranean, the natural summer landscape is composed of brown turf that greens up in fall: of course brown turf is not so appealing, does not cool the ambience, does not recuperates from damage, may create a fire hazard, among others, but may be an appropriate choice where irrigation is not feasible. It brings with a dynamic look, with well differentiated seasons, allows for most of the recreational activities, and still provides a regular green turf for most of the year. Breeders may then look for non-traditional species and features like greening up capacity and earliness, traffic resistance while dormant (i.e. underground resistant structures), or capacity to re-seed below mowing height. Definitely, for some sports, like golf, baseball, soccer or American football, if quality is important, in the playable areas turf is mandatory. Nevertheless, public education is paramount: as presented for Canada⁹, a low-maintenance-lawn may use less water than a Xeriscape. Also, even synthetic, artificial grass for sports, needs to be watered, at least in hot days, so that the players do not get dehydrated and suffer sunstrokes from the increased temperature. Synthetic turf has also maintenance requirements such as vacuum cleaning, algae and moss treatments depending on the climate, brushing and even irrigating (to subsidize the topdressing materials). And this leads us to think about turf substitutes.

Bare ground does not prevent erosion, does not cool off the environment, increases water runoff, does not purify water or air, and does not increase biodiversity. Paving results in permanently impervious surfaces, does not help on biodiversity, does not purify water or air, warms up on hot days and does not store carbon. Wood chip or pebbles ground cover in conjunction with a few drought resistant shrubs, does not help biodiversity, nor cools off the environment and may warm up, nor helps storing carbon, nor allows recreational activities. Woody ground covers store as much carbon per square meter as a Kentucky bluegrass lawn and do not provide a recreational area. A mix of shrubs and trees without a turf area, does not provide a recreational surface, although may use less water and provide for an increased biodiversity, as well as a good carbon storage.

Carbon footprint has been considered positive in properly managed lawns. For standard managed home lawns in the U.S., with clippings returned, accumulation of organic carbon in the soil will proceed for a period of 66 to 199 years depending on soil and climatic conditions before it reaches equilibrium¹⁰. Practices that decrease soil organic matter may decrease or nullify this positive effect, as in athletic fields¹¹. The best estimates produced, consider that clippings should be returned to the lawn, and permitted to decompose, so that a higher plant growth is achieved without exaggerated N fertilization¹⁰. But this is a process with a low efficiency since most of the carbon is respired back to the atmosphere. It seems that research, as well as public education, should be done on lawns with a mix of grasses and legumes: the presence of legumes may avoid N fertilization¹² and, may allow for clippings removal and usage as biofuel, while keeping the soil accumulation of carbon. Grain production straw has been considered for biofuel and the annual yield of lawn clippings by hectare is similar to that of cereal's straw: this way, lawns would not only sequester carbon but would also assist on reducing emissions of fossil carbon.

Addition of some trees and shrubs to the landscape, as it already happens in several circumstances, leaving some areas without being mowed, allowing appropriate native plants to get in, refraining from the use of fertilizers, herbicides and pesticides, would add vertical and horizontal structure, provide habitat diversity, as well as food and shelter, and, a healthier environment.

⁹ CMHC. 2000. Definitely in my backyard: making the best choices for you and the environment. Canada Mortgage and Housing Corp. <http://www.cmhc-schl.gc.ca/publications/en/definitely>. Viewed 2008.

¹⁰ Selhorst, A., Lal, R. 2013. Net carbon sequestration potential and emissions in home lawn turfgrasses of the United States. *Environ. Managmt.* 51:198–208.

¹¹ Townsend-Small, A., Czimczik, C.I. 2010. Carbon sequestration and greenhouse gas emissions in urban turf. *Geophysical Res. Letters*, 37: L02707.

¹² Kryževičienė, A., Jasinskas, A., Gulbinas, A. 2008. Perennial grasses as a source of bioenergy in Lithuania. *Agronomy Res.* 6 (Special issue): 229–239.

COMPARING FOUR BERMUDAGRASS AND FIVE ZOYSIAGRASS CULTIVARS UNDER HEAVY USE AT KINDERGARTENS

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Introduction

In urban cities of Japan, land space is limited. Because of that, the area of many kindergartens' playgrounds is small which is sometimes less than 5 m² per child. Maintaining healthy turf in such heavily used area is challenging¹. Thus, most of playgrounds are left as bare ground. Since 1973, the Japanese government of Ministry of Education, Science and Culture had supported projects for promoting greening at schools. After that, some schools have tried to convert bare ground to turf. This effort was accelerated recently due to the movement of attracting Tokyo Olympic and Paralympic Games in 2020. However only few schools have succeeded for maintaining turf in adequate quality, possibly due to lack of maintenance skills and inappropriate selection of turfgrass species and cultivars. In the past, schools unsuccessfully utilized cool season grasses. Currently most schools use warm season turfgrasses and mainly bermudagrass (*Cynodon* spp.) and zoysiagrass [*Zoysia* spp. (willd.)]. The use of warm season turfgrasses, enabled schools to successfully maintain the turfed areas, even though some of them still confront difficulties. Due to lack of research it is hard to identify successful methods of maintaining turfgrasses of acceptable quality at kindergarten's playgrounds.

The objectives of this study were to: (i) evaluate turfgrass establishment methods and (ii) evaluate bermudagrass and zoysiagrass cultivars with same management theme under heavily used kindergartens' playgrounds.

Methods

The field studies were conducted at two kindergartens in Shizuoka prefecture during 2013-2015 under normal use of the playgrounds. The two kindergartens were Kounotori Toyoda Hoikuen (KTH), Iwata-shi, Shizuoka and Yuho No Oka Hoikuen (YNOH), Hamamatsu-shi, Shizuoka. The soil at KTH was coarse sandy loam with a pH of 6.4, while the soil type at YNOH was sandy loam with a pH of 5.3. Size of playground of KTH was 638 m² and 120 children were attended which made 4.7m² of playground available for each child, and that of YNOH was 322 m² and 107 children which made 2.3m² of playground available per child. Before the establishment, plots were broadcasted with 4 kg m⁻² of compost rotor tilled at a depth of 10 cm. During the first year, N, P, and K fertilizers were applied at a rate of 151, 237, and 30 g m⁻², respectively including the nutrients contained in the compost. In the second year, 60 g N m⁻² were applied while P, K, and other nutrients were maintained at sufficient levels according to soil tests. Plots were maintained at a cutting height of 5.0 cm. At KTH turf was mowed daily with robotic lawn mower (Husqvarna Zenoah, Kawagoe, Saitama), and at YNOH turf was mowed with walk behind rotary mower (Honda, Minato-ku, Tokyo) in approximately twice a week. Clipping were returned at the both sites. Throughout the studies, plots were maintained by the kindergartens teachers. Because of that, irrigation was applied as needed to prevent drought, however at YNOH, plots were more frequently irrigated compare to KTH.

Plots measuring 2.0 m by 2.0 m were established in July 2013 using three hybrid bermudagrass cultivars (*Cynodon dactylon* (L.) Pers. X *C. transvaalensis* Burttt Davy): Tif419, Tifway(J)² and Musashi, two common bermudagrass cultivars (*C. dactylon* (L.) Pers. var. *dactylon*): Yukon and Riviera, and five zoysiagrass cultivars (*Zoysia japonica* Steud.): Fuji compact, Himeno, Asagake, Asamoe, and Zenith. Cultivars were established either by sod, seed, or plugs depending on their commercial establishment methodology. Tif419, Fuji compact, Himeno, Asagake, and Asamoe were sodded, Yukon, Riviela, and Zenith were seeded, and Tifway(J) and Musashi were plugged. Seeding rates of Yukon and Riviera were 15 g m⁻², and Zenith was 20 g m⁻². Seeded plots were planted under geotextile fabrics to enhance germination which were removed after gemination. Plugging was accomplished using 5 x 5 x 5 cm plugs spaced at 25 cm intervals and at a depth of 5 cm. During the 2 months' establishment period, traffic was limited. Utilization of the playgrounds occurred after the collection of the first data set.

¹ Organization for Landscape and Urban Green Infrastructure. 2013. Kouteishibafuka no Q&A. Kashima, Tokyo, p 24-25.

² Fujita, K. 2012. Comparing bermudagrass cultivars. College of Bioresource Science, Nihon University Research Project and Thesis, 129-132.

Percent green cover and turfgrass quality were visually estimated at monthly intervals. Percent green cover was estimated 0 to 100 scale, in which 0 indicate bare soil or there was no designated cultivar present and 100 indicates complete cover with designated cultivar.

The experimental area was designed following the randomized complete block design separated into four blocks with four replications for both sites. Data were analyzed using JMP. A one-way analysis of variance (ANOVA) was used for analysis and Fisher's Protected LSD test was used to separate main effect means.

Results and Discussion

There was a kindergarten X cultivar interaction for percent green cover, so cultivar differences in percent green cover were presented within kindergarten (Table 1). At the establishment of KTH, plugging and sodding had better green coverage than seeding. At YNOH, plugging and sodding didn't have a significant difference, however sodding had better green coverage than seeding. At the end of two years of studies only Tifway(J), Tif419 and Riviera had acceptable turf quality for both kindergartens (Data not shown). At KTH, Tifway(J) had best green coverage among all cultivars (Table 1). At YNOH, Tifway(J), Tif419, and Riviera had better green coverage than other cultivars. The results conflict with some of the previous reports which traffic tolerance of zoysiagrass have similar to superior to bermuagrass^{3,4}. This might be due to zoysiagrass has slow rate of growth⁵. In our experiments, the experimental fields had heavy traffic and turf were considerably damaged. Also, zoysiagrass showed significantly slower spring green up. This might be a suggestion of traffic tolerance under spring green up period differ between bermudagrass and zoysiagrass. Combined with establishment results, we conclude establish from seed was difficult especially when a playground was maintained by the kindergarten's teachers. We recommend Tifway(J), Tif419, or Riviera bermudagrass for intensively used kindergarten's playground. Riviera is mainly distributed by seed in Japan, however it would be better to obtain either plug or sod for the establishment. Results and recommendations would likely vary under different climatic zones, soil types, management regimes, or frequencies and amount of playground use. Also, the size of playground compare to the number of children can only be used as an estimate of playground use. Further research is needed specially to quantify amount of playground use.

Table 1 Percent of green coverage of various bermudagrass and zoysiagrass grown in kindergartens.

Cultivar	Species‡	KTH†		YNOH	
		Establishment	After 2 years	Establishment	After 2 years
		-----%-----			
Tifway(J)	CDCT	99 a [¶]	98 a	99 a	100 a
Tif419	CDCT	100 a	70 b	100 a	100 a
Riviera	CD	59 c	63 b	95 a b	98 a
Musashi	CDCT	99 a	28 c	100 a	73 b
Himeno	ZI	100 a	13 c	100 a	13 c d
Fuji compact	ZI	100 a	13 c	100 a	28 c
Zenith	ZI	13 d	10 c	75 b	0 d
Yukon	CD	60 c	10 c	98 a	0 d
Asamoe	ZI	84 a b	6 c	100 a	13 c d
Asagake	ZI	74 b c	6 c	99 a	0 d
Plug		99 a		99 a b	
Sod		92 a		100 a	
Seed		44 b		89 b	

†IWA =Iwata-shi Hoikuen; HMA = Hamamatsu-shi Hoikuen.

‡CD = *Cynodon dactylon* (L.) Pers.; CDCT = *C. dactylon* (L.) Pers. X *C. transvaalensis* Burt Davy; ZI = *Zoysia japonica*.

¶Value within columns followed by the same letter are not significantly different from another (LSD, $\alpha = 0.05$).

³ Trappe, J. M., Karcher, D. E., Richardson, M. D., and Patton, A. J. 2011. Shade and traffic tolerance varies for bermudagrass and zoysiagrass cultivars. *Crop Sci.* 51:870-877.

⁴ Youngner, V.B. 1961. Accelerated wear tests on turfgrasses. *Agron. J.* 53:217-218.

⁵ Beard, J. B. 1973. *Turfgrass science and culture*. Prentice-Hall, Inc. Englewood Cliffs, NJ. p.143.

SHADE RESPONSE OF BERMUDAGRASS ACCESSIONS UNDER VARYING MANAGEMENT PRACTICES

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Introduction

Bermudagrass (*Cynodon* species) is one of the most commonly grown turfgrass genera in the southern United States, providing a quality turf stand for recreational areas and home lawns. Its aggressive nature and excellent drought tolerance provide resistance to weed encroachment and rapid recovery from wear and disease injury during summer stress. These advantageous characteristics can be often overlooked due to its poor shade tolerance. Developing cultivars tolerant to shade would allow bermudagrass to be used in areas where trees dominant the landscape. In addition to cultivar choice, a number of management factors have been shown to affect the performance of turfgrasses under shade stress. Raising the mowing height, reducing nitrogen levels and applying plant growth regulators have all been recommended to provide competitive turf under shaded environments. The continual evaluation of germplasm for shade tolerance is critical in improving overall shade tolerance in bermudagrass; however, each genotype behaves differently when management practices are implemented. The purpose of this study is to determine the effects of varying cultural practices on four commercial cultivars and two germplasm accessions, 'WIN10F' and 'STIL03', under perpetual shade.

Methods

Treatments were designed to determine the effects of the proposed cultural practices on four cultivars and two South African accessions that were previously reported to show some level of shade tolerance¹ (Table 1). The experimental design was a strip-strip-split plot design with three replications and was established from strips of sod under full sunlight in June 2013. Once the plots were completely established, artificial shade fabric was applied over the plots to limit light to 63% in both years of the study.

Table 1. List of treatments and factors for evaluation of cultural practices on bermudagrass when grown under 63% perpetual shade.

Entries	Mowing Heights	Plant Growth Regulator	Nitrogen Fertility Applications
<i>Celebration</i> <i>Tifgrand</i> <i>Tifway</i>	1.27 cm (0.75 inches)	No Primo Maxx	Low 48.8 kg N ha ⁻¹ Applied every other month
<i>Discovery</i> <i>STIL03</i> [†] <i>WIN10F</i> [†]	5.1 cm (2 inches)	Primo Maxx L ha ⁻¹ 2 applications monthly	High 48.8 kg N ha ⁻¹ Applied monthly

[†] South African accessions

After the first year of data collection, the shade fabric was removed over the winter to mimic normal growth conditions when leaves are not present on surrounding deciduous trees. The shade fabric was replaced in the spring when the plots came out of dormancy. In both years, images were taken weekly for image analysis using a Fiji image processing macro (Fig. 1). Each treatment (mowing height, PGR, and fertility as shown in Table 1) was initiated at the same time the shade cloth was replaced. Plots were mowed once weekly using a rotary mower and a reel mower to achieve the 5.1 cm height and 1.27 cm height, respectively. In order to compare the relative shade tolerance of the South African accessions under each of the management practices relative to the controls, NDVI values along with percent plot cover (decline), percent cup-cutter fill (recovery) and percent divot fill (recovery) were taken beginning on 1 Aug 2014 through 17 Oct in 2014 and from 1 July 2014 through 10 Oct 2015 (Fig. 1). The cup-cutter and divot fill were analyzed by selecting a shape and size and analyzing percent cover within the shape. Likewise, to determine total plot cover of the image, the same shape

¹ Dunne, J.C., W.C. Reynolds, G.L. Miller, C. Arellano, R.L. Brandenburg, A. Schoeman, F.H. Yelverton and S.R. Milla-Lewis (2015). Identification of South African Bermudagrass Germplasm with Shade Tolerance. Hort. Sci. 50(10):1419-1425.

selected for the cup-cutter and divot fill was not included and then the results were averaged together. The statistical analysis was done using Proc GLM in SAS version 9.4.

Results and Discussion

All results presented are from data collected on 5 Sept 2014 (2014) and 2 August 2015 (5-weeks after cup-cutter was removed). Furthermore, the entry ‘Discovery’ was not included due to the differences in the establishment process when compared to the other entries in the study. Based on the analysis of variance, differences were observed between entries across all response variables and years. Treatment differences between the PGR applications were observed for total plot cover and NDVI in 2014 and for total plot cover and cup-cutter recovery in 2015. In addition, differences in the mowing heights were observed in percent cup-cutter fill and NDVI in 2014 and across all response variables in 2015. Two interactions were detected between entry and the PGR applications for NDVI and between mowing and the PGR applications for total coverage in 2014. Only the interaction between mowing and the PGR applications was detected in 2015. Fertility applications or the interactions with other management practices were not significant in either year. The differences observed in the PGR treatments were consistent for NDVI and total plot cover, showing the ‘Primo’ applications having greater values when compared to the ‘No Primo’ applications. These findings were consistent for total plot cover and cup-cutter recovery in 2015. However, this was not the case when comparing mowing treatments. The 5.1 cm mowing height provided the highest NDVI values when compared to the 1.27 cm treatment. On a recovery basis, the 1.27 cm treatment had a higher percent cup-cutter fill than the 5.1 cm treatment. In 2015, these findings were consistent for NDVI and percent cup cutter recovery. Furthermore, the total plot cover showed a higher percent cover for the 5.1 cm mowing height and the 1.27 cm plots showed higher divot recovery. Similar to the PGR applications, the entries had a consistent order across all response variables with only the significance between the entries varying (Table 2). In all instances, Celebration was the top performing entry followed by STILO3, WIN10F, Tifgrand and then Tifway. Although the results presented are from 5 Sept 2014 only, the analysis will be carried out across all weeks tracking changes in each response variable over the time of the data collection.

Table 2. Means comparisons for Normalized Difference Vegetation Index (NDVI), total cover, cup cutter and divot coverage rate between entries from data collected on 5 Sept 2014 (2014) and 2 Aug 2015 (2015) at the Lake Wheeler field lab, Raleigh, NC.

Entry	NDVI		Total Cover		Cup Cutter		Divot	
	2014	2015	2014	2015	2014	2015	2014	2015
<i>Celebration</i>	0.678 A†	0.684 A	82.1 A	83.8 AB	76.3 A	84.7 A	79.0 A	81.9 A
<i>STILO3</i>	0.651 AB	0.685 A	77.6 A	85.2 A	72.7 A	77.0 B	73.0 A	82.0 A
<i>WIN10F</i>	0.636 B	0.685 A	77.3 A	84.8 A	70.3 A	76.7 B	69.6 A	79.5 AB
<i>Tifgrand</i>	0.628 B	0.645 B	61.5 B	81.5 B	50.1 B	68.7 C	45.7 B	78.0 AB
<i>Tifway</i>	0.581 C	0.613 C	58.1 B	75.9 C	36.4 B	62.9 C	36.6 B	72.9 B

† Entries that share the same letters within columns are not significantly different according to Fisher’s protected LSD ($\alpha = 0.05$)



Figure 1. Process of image analysis using Fiji (ImageJ) to determine total plot coverage and cup-cutter fill from the images taken weekly.

THE POTENTIAL TO INCREASE DELIVERY OF MULTIPLE ECOSYSTEM SERVICES OF URBAN GRASSLANDS

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Introduction

Urban greening is the primary tool to provide local ecosystem goods and services to urban populations and compensate for some of the service deficit in urban areas. To design and maintain urban landscapes for targeted delivery of ecosystem services represents an opportunity to reduce the environmental impact and improve quality of life in urban areas¹. Urban green areas are mainly distinguished by their tree or shrub cover and level of soil sealing², attributes that have large effects on the types of services provided³. Grasslands are among the most common urban green infrastructure and sum to large areas, but also considerable maintenance costs. These urban grasslands deliver a range of ecosystem services, still it is possible to build in more ecological processes and increase the multifunctionality at the level of the individual grassland. Based on literature and our own results, we here discuss some approaches to achieve this. Such optimal use of urban greening for ES delivery requires a thorough understanding of ecological processes in urban areas, how they interact and respond to management and use. It is all about how to manage processes to deliver services in a cost- and area efficient way.

Urban grasslands differ considerably from natural and semi-natural grasslands, differences that have consequences for service provision. Contrasting origin, historic and current management contributes to differences while urbanization filters further modify habitat availability, spatial arrangement of habitats, matrix permeability, plant species pools, and the evolutionary selection pressures on local populations⁴. Urban grasslands can provide a set of supporting, provisioning, regulating and cultural ES based on abiotic and biotic resources and ecological, hydrological and biochemical processes. Urban grasslands contribute to provisioning services like water supply through infiltration and recharging of aquifers, genetic resources of grassland organisms, and raw materials and biomass for forage or processing to compost, ground cover or bioenergy. Supporting services like habitat provision, soil formation, primary production and biogeochemical cycling are also provided. Yet, the regulating and cultural services usually are of higher importance to the urban population. Urban grasslands provide cultural services like recreation and aesthetics, social meeting ground, education, research, teaching and experience. Among the regulatory services the vegetation cover of urban grasslands contributes to the regulation of microclimate, gas exchange and carbon sequestration. The soils further contribute to water retention/flood attenuation, water infiltration, and pollution mitigation. These ecosystem services are connected through ecological, biogeochemical and hydrological processes causing both synergies and trade-offs. As a consequence, improving delivery of a set of services may reduce the delivery of other services both at large and small spatial scales⁵. There is often a positive correlation between certain ES as they are provided by the same type of urban green infrastructure with processes operating at similar scales^{6, 7}. Such bundles of spatial correlated or aggregated ecosystem services are observed in both urban and rural

¹ Lovell, S.T., Johnston, D.M. 2009. Creating multifunctional landscapes: how can the field of ecology inform the design of the landscape? *Frontiers in Ecology and the Environment* 7: 212–220.

² Pauleit, S., Duhme, F. 2000. Assessing the Environmental Performance of Land Cover Types for Urban Planning. *Journal of Landscape and Urban Planning*, 52: 1-20.

³ Lehmann, I., Mathey, J., Rößler, S., Bräuer, A., Goldberg, V. 2014. Urban vegetation structure types as a methodological approach for identifying ecosystem services – Application to the analysis of micro-climatic effects. *Ecological Indicators* 42:58–72.

⁴ Williams, N.S.G., Schwartz, M.W., Vesk, P.A., McCarthy, M.A., Hahs, A.K., Clemants, S.E., Corlett, R.T., Duncan, R.P., Norton, B.A., Thompson, K., McDonnell, M.J. 2009. A conceptual framework for predicting the effects of urban environments on floras. *Journal of Ecology*, 97:4–9.

⁵ Bennett, E.M., Peterson, G.D., Gordon, L.J. 2009. Understanding relationships among multiple ecosystem services. *Ecology Letters* 12:1-11.

⁶ Demuzere, M., Orru, K., Heidrich, O., Olazabal, E., Geneletti, D., Orru, H., Bhave, A.G., Mittal, N., Feliu, E., Faehnle, M. 2014. Mitigating and adapting to climate change: Multi-functional and multi-scale assessment of green urban infrastructure. *Journal of Environmental Management* 146:107-115.

⁷ Derksen, M.L., van Teeffelen, A.J.A., Verburg, P.H. 2015. Quantifying urban ecosystem services based on high resolution data of urban green space: an assessment for Rotterdam, the Netherlands. *Journal of Applied Ecology*, 52:1020–1032.

areas⁸. To combine processes with trade-offs in urban areas has parallels to the land-sparing vs .land-sharing discussion in agriculture⁹, where trade-off between provision and regulation services are frequent.

Although green infrastructure has the potential to deliver a range of ES, the documentation of this delivery at appropriate scales is far from solid^{10, 7} and in some cases the efficiency is exaggerated. To ensure that service delivery of urban green areas is more than a set of good intentions, the knowledge of interactions between processes, their trade-offs and disservices and how green infrastructure can be used in combination with technological solutions has to be strengthened. The contributions to processes like C storage, air quality improvement, noise reduction and cooling per surface area are of less relevance for grasslands and herbaceous vegetation⁷. Instead of expecting individual grasslands to provide a full suite of services, we have a rather limited bundle of services to optimize centred at biodiversity and habitat provision, recreation and stormwater management. We present some approaches to include and secure these functions of green infrastructure, especially how to improve different aspects of biodiversity as pollination services using management regimes, the trade-off but also positive relationship between invasive species control and biodiversity, and the underexploited use of urban grasslands for stormwater management turning water from a problem to a resource.

⁸ Yanga, G., Ge, Y., Xue, H., Yang, W., Shi, Y., Peng, C., Du, Y., Fan, X., Ren, Y., Chang, J. 2015. Using ecosystem service bundles to detect trade-offs and synergies across urban–rural complexes. *Landscape and Urban Planning* 136:110–121.

⁹ Ekroos, J., Ödman, A.M., Andersson, G.K.S., Birkhofer, K., Herbertsson, L., Klatt, B.K., Olsson, O., Olsson, P.A., Persson, A.S., Prentice, H.C., Rundlöf, M., Smith, H.G. 2016. Sparing Land for Biodiversity at Multiple Spatial Scales. *Frontiers in Ecology and Evolution* 3:145.

¹⁰ Pataki, D.E., Carreiro, M.M., Cherrier, J., Grulke, N.E., Jennings, V., Pincetl, S., Pouyat, R.V., Whitlow, T.H., Zipperer, W.C. 2011. Coupling biogeochemical cycles in urban environments: ecosystem services, green solutions, and misconceptions. *Frontiers in Ecology and the Environment* 9:27–36.

TURF QUALITY AND SPECIES SUCCESSION OF BERMUDAGRASS AND KENTUCKY MIXTURES

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Introduction

Climatic changes and the need to reduce water consumption for irrigation led to consider the use of water-efficient turfgrass species in transition zones. Turfgrass managers and home lawn owners are often hesitant to use warm-season turfgrasses in the transition zone because of the long winter dormancy. Dormancy consists of a physiological rest followed by leaf desiccation, resulting in reduced or no color¹, which can persist for several months during the winter. These issues could be addressed by mixing cool- with warm-season species. In the resulting mixture, cool-season species might be dominant during winter, while warm-season species should be prevalent in summer. The competition between species in a mixture can be influenced by several factors, such as the selection of the cultivar and the management practices applied to the turf². Normally, the segregation of species within the mixture causes a serious reduction of turf quality, mostly evident during the transition from one season to the other. In this scenario, cultural practices can be crucial for maintaining the balance between warm- and cool-season species in the mixture. The objectives of this study were to test the turfgrass quality and species succession of various Kentucky bluegrass (*Poa pratensis* L.) and bermudagrass [*Cynodon dactylon* (L.) Pers.] mixtures to cover the lack of information about performance and dynamics of species succession in such mixtures.

Methods

Two field studies were conducted in Legnaro (northeastern Italy) from June 2011 to October 2013 (Study 1), and in Fayetteville (northwestern Arkansas - USA) from June 2011 to December 2012 (Study 2). Both locations have a humid, subtropical climate. Bermudagrasses 'Yukon' and 'Veracruz' were seeded in June 2011 at 5 g m⁻² in Legnaro and in Fayetteville, and Kentucky bluegrasses 'Brooklawn', 'Mystere', and 'Nublu Plus' were over-seeded in September 2011 at 30 g m⁻². In Legnaro, irrigation was provided only during establishment in June and September 2011 at 5 mm d⁻¹ and in July 2013 to prevent drought stress. Each plot was mowed weekly at 30 mm height, using a rotary mower, with clippings removed. In Fayetteville, irrigation was provided at a rate of 5 mm d⁻¹ from June 2011 to April 2012, to ensure proper establishment of each species. At this location, plants were mowed weekly at 30 mm using a triplex mower, with clippings returned. For both studies, nitrogen (N) fertilization was scheduled in September, October and March using urea at a rate of 6.7 g m⁻² of N, totaling 20 g m⁻² y⁻¹ of N. Phosphorus (P), potassium (K), and micronutrients were applied prior to the onset of the study and at the end of each growing season (November) based on soil tests recommendations. To encourage regrowth of Kentucky bluegrass, the plots were subjected to scalping in September, by reducing the mowing height to 18 mm and removing the clippings. Turf quality was estimated every other week from October 2011 to September 2013 in Legnaro, and from April to December 2012 in Fayetteville, using a 1 to 9 visual scale rating. The frequencies of species in the mixtures were determined every month with a point-intercept method^{3,4}, using linear transects by recording species in 10 cm segments of four lines of 1 m each randomly placed within each plot. For each species, the frequency in the mixture was expressed on a percentage basis (0–100%). In both studies, the experimental design was a split-plot with three replicates, having bermudagrasses as main plots (4 m x 3 m) and Kentucky bluegrasses as subplots (3 m x 2 m). Turf quality and species frequency in the mixtures were subjected to a repeated measures analysis of variance using SAS Proc Mixed (version 9.4; SAS Institute, Cary, NC). Fisher's protected least significant difference test was used at the 0.05 probability level to identify significant differences among means.

¹ Rimi, F., S. Macolino, M.D. Richardson, D.E. Karcher and B. Leinauer. 2013. Influence of three nitrogen fertilization schedules on bermudagrass and seashore paspalum: I. Spring green-up and fall color retention. *Crop Sci.*, 53:1161–1167.

² Rimi, F. and S. Macolino. 2014. Mixing warm-season turf species with red fescue (*Festuca rubra* L. ssp. *rubra*) in a transition zone environment. *Europ. J. Hort. Sci.*, 79 (3):167–174.

³ Poissonet, P.S., Poissonet, J. A., Godron, M.P. and G.A. Long. 1973. A comparison of sampling methods in dense herbaceous pasture. *J. Range Management*, 26:65–67.

⁴ Glatzle, A., Mechel, A. and M.E. Vaz Lourenco. 1993. Botanical components of annual Mediterranean grassland as determined by point-intercept and clipping methods. *J. Range Management*, 46:271–274.

Results and discussion

The analysis of turf quality and species frequency revealed for both studies significant two-way interactions bermudagrass x date and Kentucky bluegrass x date.

In Study 1, turf quality was not affected by Kentucky bluegrass cultivars. During warmer months, from April to September 2012 and from June to September 2013, plots including bermudagrass 'Yukon' showed higher quality than 'Veracruz' plots. Conversely, 'Veracruz' displayed higher quality in October and November 2011, and from October to March 2012 (Fig. 1A). As regard to species frequency, 'Yukon' frequency was higher than 'Veracruz' from July 2012 throughout the study period (Fig. 1B). Significant differences among Kentucky bluegrasses occurred only in the last four months of experimentation, with 'Mystere' showing the lowest frequency (Fig. 1C).

In Study 2, differences for turf quality among bermudagrasses were limited to September 2012 (Fig. 2A). In May and June 2012, the frequency of 'Yukon' in the mixtures was higher than 'Veracruz', which showed a slight increase during summer 2012 (Fig. 2B). Throughout the entire study period, differences among Kentucky bluegrasses were observed only during fall 2012, in which 'Mystere' had the highest frequency, 'Nublu Plus' the lowest, and 'Brooklawn' the intermediate.

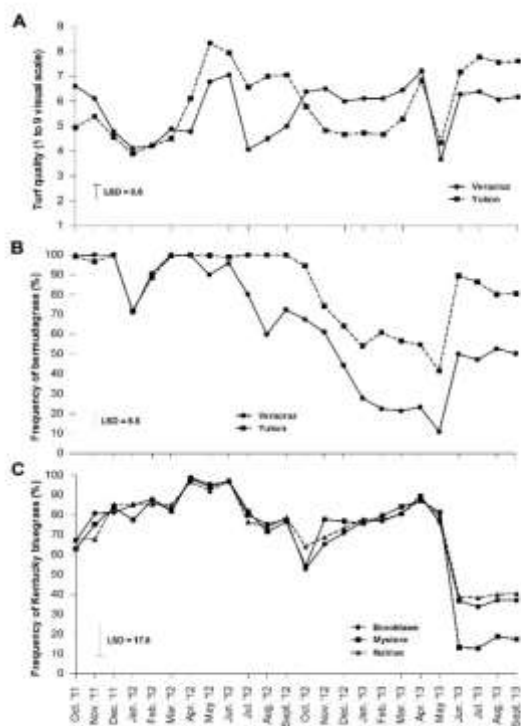


Figure 1. Legnaro, Italy (Study 1): A) Turf quality of Kentucky bluegrass and bermudagrass mixtures as affected by bermudagrass cultivars; B) Specific frequency of bermudagrasses in Kentucky bluegrass and bermudagrass mixtures as affected by bermudagrass cultivars; C) Specific frequency of Kentucky bluegrass in Kentucky bluegrass and bermudagrass mixtures as affected by Kentucky bluegrass cultivars. Vertical bar indicates LSD ($p < 0.05$).

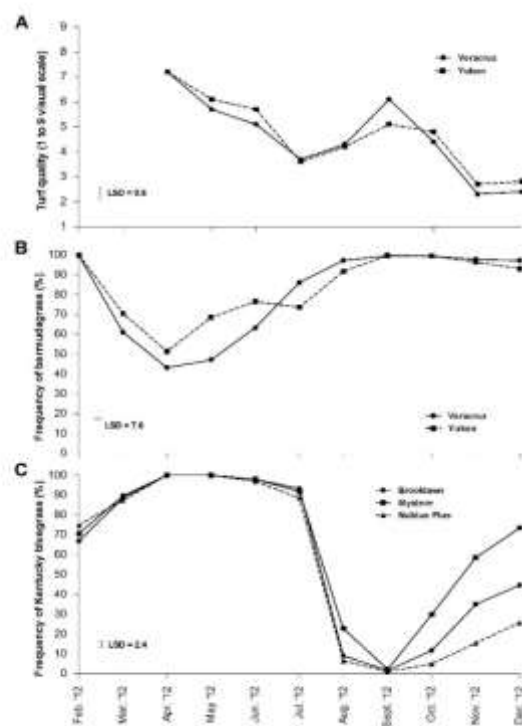


Figure 2. Fayetteville, AR, USA (Study 2): A) Turf quality of Kentucky bluegrass and bermudagrass mixtures as affected by bermudagrass cultivars; B) Specific frequency of bermudagrasses in Kentucky bluegrass and bermudagrass mixtures as affected by bermudagrass cultivars; C) Specific frequency of Kentucky bluegrass in Kentucky bluegrass and bermudagrass mixtures as affected by Kentucky bluegrass cultivars. Vertical bar indicates LSD ($p < 0.05$).

On the basis of these results, the choice of bermudagrass cultivar appears critical for establishing functional Kentucky bluegrass and bermudagrass mixtures in transition zones. Generally, 'Yukon' bermudagrass seems to be more aggressive than 'Veracruz' in such mixtures, resulting in a higher turfgrass quality during warmer periods. The Kentucky bluegrass cultivars had limited influence on the variation of plant composition. The competition between bermudagrasses and Kentucky bluegrasses in mixture and the related turfgrass quality appeared strongly dependent on adaptation to local environmental conditions of bermudagrass cultivars.

THE IMPACT OF DIFFERENT MANAGEMENT PRACTICES ON TURFGRASS QUALITY, BOTANICAL COMPOSITION, COLOUR AND GROWTH OF URBAN LAWNS

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Introduction

Return of clippings through mulching becomes increasingly the standard method used for the maintenance of low-input turfs. The reason can be seen in savings, as these maintenance systems do not include collecting, loading and transporting of clippings¹. It is also possible to reduce N fertilization and save the cost of the fertilizer. This fact becomes more significant in low-input turfs which are usually not fertilized. From this point of view, clipping mulching is beneficial as the organic matter left on the ground becomes gradually decomposed and nutrients contained therein become available to plants again. Starr and DeRoo² reported, that nitrogen from clippings remaining on the ground covers as much as 30% of annual nitrogen requirements. Higher nitrogen availability in the organic material left on the ground leads to a faster growth of the stand and hence to an increase in the dry matter weight³. The organic matter left on the ground has a positive impact on the sward colour and cause lower weed encroachment⁴.

The goal of the study was to examine the impact of clippings left on the turf in the form of mulch and also evaluate how the level of fertilization affects visual quality, botanical composition, colour and growth of urban lawns.

Methods

A small-scale trial was established at the Research Station of Fodder Crops in Vatin (MENDELU) with each experimental plot sized 4 m² and replicated three times. The station lies in the potato-growing production region with average annual temperature of 6.1°C and total annual precipitation amounting to 737 mm. The experiment was established in 2006 with a grass-legumes mixture that consisted of: *Lolium perenne* 25%, *Poa pratensis* 25%, *Festuca rubra* 30%, *Festuca ovina* 5%, *Anthoxanthum odoratum* 5%, *Cynosurus cristatus* 5%, *Trifolium repens* 3%, and *Lotus corniculatus* 2%. The study was conducted through six growing seasons during the period 2007-2012.

Treatments consisted of: a) two different clipping management techniques (removed or returned) and b) three fertilization schemes: i) without N, P, K fertilization, ii) 50 kg ha⁻¹ N +PK (in early spring) and iii) 100 kg.ha⁻¹ N + PK. (half in early spring and half after the 3rd mowing)The plots were mowed with a rotary mower (Honda HRX537) five times a year at 40 mm cutting height in the following sequence: second decade of May, second decade of June, third decade of July, second decade of September and third decade of October. Sward structure was assessed by using the projective dominance method before the first cut (May). The structure is expressed as a share of grasses, legumes, weeds and bare soil. Sward height was measured by means of a measuring rod before each cut. Turfgrass quality and colour were assessed before the first cut with point scale 1-9 (1-the worst, 9-the best). Statistical analyses were performed using repeated measures ANOVA with multiple post-hoc comparisons according to Tukey test (P-value <0.05). The Statistica 10 software (StatSoft) was used for the analysis.

Results and Discussion

Results showed, that returning of grass clippings significantly affected the botanical composition of grass-legumes mixture, where decrease in legumes proportion (from 32% to 19, 1%) was observed. In contrast the returning of clippings supported a significant increase of the turfgrass component in stand (Fig. 1) since grass

¹ Knot, P. 2013: Clipping management and its effect on the composition and height of low-input turf. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 61(6):1741–1747.

² Starr, J.L., and H.C. DeRoo. 1981. The fate of nitrogen applied to turfgrass. Crop Science, 21(4):531–536.

³ Kopp, K., and K. Guillard. 2002. Clipping management and nitrogen fertilization of turfgrass: growth, nitrogen utilization, and quality. Crop Science, 42(4):1225–1231.

⁴ Heckman, J.R., H. Liu, W. Hill, M. DeMilia, and W.L. Anastasia. 1999. Kentucky bluegrass responses to mowing practice and nitrogen fertility management. Journal of Sustainable Agriculture, 15(4):25–33.

percentage increased from 57.8% to 69.7%. This fact has also been confirmed by Busey⁵. Differences in weed presence were found to be not significant. However, Lipinska and Sykut⁶ reported a decrease in weed percentage when clippings were returned. N fertilization improved the grass component of the stand, agreeing with the results of Silverton et al.⁷. The unfertilized plots exhibited increased weed proportion level. Clippings management did not affect turfgrass quality (Table 1). However return of clippings increased the height of the sward. Nitrogen application decreased legumes and supported turfgrass component. More specifically it increased turfgrass quality, improved colour and sward height. The results showed that returning the clippings is beneficial, but it is also necessary to apply certain amount of mineral fertilizer in order to preserve the quality of urban lawns in a long-term time perspective.

Table 1: Average of height of sward, turf quality and colour in different management practices.

Management practices	Height of sward (mm)	Turf quality (points)	Colour (points)
Cutting – clippings removed	225.9a	7.2a	8.4a
Mulching - clippings returned	248.6b	7.2a	8.6a
0 kg ha ⁻¹ N +PK	226.1a	6.4a	7.8a
50 kg ha ⁻¹ N +PK	240.0a	7.4b	8.7b
100 kg ha ⁻¹ N +PK	242.8a	7.3b	8.6b

Various letters indicate significant differences in the column for each treatment ($P \leq 0.05$).

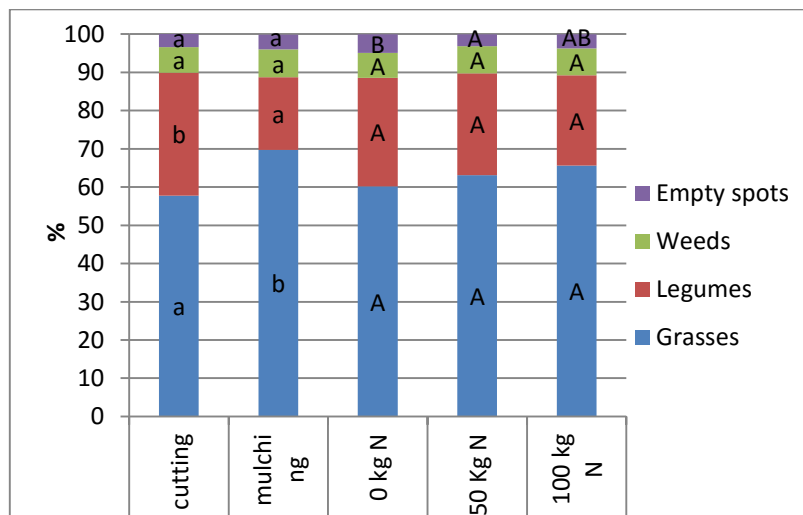


Figure 1: Effect of different management on the structure of the low-input turf. Various letters indicate significant differences between the same groups ($P \leq 0.05$).

Acknowledgement

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⁵ Busey, P. 2003. Cultural Management of Weeds in Turfgrass. *Crop Science*, 43(6):1899–1911.

⁶ Lipinska, H., and M. Sykut. 2012. The influence of cut *Festuca rubra* L. turf not removed from the lawn surface on the proportion of dicotyledons in the lawn. *Acta Agrobotanica*, 65(1).123–128.

⁷ Silvertown, J., P. Poulton, E. Johnston, G. Edwards, M. Heard, and P. Biss. 2006. The Park Grass Experiment 1856-2006: it’s contribution to ecology. *Journal of Ecology*, 94:801–814.

QUALITY ASSESSMENT OF THREE WARM-SEASON TURFGRASSES GROWING ON SHALLOW GREEN ROOF SYSTEMS WITH DIFFERENT SUBSTRATE DEPTHS

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Introduction

Green roofing is an urban greening technique that provides several environmental benefits such as amelioration of the urban heat-island effect, building energy savings, storm water management, improvement of urban landscape aesthetics and provision of new flora and fauna habitats.

Turfgrasses have the unique ability to serve aesthetics, function and recreation requirements which are demanded from urban plants¹. Turfgrasses have rarely been evaluated on extensive green roofs due to their increased water demands compared to succulents or other xerophytic plants. However, there are studies which indicate a clear preference towards usable and accessible green roof systems.

Thus, there is a need to investigate turfgrass usage on green roofs. In intensive green roofs where the buildings frame can withstand additional loading the substrate depth is adequate for turfgrass growth. In contrast, in extensive green roofs, substrate depth is minimal (2-15 cm) in an effort to reduce the load exerted to the building. Taking into account that the vast majority of city buildings are old, it is obvious that research needs to focus on retrofitted buildings using green roof systems with minimal loads. Ntoulas et al.² evaluated the establishment and growth of *Zoysia matrella* on adaptive green roof systems. They reported higher green turf cover (GTC) and normalised difference vegetation index (NDVI) values when substrate depth was 15 cm compared to a shallower substrate depth of 7.5 cm during both establishment and the water deficit periods. Ntoulas et al.³ evaluated *Zoysia matrella* performance on two different green roof substrates types and depths (7.5 and 15 cm) under two different irrigation regimes (3 mm or 6 mm every 3 days). They reported that GTC and NDVI values were mostly affected by substrate depth, moderately by irrigation regime and to a lesser extent by substrate type. Ntoulas and Nektarios⁴ reported that *Paspalum vaginatum* provided better growth at 15 cm substrate depth compared to 7.5 cm and demanded less water. They also reported that *P. vaginatum* growth at 7.5 cm substrate depth is possible but water inputs should increase by 40%. Nektarios et al.⁵ reported that *Festuca arundinacea* can grow in reduced substrate depth of 7.5 cm without being stressed compared with a substrate depth of 15 cm.

The aim of the present study is to investigate the growth response of three warm-season grasses (hybrid bermudagrass, *Cynodon dactylon* x *transvaalensis* 'Mini verde'; seashore paspalum, *Paspalum vaginatum* 'Platinum TE'; zoysiagrass, *Zoysia japonica* 'Zenith') in different shallow substrate depths of an extensive green roof system.

Methods

The study was performed from 3 Aug. until 10 Sept. 2011 and was replicated from 15 May until 11 Jul. 2012. It consisted of 18 lysimeters placed on outdoor benches equipped with a rain shelter that could be spread in the rare case of a rain event (only a single rain event occurred on the second study year). The lysimeters had an internal diameter of 30 cm. Within each lysimeter a complete layered simulation of an extensive green roof system was constructed and included: a) a protection mat at the bottom of the lysimeters; b) a drainage layer of 25 mm height with water retaining troughs and openings for ventilation placed on top of the protection layer; c) a non-woven geotextile placed on top of the drainage layer; d) a specialized green roof substrate comprised of 40% pumice, 40% thermally treated clay, 8% peat, 7% compost and 5% zeolite by volume. Half of the lysimeters had a substrate depth of 7.5 cm (shallow) and the other half had 15 cm (deep). Light

¹ Beard, J. B., and Green, R. L., 1994. The role of turfgrasses in environmental protection and their benefits to humans. *Journal of Environmental Quality*, 23(3), 452-460.

² Ntoulas, N., Nektarios, P. A., Charalambous, E., and Psaroulis, A. 2013. *Zoysia matrella* cover rate and drought tolerance in adaptive extensive green roof systems. *Urban Forestry & Urban Greening* 12(4), 522-531.

³ Ntoulas, N., Nektarios, P. A., and Nydrioti, E. 2013. Performance of *Zoysia matrella* 'Zeon' in shallow green roof substrates under moisture deficit conditions. *HortScience* 48(7), 929-937.

⁴ Ntoulas N. and Nektarios P.A. 2015. *Paspalum vaginatum* drought tolerance and recovery in adaptive extensive green roof systems. *Ecological Engineering* 82, 189-200.

⁵ Nektarios, P.A., N. Ntoulas, G. Kotopoulos, Th. Ttoulou and P. Iliia. 2014. *Festuca arundinacea* drought tolerance and evapotranspiration when grown on two extensive green roof substrate depths and under two irrigation regimes. *European Journal of Horticultural Science* 79, 142-149.

compression and leveling was applied at the substrates after their placement into the lysimeters. The zoysiagrass lysimeters (*Zoysia japonica* 'Zenith') were seeded on 13 June 2011 and the hybrid bermudagrass (*Cynodon dactylon* x *transvaalensis* 'Mini verde') and seashore paspalum lysimeters (*Paspalum vaginatum* 'Platinum') were sodded on 22 June 2011. The seeded and sodded lysimeters were maintained after the termination of the first study year and were utilized for the second study year (2012).

At the initiation of the study all lysimeters were irrigated close to saturation in order to produce uniform substrate moisture conditions. From then on, irrigation was performed at 65% of crop evapotranspiration on a daily basis, based on the evaporation of a Class-A Pan.

Measurements included: a) determination of the in situ moisture content of the substrate, just before the irrigation of the turfgrass using a dielectric moisture sensor. Measurements in substrate profiles with different depth were achieved by interchanging the sensor rods. For each rod length sensor was calibrated for the specific substrate; b) Green Turf Cover (GTC) was determined using digital image analysis. Images were acquired using a digital camera mounted on top of a sealed box, equipped with 4 fluorescent lamps that secured consistent lighting conditions throughout the image acquisition process; c) Stomatal resistance was determined using a diffusion porometer before each irrigation event, that is, at noon on cloudless days. Measurements were made on the abaxial side of young fully expanded leaves. In the first study year (2011) measurements initiated 6 weeks after establishment and lasted for another 6 weeks. In the second study year (2012) measurements started 6 weeks after the end of winter dormancy and lasted for 2.5 months.

Results and Discussion

Significant differences were observed between GTC values of the three turfgrass species and the two substrate depths. Zoysiagrass exhibited the best adaptation in the reduced depths of extensive green roof systems. At the deeper substrates (15 cm) zoysiagrass managed to sustain growth for the whole duration during both study years. In addition, it was the only turfgrass species that managed to perform well under the reduced substrate depth of 7.5 cm. During the first study year (2011) zoysiagrass at 7.5 cm substrate depth performed equally well with seashore paspalum and hybrid bermudagrass growing at 15 cm substrate depth.

During the first study year (2011) seashore paspalum and hybrid bermudagrass performed well only when grown at the deeper substrates of 15 cm. In contrast they lost their GTC values fast at the substrate depth of 7.5 cm. The same pattern was observed in the second study year (2012) for hybrid bermudagrass. However, in 2012 seashore paspalum exhibited a poor performance at both deep and shallow substrate depths.

Conclusions

Differences observed in GTC between the three turfgrass species highlight the significance of research that focuses on determining the capacity of each turfgrass species to adapt and provide sustainable growth in shallow green roof systems. Zoysiagrass seems to be one of the best options for providing an aesthetic and functional surface on the roof tops of existing and new buildings. Its growth was not hindered as much as of the other turfgrass species tested, which implies adaptation based on a potential metabolic mechanism. Seashore paspalum and hybrid bermudagrass were not as effective as zoysiagrass and actually they demand at least 15 cm of substrate depth in order to provide sustainable growth.

GOLF COURSES AS A PART OF URBAN TURF GRASS PHENOMENON: RESULTS OF SWEDISH INTERDISCIPLINARY RESEARCH

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Introduction

Golf has become a very popular game in Nordic countries during recent decades. Swedish golf courses are often located in attractive landscapes in the vicinity of nature reserves, lakes and forest margins.

The group of scientists from Swedish University of Agricultural Sciences have researched golf courses within the interdisciplinary project “Lawn as the cultural and ecological phenomenon” funded by the Swedish Research Council (FORMAS). One of the goals of this project was to study differently managed lawns, from the most intensively managed parterre type of lawns to the higher grass meadow like lawns, in social, ecological, cultural and historical perspectives¹. Due to environmental policies, the use of pesticides and herbicides has been almost totally abandoned in Swedish lawns since about 15 years ago. Today, there are a very few intensively managed parterre type of lawns left in Swedish cities. That is why golf courses due to their highest level of management intensity were the ideal final point in our urban intensity gradient of lawn’s research. However, golf courses also consist of grasslands along the whole management intensity gradient, which allowed us to compare the effect of management intensities at the same sites.

We consider golf turfs as planted grass communities (lawns) of different management intensity (fairway, rough and high rough). Due to the location of Swedish golf courses within the urban areas or in their fringes, there is a growing demand of creating multifunctional golf courses which are accessible to the public and can provide a whole range of ecosystem services. The Scandinavian Turfgrass and Environmental Research Foundation (STERF) is one of the forerunners of this modern movement in golf “ecologisation” emphasizing biodiversity in the design and management of the courses as well as lower application rates of pesticides and fertilizers. Since it is to large extent unknown how golf course management effects ecosystem services, STERF supported our interdisciplinary research for shedding light on this issue.

Methods

In this study, six golf courses in three cities in Sweden (Uppsala, Malmo and Gothenburg) have been investigated. From these courses, six holes have been sampled for both environmental (soil carbon sequestration) and ecological parameters (plant and pollinator abundance and diversity). The study also includes an inventory of maintenance activities on golf courses such as mowing, aeration, dressing, irrigation and fertilization. The project has also completed a sociological part based on surveys, interviews and observational studies of players, golf course manager and green keepers. A total number of 180 golf players and 12 golf course employees were included in the study. The observational studies in the golf environment were aimed to investigate where the visitors moved around when they were not playing golf. The interviews were aimed to ask players about their opinions, attitudes, needs and expectations on golf and golf courses.

The main hypotheses for this study were:

Soil carbon sequestration potential increases with plant biomass production.

Abundance and diversity of plants and pollinators are correlated to management intensity, with higher biodiversity in less intensively managed lawns.

Biodiversity of the golf course is related to species richness in its surroundings.

More intensive management with more inputs shows trade-offs between carbon sequestration and biodiversity but not necessarily with grass properties and recreational values for golf players.

Golf players also want the golf courses area to be multifunctional.

¹ Ignatieva, M., Ahrné, K., Wissman, J., Eriksson, T., Tidåker, P., Hedblom, M., Kätterer, T., Marstorp, H., Berg, P., Ericsson, T., Bengtsson, J. 2015. Lawn as a cultural and ecological phenomenon: A conceptual framework for transdisciplinary research, *Urban Forestry & Urban Greening*, 14: (383-387).

Results and Discussion

Social studies

The interviews indicated that the golf course and time spent on the golf course include so much more than the sport itself. For many players visits to the golf course also acted as experience of nature and beautiful surroundings as well as a meeting place in a social context, a way to stay in shape (fitness) as well as a way to relax (recreation)²). Many interviewed also stressed the importance of having golf facilities which are designed in more environmentally friendly manner. The golfers often mentioned that golf courses also need to offer something nice for the senses.

Ecological studies

There is a correlation between management type and ecological properties. Plant species richness, number of flowers and number of visiting pollinators all increased with decreasing management intensity, from the fairway to the high rough. This shows that pollinators are attracted to the flower-rich parts of the golf course. Flower-rich areas can be achieved either by constructing such areas or by ensuring that management can be performed in a way which allows for an increase in floral wealth. A higher abundance of flowers would benefit several groups of organisms.

Soil carbon sequestration

Biomass production was lowest on the greens, followed by fairways and roughs. The difference between roughs and fairways was thereby significant³. Soil organic carbon concentrations (0.93%) were significantly lower in greens than in fairways (4.0%) and roughs (4.5%). The difference between roughs and fairways was observed in the same direction as above-ground production. On average, roughs had about 10 tons more soil carbon per hectare than fairways. Alternative cutting regimes on greens and fairways are probably not feasible but the management of the rough may be optimized in order to maximize carbon sequestration.

Importance of golf from social, ecological and cultural perspectives

Golf courses have great potential to support multiple values such as biodiversity, carbon sequestration and social wellbeing. Most of the interviewees were not just dedicated golfers who enjoy the game; they combine golf as an exercise with a lot of other activities. Our results show that the golf course is often seen as a multidimensional and valuable environment.

Golf courses include large areas of land in their outskirts that are not used for the game of golf per se. Therefore, there should be potential for the use of golf courses to provide new opportunities for other social groups in addition to golfers. The development of environmentally-friendly multifunctional golf courses has to be considered in urban planning of green infrastructure. Golf courses could also have the potential to support and maintain wild flora and fauna, e.g., through wetland creation, particularly urban settings where natural habitats for rare species that are declining⁴.

² Eriksson, F., Eriksson, T. & Ignatieva, M.. 2015. Golf courses as part of urban green infrastructure: social aspects of golf courses and extensively managed turfgrass areas from Nordic perspective., Proceedings from 52nd IFLA Congress, June 6-7 2015, St. Petersburg Russia: Peter the Great Saint-Petersburg State Polytechnic University Polytechnic University Publishing House, St. Petersburg; 474-478

³ Poeplau, C., Marstorp, H., Thored, K., Kätterer, T. 2016. Effect of grassland cutting frequency on soil carbon storage - A case study on public lawns in three Swedish cities. SOIL Discuss., doi:10.5194/soil-2015-78, in review, 2016.

⁴ Strandberg, M., Blombäck, K., Dahl Jensen, A.M. & Knox, J.W. 2012. The future of turfgrass management: challenges and opportunities. *Acta Agriculturae Scandinavica Section B – Soil and Plant Science*, 1: 3-9.

TURFGRASS LANDSCAPE

POSTER PRESENTATIONS

SEASHORE PASPALUM IN THE MEDITERRANEAN TRANSITION ZONE: PHENOTYPIC TRAITS OF TWELVE ACCESSIONS DURING AND AFTER ESTABLISHMENT

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Introduction

The use of warm season turfgrasses is a consolidated trend in the transitional areas of Mediterranean countries. Several experimental trials account for the adaptation of the main species at different latitudes of Italy, Spain and Turkey for applications ranging from park lawns to golf greens^{1, 2, 3, 4, 5, 6, 7, 8, 9, 10}. While hybrid bermudagrasses have reached general acceptance in soccer pitches and golf courses, most of the turf managers consider seashore paspalum mainly for its tolerance to salt stress and limit its application to salt affected ornamental areas^{11, 12}. Results reported by Trenholm et al.^{13, 14} and Brosnan and Deputy¹⁵ have instead demonstrated that some cultivars of seashore paspalum can provide lawns with quality, wear resistance and damage recovery that are superior or comparable to some hybrid bermudagrass cultivars that are recognized reference standards for the turfgrass industry. The same studies report that shoot density seems consistently associated with traffic tolerance.

The species displays a high degree of interspecific variability with ecotypes giving a broad range of performances as to quality, tolerance to mowing, traffic, wear and other biotic and abiotic stresses¹⁶, hence assessing the relative performance of different cultivars in the Mediterranean environment may support turf managers selecting the right cultivar.

The aim of the research is to compare growth characteristics and morphological traits of eight commercial cultivars of seashore paspalum during establishment and on mature turf, grown in two locations of the Mediterranean area, in order to assess their relative turf performances and to preliminarily estimate their traffic tolerance. Furthermore, four wild ecotypes were included in the trial in order to evaluate their

¹ Volterrani, M., Grossi, N., Pardini, G., Miele, S., Gaetani, M., Magni, S., 1997. Warm season turfgrass adaptation in Italy. *International Turfgrass Society Research Journal* 8, 1344-1354

² Croce, P., De Luca, A., Mocioni, M., Volterrani, M., Beard, J.B., 2004. Adaptability of warm season turfgrass species and cultivars in a Mediterranean climate. *Acta Horticulturae* 61:365-368

³ Gomez de Barreda, D., Rivero, S., 2008. Bermudagrass overseeding with 13 cool season turfgrasses in Spain. In: Magni, S. (Ed.), *Proceedings of 1st European Turfgrass Society Conference*. Stamperia Editoriale Pisana, Pisa, Italy, pp. 85-86

⁴ De Luca, A., Volterrani, M., Gaetani, G., Grossi, N., Croce, P., Mocioni, M., Lulli, F., 2008. Warm season turfgrass adaptation in Europe North of the 45° parallel. In: Crews, D., Lutz, R. (Eds.), *Science and Golf V: Proceedings of the World Scientific Congress of Golf*, Energy in Motion, Inc., Mesa, AZ. 496-501

⁵ Geren, H., Avcioglu, R., Curaoglu, M., 2009. Performances of some warm-season turfgrasses under Mediterranean conditions. *African Journal of Biotechnology* 8, 4469-4474

⁶ Volterrani, M., Magni, S., Gaetani, M., De Luca, A., Croce, P., Mocioni, M., 2010. Bermudagrass evaluation trial in Italy. *Proceedings of 2nd European Turfgrass Society Conference*. Angers, France, April, pp. 222-224

⁷ Severmutlu, S., Mutlu, N., Shearman, R.C., Gurbuz, E., Gulsen, O., Hocagil, M., Karaguzel, O., Heng-Moss, T., Riordan, T.P., Gaussoin, R.E., 2011. Establishment and turf qualities of warm-season turfgrasses in the Mediterranean region. *HortTechnology* 21(1), 67-81

⁸ Volterrani, M., Magni, S., Lulli, F., Mocioni, M., Croce, P., De Luca, A., Grossi, N., 2012. Converting bentgrass greens to hybrid bermudagrass by transplant of single potted plants. In: Zuin, A., Aamlid T. (Eds), *Fokus*. Reviewed abstracts presented at the 3rd European Turfgrass Society conference. Bioforsk, Ås, Norway, 8, pp. 102-103

⁹ Grossi, N., Magni, S., de Bertoldi, C., Lulli, F., Gaetani, M., Caturegli, L., Volterrani, M., Croce, P., Mocioni, M., De Luca, A., 2014. Establishment and winter management of "Miniverde" Bermudagrass for putting greens in Italy. *European Journal of Turfgrass Science* 45(2), 67-68

¹⁰ Magni, S., Gaetani, M., Caturegli, L., Leto, C., Tuttolomondo, T., La Bella, S., Virga, G., Ntoulas, N., Volterrani, M., 2014. Phenotypic traits and establishment speed of forty-four turf bermudagrass accessions. *Acta Agriculturae Scandinavica* 64, 722-733

¹¹ Shahba, M.A., 2010. Comparative responses of bermudagrass and seashore paspalum cultivars commonly used in Egypt to combat salinity stress. *Horticulture Environment and Biotechnology* 51(5), 383-390

¹² Duncan, R.R., Carrow, R.N., 2000. *Seashore paspalum: The environmental turfgrass*. J. Wiley & Sons, Hoboken, NJ, 281 pp

¹³ Trenholm, L.E., Duncan, R.R., R.N. Carrow., 1999. Wear tolerance, shoot performance, and spectral reflectance of seashore paspalum and bermudagrass. *Crop Science* 39, 1147-1152

¹⁴ Trenholm, L.E., Carrow, R.N., Duncan, R.R., 2000. Mechanisms of wear tolerance in seashore paspalum and bermudagrass. *Crop Science* 40, 1350-1357

¹⁵ Brosnan, J.T., Deputy, J., 2009. Preliminary Observations on the traffic tolerance of four seashore paspalum cultivars compared to hybrid bermudagrass. *HortTechnology* 19(2), 423-426

¹⁶ Trenholm, L.E., Carrow, R.N., Duncan, R.R., 1998. Paspalum vs. bermudagrass: which is more traffic tolerant? *Golf Course Management*, July, 61-64

performance in comparison with improved commercial cultivars and potentially identify new plant material with desired turf characteristics.

Materials and Methods

Commercial cultivars of Seashore Paspalum (*Paspalum vaginatum* Swarz.) were both seed propagated (Marina and Seaspray) and vegetatively propagated (Platinum, Salam, Seadwarf, Sea Isle 1, Sea Isle 2000 and Velvetene). Ecotypes were collected from naturally occurring populations found in Italy (CeRTES 101 = warm temperate, brackish water environment), Argentine (CeRTES 102 = warm temperate, dune slacks; CeRTES 103 = warm temperate, brackish water environment) and Egypt (CeRTES 104 = Sinai peninsula, warm arid seashore sand). Trial locations were Pisa (43°40'N, 10°19'E, 6 m a.s.l.) and Palermo (38°06'N, 13°20'E, 50 m a.s.l.). Experimental plots were 1.5 by 1.5 m with 0.5 m bare soil pathways and arranged in a randomized complete-block design with four replications. Entries were established as single plants in June 2013 and left unmown during all growing season. During 2014 the plots were maintained with regular mowing at 2.5 cm.

During establishment (2013) ground cover was determined processing digital images of plots to obtain green surface percentage. The end of active growth was assumed to be at 133 DAP (Days After Planting) when no further green coverage expansion was detectable. Stolon elongation was measured weekly from 28 to 56 DAP on two representative stolons per plot. Internode diameter and length were measured on ten node-internode units per plot randomly collected at 84 DAP. Growing Degree Days (GDD) were calculated using the formula $GDD = \{[\text{Max. Temperature } (^{\circ}\text{C}) + \text{Min. Temperature } (^{\circ}\text{C}) / 2]\} - 5^{\circ}\text{C}$ ^{17, 18}, and Cumulated GDD at 133 DAP were calculated to compare growth data of the two locations.

The year following establishment (2014) spring green-up was weekly estimated from 15 March to 30 April 2014 when some of the entries first exceeded the 90% coverage threshold. Color (with a rating scale of 1=light green and 9=dark green), and quality (with a rating scale of 1 = poorest and 9 = best) were visually assessed at 30-day intervals from 1 May to 1 October. In the second week of October 2014, at both testing sites, on one 50 cm² core sample per plot, the following parameters were determined: leaf width (20 fully expanded), shoot density (direct counting), horizontal stem density (stolons and rhizomes collected were measured after soil washing) and node density (nodes of stolons and rhizomes collected from the core samples were counted). Fall color retention was weekly estimated from 15 November to 30 December when one of the entries first reached full dormancy.

Data were subjected to analysis of variance and significant different means were separated using Fisher's Least Significant Difference (LSD) at the t-probability level of 0.05.

Results and discussion

The study has highlighted a noticeable variability of aesthetic and morphological traits among the compared entries. During the establishment year, stolon growth rate was on average faster in Palermo, probably due to the higher mean minimum temperatures. Green cover values at 133 DAP were also higher in Palermo while morphological traits such as internode length and diameter had on average higher values in Pisa.

Vegetatively propagated entries gave in general the best performance as to quality and fall color retention. Platinum, Sea Isle 1 and Sea Isle 2000 proved also relatively quick to establish and ranked among the denser entries. Based on density data, these cultivars might reasonably replace some bermudagrasses in trafficked turfs¹⁹. Sea Isle 2000 also ranked first for texture and had an early spring green up.

Seed propagated entries Marina and Seaspray were as quick to establish as the quickest vegetative cultivars, however color, density and quality of the mature turf were lower.

CeRTES 104 was the only wild type showing several desirable traits. While relatively slow to establish, its density and color ranked among the top performing cultivars, this producing a turf quality comparable to the best vegetative accessions with potentially superior wear tolerance.

¹⁷ Baskerville, G.L., Emin, P., 1969. Rapid estimation of heat accumulation from maximum and minimum temperatures. Ecology 50(3), 514-517

¹⁸ Serena, M., Leinauer, B., Sallenave, R., Schiavon, M., Maier, B., 2012. Turfgrass establishment from polymer-coated seed under saline irrigation. HortScience 47(12), 1789-1794

¹⁹ Trenholm, L.E., Duncan, R.R., R.N. Carrow., 1999. Wear tolerance, shoot performance, and spectral reflectance of seashore paspalum and bermudagrass. Crop Science 39, 1147-1152

GOLF COURSE MANAGEMENT STRATEGIES FOR IMPROVING BIODIVERSITY IN NATURALIZED ROUGHS

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Introduction

Many golf courses are interested in reducing turfgrass mowed areas and replace them with low-maintenance habitats such as mulched beds, naturalized roughs, and wildflower banks in order to reduce inputs and increase biodiversity^{1,2,3}. Golf courses are mainly characterized by plant communities with simplified botanical composition thus improving naturalized rough would help to enhance biodiversity as well as the aesthetic value of the site. Few studies investigated the importance of naturalized roughs in plant⁴ and wildlife⁵ biodiversity and especially their role in improving the complexity of golf course habitat and the surroundings.

Field surveys were performed in 2013 and 2014⁴ to investigate vegetation structure and botanical composition of recently naturalized roughs in La Montecchia Golf Course, located in the Venetian Valley of northern Italy. In addition, a plot-trial was conducted for three years (2013-2015) in two selected areas of the naturalized roughs to evaluate the efficacy of different cultural practices in increasing plant population. The areas were characterized by reduced plant biodiversity due to excessive competitiveness of pre-existing or invasive species and residual soil fertility.

Methods

A field study was conducted at La Montecchia golf course in Selvazzano, north-eastern Italy (45°23'N, 11°45'E, and elevation 18 m) from April 2013 to May 2015 to determine number and abundance (%) of plant species of recently naturalized roughs.

Botanical surveys were performed with modified Braun-Blanquet method. Effective species numbers of order $q=0$ and $q=1$ were calculated for each homogeneous section of the naturalized roughs; effective number of order $q=0$ corresponds to species richness, while the order $q=1$ takes into account the influence of species rarity⁶ down-weighting species with low abundance. Each section was classified on the base of its vegetation structure considering distribution in space of herbaceous, shrubby and woody layers: A) herbaceous layer without shrubs and trees; B) herbaceous layer and scattered trees; C) herbaceous and shrubby layers eventually with scattered trees; D) dominant woody layer (ground cover of 100%), referable to a forest structure; E) ditches and ponds. Matrix of species was subjected to hierarchical cluster analysis: the distance matrix was computed with the Euclidean method and average linkage method⁷ was used to group data.

With the aim to rise plant biodiversity, a three-year study (2013-2015) was conducted in two selected areas of the naturalized sections dominated by *Agropyron repens* L. (Site 1) and *Festuca rubra* L. (Site 2). Three cultural practises were compared in a randomized complete block design with three replicates (plot size 12 m²): a) mowed with biomass removal followed by verticutting; b) mowed with biomass removal followed by verticutting and supply of late-cut hay deriving from nearby permanent meadows, to provide seeds of local species; c) unmowed (control). For both sites cuts were made once in spring 2013, for treatment b the hay was distributed immediately after cut. The botanical surveys were performed in each spring season of 2013, 2014, and 2015.

The number of species and the percentage of dominant species were subjected to ANOVA performed separately for each site, and species matrices were used for principal component analysis (PCA). All statistical analysis was performed using R 3.1.3 (R Development Core Team) software.

¹ Brame, R.A. 2012. Tall grass rough... or natural rough? <http://www.usga.org/course-care/2012/06/tall-grass-rough-or-natural-rough-21474847128.html>. USGA.

² Gross, P., T. Eckenrode. 2012. Turf reduction template: A guideline for reducing turf acreage while maintaining golf course quality. USGA. Green Section Record, 50:1-5.

³ Swift, S. 2012. Golf: Operation Pollinator. Griffin Gate Newsletter, pp.4

⁴ Pornaro, C., S. Macolino, and A. De Luca. 2014. *Acer*, 3:39-43.

⁵ Dobbs, E.K. 2013. Enhancing beneficial insect biodiversity and biological control in turf: mowing height, naturalized roughs, and operation pollinator. University of Kentucky, Lexington, Kentucky.

⁶ Pornaro, C., M.K. Schneider, and S. Macolino. 2013. Plant species loss due to forest succession in Alpine pastures depends on site conditions and observation scale. *Biological Conservation*, 161:213-222.

⁷ Ward, J.H. 1963. Hierarchical grouping to optimize an objective function. *Journal of the American Statistical Association*, 58.301:236-244.

Results and discussion

The golf course surface is approximately 64 ha, of which 19% are naturalized roughs having a total of 131 species. Vegetation structure was mainly referable to type D including 40 homogeneous rough sections, while types A, B, C, and E counted respectively 16, 11, 22 and 8 sections. The cluster analysis returned 4 groups with group 1 clearly separated from the others and including sections referring to type D. The other groups were less clearly separated and included different vegetation types (data not shown). However, botanical composition of sections referring to group 2 was dominated by *A. repens* with a great abundance (30%) of *Cynodon dactylon* (L.) Pers., while group 3 included sections dominated by *C. dactylon*, *A. repens*, and *F. rubra*, and group 4 sections dominated by *F. rubra* with a percentage of *Festuca arundinacea* Schreber and *C. dactylon* ranging from 10 to 60. Studied sections differed for dimension and effective species number of both orders ($q=0$ and $q=1$). Number of species ranged from 5 to 43, with lower values in group 1. Since for the calculation of effective species number of order 1 the species with low abundance were sequentially down-weighted, the botanical composition resulted simplified in group 1 with few species having high percentage cover, and more complex in the other groups, although dominated by one or two species only.

Regarding plot trials, results of ANOVA for Site 1 revealed a significant effect of treatment and time on number of species, displaying higher values for treatments *a* than treatment *b* (9 vs 5) and highest values in the last year of investigation. The percentage of dominant species were affected only by time, revealing that treatments were not able to limit the dominance of *A. repens*. For Site 2, a significant interaction between treatment and time was found for species richness while, similarly to Site 1, the percentage of dominant species was affected only by time. Treatment *a* showed higher number of species in 2014 and 2015, demonstrating that the introduction of external seeds is able to improve biodiversity, but not to reduce the dominance of *F. rubra* (data not shown). The PCA showed that, in 2013, botanical composition was similar in all plots for both sites, while in 2014 a shift was observed in plots where treatments *a* and *b* were applied (Fig. 1). Marked changes were observed for treatment *a* of Site 2. In the last year of investigation (2015), the botanical composition returned to its original status.

According to other studies^{8,9,10,11,12} our results suggest that cultural practices are necessary to increase the number of species and to promote the complexity of botanical composition. However, a single cut was not sufficient to reduce the abundance of dominant species (*A. repens* and *F. rubra*), even when local hay was added as external seed source.

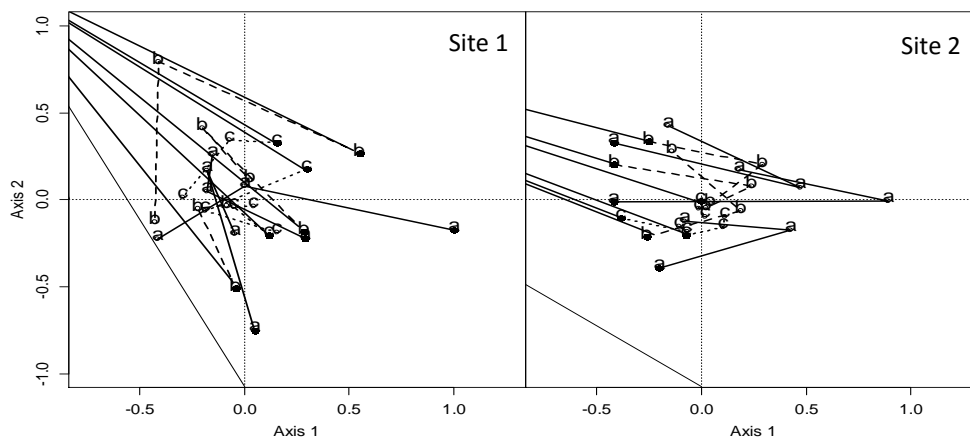


Figure 1. Principal component analysis (PCA) of Sites 1 (on the left) and Site 2 (on the right) along the first two axes. Full dots are plots surveyed in 2013; empty dots are plots surveyed in 2014 and 2015. Letters *a* (mowed and hay added, solid line), *b* (mowed, dashed line) and *c* (control, dotted line) indicate cultural practices. Dots of each block are joined with a line along time gradient.

⁸ Voigt, T. 1993. Ornamental native grasses. *Grounds Maintenance*, 28(3):48-57.

⁹ Voigt, T. 1996. Native grasses flourish on Midwestern golf courses. *Golf Course Management*, 64(11):58-62.

¹⁰ Voigt, T. 2000. Native Midwestern plants for golf course landscapes. *Ergenia*, 18:56-63.

¹¹ Voigt, T. 2001. Native plants for Midwestern golf courses. *Golf Course Management*, 69(12):63-67.

¹² Voigt, T., and J. Tallarico. 2004. Turf and native grasses for out-of-play areas. *Golf Course Management*, 72(3):109-113.

MULTIFUNCTIONALITY ON URBAN GOLF COURSES – A CASE STUDY IN THE STOCKHOLM AREA

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Introduction

Urban landscapes are today home to the majority of the world population and it is prognosed that 66% of the world's population will live in cities by 2050¹. The Stockholm region is no exception, with an expected population increase of 32% between 2014 and 2045². Consequently, urban green areas are decreasing due to exploitation and the remaining green areas for recreation will be used by more people³. The decreasing amount of green area per capita increases the demands on these areas to provide multiple ecosystem services. These general challenges require new strategies and solutions on how to plan, design and manage green space in urbanizing landscapes. Multifunctional urban golf courses have a large, mostly untapped potential to provide ecosystem services in our future cities^{4,5}.

Managed turfgrass areas such as golf courses, sport fields, landscaped amenity areas and public parks all provide an important social, environmental and economic resource for both urban and rural communities. These areas serve a multifunctional purpose, by offering valuable open spaces for recreation, helping to improve the health and quality of life for individuals, and when designed and managed appropriately, can enhance biodiversity and support regulatory targets for environmental protection. Conversely, where turfgrass management practices are inadequate or inappropriate, their services to society are reduced, and their impacts on the environment can be damaging and costly⁶.

Of the 6,740 golf courses in Europe, covering approximately 400,000 hectares of land, many can provide important wildlife habitat⁷ while also offering green space for local communities⁸. However, it is an ongoing debate whether golf courses limit public access to green spaces, and thereby the possibility for outdoor recreation, or help protect green space for urban residents. In Scandinavia, this discussion has been framed by a strong legal support for the Right of Public Access, which provides general access to the countryside. In areas with restrictions on the freedom to roam and infrequent recreational use prior to the golf course establishment, the introduction of inviting elements such as information signs, paths and public resting areas may actually increase the use⁵. The financial crisis of recent years has put many golf courses under pressure, and a broader use could improve the economy of the clubs and strengthen local partnerships as well as the role of golf in society⁹. In order to preserve golf courses from exploitation it is important that the golf course host other activities apart from the game of golf and that the land is used and be accessible to a diversity of actors.

The aim of this study was to identify important prerequisites for multifunctional activities for example outdoor recreation, sports, education, and experience of nature on urban and peri-urban golf courses and in their surroundings.

Methods

The case study was conducted in May 2015¹⁰ at two golf courses in the urbanizing southeastern part of Stockholm, Sweden; Nacka golf club and Björkhagen golf club. Nacka golf course is an 18-hole course

¹ UN. 2014. World Urbanization Prospect. United Nations.

² Statistiska Centralbyrån (SCB). 2014. Stockholms län – Huvudrapport; Befolkningsprognos 2014-2023/45, 2014:4, Stockholm: Statistiska centralbyrån.

³ Civilutskottet, Trafikutskottet & Jordbruksutskottet. 2011. Hållbara städer – med fokus på transporter, boende och grönområden, Stockholm: Riksdagstryckeriet. 2010/11: FRF3.

⁴ Colding, J. and C. Folke. 2009. The role of golf courses in biodiversity conservation and ecosystem management. *Ecosystems*, 12:191-206.

⁵ Sandberg, O.R., H. Nordh and M.S. Tveit. 2016. Perceived accessibility on golf courses – Perspectives from the golf federation. *Urban Forestry & Urban Greening* 15: 80–83.

⁶ Strandberg, M., K. Blombäck, A.M. Dahl Jensen, J.W. Knox. 2012. The future of turfgrass management: challenges and opportunities. *Acta Agriculturae Scandinavica Section B – Soil and Plant Science*, 1: 3-9.

⁷ Saarikivi, J. 2016. Biodiversity in golf courses and its contribution to the diversity of open green spaces in an urban setting. Helsinki University, Doctoral dissertation.

⁸ Golf Europe. 2016. A plan for sustainable development – people, passion, purpose. Golf Europe Place de la Croix-Blanche 19, P.O. Box 110, 1066 Epalinges, Switzerland. 47 p.

⁹ Strandberg, M. 2011. Multifunctional golf courses an underutilised resource. STERF, Box 84, SE 182 11 Danderyd, www.sterf.org 31 pp.

¹⁰ Acknowledgement to research assistants: Maria Isaksson, Maria Sima, Emma Hillström, Patrik Isacson and Jonas Andersson

partly located in a nature reserve. The club was established in 1989, the golf course was completed in 1994 and then expanded in 1997. Nacka golf club has been recognized by the Golf Environment Organisation (GEO), which certifies environmental friendly management. Björkhagen golf course was built 1972 and has nine holes embedded in the Nacka nature reserve. The golf course is managed by the golf club, which leases the land from Stockholm municipality. Approximately three times as many people live around Björkhagen as Nacka; 18 000 inhabitants and 6000 inhabitants respectively. Data on present multi-functional uses and the landscape elements supporting them was collected by site observations. Attitudes to different uses were collected by structured interviews with 10 golfers and 10 non-golfers per area, selected randomly on site. In addition, semi-structured interviews were performed with representatives from the two golf clubs regarding their work and views about multi-functionality.

Results and conclusions

Both Nacka and Björkhagen golf courses are accessible to other visitors than golfers. There were more visitors and a higher number of different activities at Björkhagen Golf course than at Nacka golf course. This can be explained by a larger population, better communications, and a lake located in the Björkhagen area. Members at Nacka Golf Club were slightly more positive than members at Björkhagen Golf Club to the golf course hosting other activities in addition to golf. The reason for this could be the very high number of non-golfers visiting the golf course at Björkhagen, creating tensions between different users. In general, non-golfers generally saw the areas as available for recreation. Over all, differences in golfer’s attitude to multi-functional use of golf courses have low impact on the number of visiting non-golfers.

In the Björkhagen area the most important use aspect for visitors and golfers was the proximity of the golf course to the city of Stockholm (Fig. 1). Björkhagen has several public transportation nodes with frequent service within 1 km from the golf course, while public communications in the Nacka area are limited. Both Björkhagen golf club and Nacka golf club have landscape elements that attract visitors. A lake with a beach located in the Björkhagen area could explain the high number of different activities on the golf course. Similar landscape elements can be found in both courses. Seventeen out of 20 respondents in Nacka mentioned the beautiful landscape as one – or the - reason why they visited the area, which is nearly three times as many as in the Björkhagen area (Fig. 1). Since the number of multifunctional activities is lower in Nacka, a conclusion could be that a beautiful landscape as such does not promote multifunctional activities, but does attract visitors. This study shows that prerequisites for multi-functional activities and non-golf visitors on golf courses are more related to the surrounding landscape elements and a high accessibility, rather than to the attitudes of golfers and non-golfers using the peri-urban golf courses and green areas.

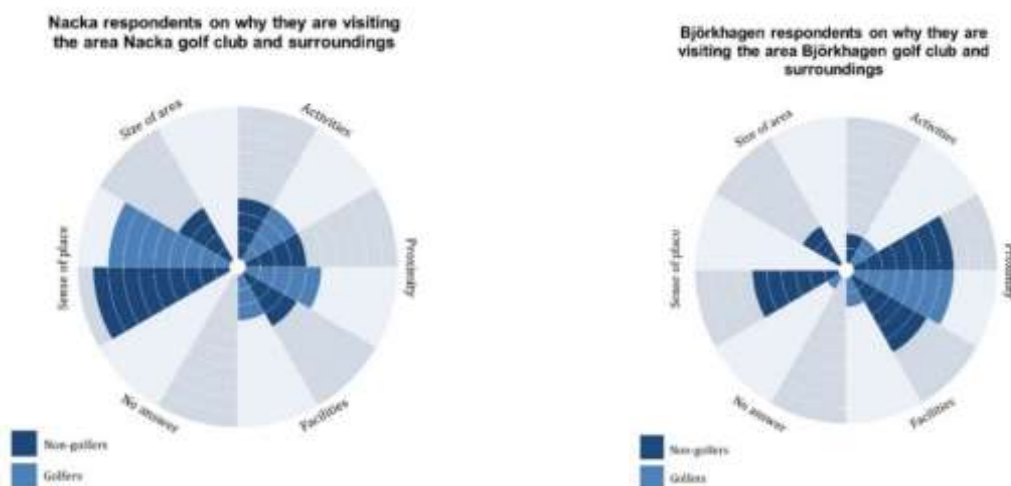


Figure 1. Respondents answers on why they are visiting the area. Visitors to Björkhagen study area are more attracted by the proximity of the area while visitors to Nacka study area are more attracted by the sense of place.

HARD AND CHEWING FESCUES SUIT THE BEST FOR ALLEY GRASSING IN FRUIT ORCHARD UNDER DRY CONDITIONS

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Introduction

Grassing of alleys in fruit orchards has become a standard management practice. Permanent vegetation cover protects soil against erosion, increase soil fertility, facilitates traffic, and improves plant species diversity and pollination. However in areas with lack of precipitation a serious competition for water may occur between orchard trees and alley grass plants. Since there is a correlation between water consumption and biomass production, the current study evaluated the height and compressed height of different turfgrass species and one commercial mixture as well as weed encroachment and proportion of bare soil in an effort to determine turfgrass persistence and competition capacity.

Methods

The study was established at the University Farm Žabčice (49°1'N, 16°35'E), Czech Republic. The climate is warm and dry with an average yearly precipitation of 480 mm and average temperature of 9.2 °C. The soil is classified as haplic chernozem arenic, and contains 60% sand, 25% silt and 15% clay. The trial was sown on 28 April 2009 in alleys of sour cherry orchard (trees spaced at 5 m x 3 m) without irrigation system. The experimental plots were established in three replicates, having a plot size of 6 m x 3 m. Individual plots were divided by fallows having an area of 3 x 3 m.

Five different species or mixtures were tested along with an unseeded control: a) Hard fescue (*Festuca trachyphylla*), cv. Štěpánka, at seeding rate of 5 g m⁻²

b) Chewing fescue (*Festuca rubra*), cv. Protenza (dwarf variety with blue-grey leaves), at a seeding rate of 5 g m⁻²

; c) Tall fescue (*Festuca arundinacea*), cv. Barfelix, at a seeding rate of 8 g m⁻²

; d) White clover (*Trifolium repens*), cv. Klement, at a seeding rate of 2 g m⁻²

; e) a commercial mixture of strong creeping red fescue (cv. Tagera) 40%, perennial ryegrass (cv. Sport) 35%, smooth stalked meadow grass (cv. Lato) 10%, *xFestulolium krasanii* (cv. Bečva) 10% and white clover cv. Huia) 5%. Although the mixture was offered as turfgrass mixture for alleys in fruit orchard, forage varieties were included. The seeding rate of the mixture was 5 g m⁻²

and f) Fallow land – no seeded bare soil.

Fertilizers were applied in sowing year using Entec (Compo) and ammonium sulphate totalling 103 kg N, 23 kg P and 23 kg K ha⁻¹. In 2010 a single application of ammonium sulphate at 40 kg N ha⁻¹ was applied in. No other fertilisers were utilised until 2015.

During the first year 3 mowings were performed and clippings were returned. During the first year the stands were not evaluated. In following years turfgrasses were mowed 2–5 times per year depending on the precipitation and the growth rate of the turfgrasses. The first mowing was performed usually in the middle of May during the heading stage of early hard fescue. Cutting height was 70 mm. Stands height was measured at 10 locations in each plot and their mean value was used for statistical analysis. Weed coverage and areas without vegetation were estimated visually. Dry matter production of above ground biomass was measured by cutting an area of 1 m² per plot and drying biomass at laboratory temperature for 5 days.

The compressed height (comprising of plant height and productivity of sward) of the turfgrasses was measured by rising-plate meter¹ immediately before the first cut. Statistical analyses were performed using one-way ANOVA (Statistica 9.1, StatSoft) with multiple comparisons according to Fisher LSD test (P < 0.05).

Results and Discussion

Hard fescue provided the best visual quality from an aesthetical point of view, with low aboveground growth and minimal weed encroachment. White clover almost disappeared in 2013 and this it is not considered as a suitable species as monostand for more than 3 years. Commercial mixture recommended for vineyards contained forage varieties of grasses and produced the most biomass with red fescue dominating from 2012

¹ Castle, M.E. 1976. A simple disc instrument for estimating herbage yield. Journal of the British Grassland Society, 31:37–40.

and on. Tall fescue was a persistent species but exhibited increased growth along with deep rooting that resulted in increased competition for water. The red fescue cultivar 'Protenza' was bred for extensive use and was the least productive but provided a thin stand with increased weed encroachment and open spaces indicating a low competitiveness ability. It is an early blooming variety which flowered usually at the beginning of May and can produce seeds before the first cut what influences the stand quality.

The main weed and unsown species which occupied fallow and bare soil in tested plots were: *Artemisia vulgaris*, *Bromus molis*, *Bromus sterilis*, *Bromus tectorum*, *Calamagrostis epigejos*, *Cirsium vulgare*, *Convolvulus arvensis*, *Hordeum murinum*, *Lolium perenne* and *Taraxacum sect. Ruderalia*. The fallow plots were covered mostly by annual grasses which did not cover the soil equally and did not ensure sufficient stability for traffic through the alleys after rain events. Turfgrasses regularly entered into summer dormancy during drought periods. The stands characteristics of the new and mature turfs are presented in Tables 1 and 2.

Table 1: Stands characteristics in the 1st growth during the 1st growth (8 May 2015, 6 years after establishment). The letters indicate statistically significant differences among values within columns.

Treatment	Sward height (mm)	DM production (g m ⁻²)	Weeds (%)	Empty areas (%)
Hard fescue	276 ^b	290 ^b	5	6.3 ^{ab}
Red fescue	337 ^c	273 ^{ab}	12,7	13.3 ^b
Tall fescue	273 ^b	315 ^{bc}	2,3	4.3 ^a
White clover	233 ^a	226 ^a	12.0	5.7 ^a
Forage mixture	398 ^d	346 ^c	0	2.7 ^a

Table 2: Stands characteristics during the 1st growth (17 May 2015, 6 years after establishment). The letters indicate statistically significant differences among values within columns.

Treatment	Height of inflorescens (mm)	Height of leaves (mm)	Compressed height (mm)	Weeds (%)	Empty areas (%)
Hard fescue	471 ^a	311 ^b	181 ^{ab}	3.3 ^a	3.3 ^a
Red fescue	441 ^a	238 ^b	155 ^a	21.7 ^b	5.3 ^a
Tall fescue	570 ^b	390 ^c	215 ^{bcd}	1.7 ^a	6.3 ^{ab}
White clover	664 ^c	112 ^a	251 ^{cd}	53.3 ^c	34.7 ^c
Forage mixture	646 ^c	397 ^c	262 ^d	1.0 ^a	5.3 ^a
Fallow	668 ^c	X	201 ^{abc}	61.7 ^c	38.3 ^c

Conclusions

There are significant differences among turfgrass species used in fruit orchards alleys in terms of aboveground biomass production, stand height, weeds encroachment and coverage. The most suitable species appear to be the hard and chewing fescues whilst mixture of forage varieties and tall fescue provide lush growth which will probably compete markedly for water with orchard trees. White clover can be used in mixture with grasses but its persistence in cutting management is short-lived.

HEAVY METAL LEACHING FROM ADAPTIVE GREEN ROOF SYSTEMS SODDED WITH TALL FESCUE

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Introduction

Over the last decades several scientific studies have been accomplished worldwide concerning green roofs utilization and their prolific environmental benefits in degraded urban landscapes. Nowadays the integrated and multifaceted design of green roofs seems to be an appropriate solution for the major problem of air pollution that negatively affects human health. Green roofs have several advantages such as noise absorption, improvement of the roof thermal insulation, fire resistance, protection against radiation, urban microclimate improvement and urban heat island abatement and amelioration. Their capacity to act as elevated urban biofilters has been long been identified and investigated. Rowe (2011)¹ provided a thorough review of green roof potential to retain and/or absorb air pollutants such as organic compounds, nitrous oxides (NO_x), sulfur dioxide (SO₂), carbon dioxide (CO₂), particle matter smaller than 10 μ (PM₁₀) and others. Yang et al. (2008)² utilized a dry deposition model and estimated that Chicago green roofs are capable of removing annually 1675 kg of air pollutants including O₃, NO₂, PM₁₀, and SO₂.

Research has been shown that plants exhibit variable capacity in retaining and/or absorbing air pollutants. Speak et al. (2012)³ reported that two turfgrass species (*Agrostis stolonifera* and *Festuca rubra*) were more effective in capturing particulate matter smaller than 10 μm (PM₁₀) compared with *Plantago lanceolata* and *Sedum album*. The use of turfgrass species on green roof systems has been extensively researched in the recent years (Ntoulas and Nektarios, 2016⁴; Ntoulas et al., 2012⁵, 2013a⁶, 2013b⁷). Turfgrasses have the unique capacity to meet all three requirements for plants used in urban environments, namely aesthetics, function and recreation (Beard and Green, 1994)⁸. Moreover, their use on urban green roofs might improve the entrapment of air pollutants. Therefore, the aim of the present study is to evaluate the combined effect of turfgrasses and green roof systems to retain heavy metals resulting from air depositions.

Material and Methods

The outdoor study was conducted from 25 July to 30 Nov. 2014. Fourteen (14) orthogonal lysimeters having dimensions of 110 cm wide × 210 cm long × 35 cm height were on the roof of the library building at the Agricultural University of Athens, Greece (37°59' lat., 23°42' long.). Each lysimeter was thermally insulated at the bottom and from all four sides by 5 cm extruded polystyrene slabs. Taking into account the width of the polystyrene slabs, the remaining space within each lysimeter was 100 cm wide × 200 cm long × 30 cm in height. On top of the polystyrene slabs a waterproofing membrane was lined with the use of heated air. The containers were set at an inclination of 5° with the use of a laser leveling instrument. An outflow opening was constructed in the middle of their lowest part. The outflow opening was lined with the same waterproofing material and was connected to a PVC pipe leading the runoff to a tipping bucket system.

A complete extensive green roof layering system was simulated within each lysimeter, starting with a protection mat placed on top of the water proofing membrane. The 3 mm protection mat was made of non-rotting synthetic polyester fibers, weighing 0.30 kg m⁻², able to retain 3 L m⁻² water as manufacturing

¹ Rowe, D.B. 2011. Green roofs as a means of pollution abatement. *Environmental Pollution* 159:2100-2110.

² Yang, J., Q. Yu, P. Gong. 2008. Quantifying air pollution removal by green roofs in Chicago. *Atmospheric Environment*. 42:7266-7273.

³ Speak, A.F., J.J. Rothwell, S.J. Lindley, C.L. Smith. 2012. Urban particulate pollution reduction by four species of green roof vegetation in a UK city. *Atmospheric Environment*. 61:283-293.

⁴ Ntoulas N. and Nektarios P.A. 2015. *Paspalum vaginatum* drought tolerance and recovery in adaptive extensive green roof systems. *Ecological Engineering* 82:189-200.

⁵ Ntoulas, N., Nektarios, P. A., Spaneas, K., Kadoglou, N. 2012. Semi-extensive green roof substrate type and depth effects on *Zoysia matrella* 'Zeon' growth and drought tolerance under different irrigation regimes. *Acta Agriculturae Scandinavica, Section B-Soil & Plant Science* 62:165-173.

⁶ Ntoulas, N., Nektarios, P. A., Nydrioti, E. 2013a. Performance of *Zoysia matrella* 'Zeon' in Shallow Green Roof Substrates under Moisture Deficit Conditions. *HortScience* 48:929-937.

⁷ Ntoulas, N., Nektarios, P. A., Charalambous, E., Psaroulis, A. 2013b. *Zoysia matrella* cover rate and drought tolerance in adaptive extensive green roof systems. *Urban Forestry & Urban Greening* 12:522-531.

⁸ Beard, J. B., Green, R. L., 1994. The role of turfgrasses in environmental protection and their benefits to humans. *Journal of Environmental Quality*, 23:452-460.

instructed. A 25 mm high, 1.36 kg m⁻² drainage board with 11.8L m⁻² water storing capability was laid over the protection cloth. Then, the drainage layer was covered by a non-woven geotextile made of thermally strengthened polypropylene. The substrate for plant growth was placed on top of the non-woven geotextile. Half of the lysimeters were filled with 8 cm substrate depth, while the other half with 16 cm substrate depth. The substrate comprised of pumice, attapulgite clay, zeolite and grape marc compost at volumetric proportions of 65:15:5:15 respectively (Patent# 1008610). Each lysimeter was equipped with autonomous automated subsurface drip irrigation system.

On 19 March 2014 eight lysimeters were sodded with *Festuca arundinacea* from which four had 8 cm and the other four had 16 cm substrate depth. In addition, four lysimeters were left unplanted with bare substrate from which two had 8 cm and two more had 16 cm substrate depth. These lysimeters were used as controls to compare the effects of vegetation between planted and non-planted green roofs. The reported data from the current study is the average of the available replications of each treatment. In addition, two lysimeters were left empty without any green roof layering over the water proofing membrane in order to simulate a common building roof top without any green roof technology. These lysimeters were utilized as controls for comparing traditional roof tops with green roof systems. During the study period, rainfall was recorded with a tipping bucket rain gauge. The data series obtained from the two empty control lysimeters were compared with the rain gauge data series and it was found that they were almost identical.

The runoff from each lysimeter was guided towards the outflow opening and through a pipe to a tipping bucket system that recorded continuously the runoff volume. The volume needed to turnover each tipping bucket was predetermined and the number of tips was logged with the use of inductive proximity sensors to a PLC/Programmable Logic Controller. Data could be accessed in real time from the PLC with a PC through the internet. From each tipping bucket a specialized system retained 5% of the runoff of each tip in a container. The collected subsamples were filtered through 0.20 µm polyester filters. Ultra-trace nitric acid (≥69%) was added in order to obtain 1% nitric acid for each sample. Then, the samples were refrigerated at 5°C until analysis. Elemental profile of copper (Cu), antimony (Sb), vanadium (V) and zinc (Zn) was obtained through inductively coupled plasma mass spectroscopy (ICP-MS, Perkin Elmer SCIEX, 9000 Series) due to its capabilities for rapid ultra-trace level multi-element determinations, in comparison to AAS and ICP-AES techniques¹. LOQ's (limits of quantification) are lower than the measured values for all the elements. In order to assess the accuracy of the process the following standard reference material was used, ERM-CA713 wastewater. The obtained recoveries of the procedure were regularly in the range of 70-120%.

Results and Discussion

The concentration of all four tested heavy metals was substantially increased in the effluent runoff from the conventional roof compared with all green roof systems both with and without tall fescue sod. The increased concentration of the heavy metals was more pronounced at the first rainfall events substantiating a "first flush effect" phenomenon. This was expected since the first rainfall was moderately intense (13.5 mm) and occurred 53 days after the previous rainfall, thus permitting the accumulation of air born pollutants. The concentration of the heavy metals was constantly decreasing after the first flush event, reaching very low values after the occurrence of 5 rainfall events reaching. This concentration decrease lasted 48 days from the first intense rainfall. In contrast to the other heavy metals, Zn exhibited a similar decrease with the other heavy metals only until the fourth rainfall event. On the fifth rainfall event a substantial increase was observed that presumably was cause by an incoming air pollutant containing Zn. The cause of this increase has not been yet identified.

The behavior of the four heavy metals differentiated through the various green roof systems. More specifically, Cu exhibited a similar concentration in runoff between bare and sodded lysimeters despite the depth of the substrate except from 2 cases when the sodded and the bare substrate having a depth of 16 cm provided increased concentration compared with the shallower substrates of 8 cm depth. Similar results were obtained for Zn. In contrast Sb and V provided different concentrations between shallow (8 cm) and deep (16 cm) substrate depths. More specifically after the initial rainfall the lysimeters that were sodded with tall fescue and had a substrate depth of 16 cm provided increased Sb and V concentration compared with the lysimeters that were sodded but with 8 cm substrate depth.

Conclusions

Based on all the above findings, all tested green roof systems were able to retain substantial amounts of air pollutants compared with a conventional roof.

TURFGRASS FOR SPORTS

ORAL PRESENTATIONS

MAXIMIZING TURFGRASS PERFORMANCE: A LOOK AT MICRONUTRIENTS AND TURF NUTRITION

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Introduction

Proper fertilization of turfgrass systems is required to maintain healthy stands of turf that are capable of withstanding the use they receive. A significant amount of work has been conducted throughout the years looking at the nitrogen, phosphorus, and potassium requirements of various turfgrasses. Of these, N is the most important, having the greatest influence on color, shoot density, and growth^{1,2}. The nutrient needs of turfgrass plants for other nutrients is highly dependent on soil conditions, particularly in the case of micronutrients. With recent emphasis being placed on tissue testing for nutrient deficiencies in turfgrass systems it has become easier to gain assess the micronutrient status of their turfgrass stands. While it is important that adequate levels of micronutrients be present for plant growth and development, it is also important to recognize that the choice of fertilizer material used to make a micronutrient application can impact the expected results.

When considering micronutrient fertilizer applications, a decision is made to use either a foliar or a granular formulation. The relatively low cost of adding a micronutrient package to a granular blend has resulted in many turfgrass managers choosing to simply add them in regardless of demonstrated need, almost as if they view the decision as some sort of insurance against future problems. When including a granular micronutrient package in a fertilizer blend it is important to consider the particle distribution, distribution uniformity, and plant availability of those fertilizer materials once spread on your site.

Particle distribution is the number of fertilizer particles per area of turf. When blended with other fertilizer components such as nitrogen and potassium, the number of micronutrient particles per pound of blended fertilizer can be very low. To assess this, we counted the number of fertilizer particles per pound of material of various fertilizer raw materials. We concluded that for fertilizer particles sizes used on sport turf (SGN 220), the number of fertilizer particles in a 15-0-15 with 1% Fe, Mn and Mg (derived from Fe sulfate, Mn sulfate and sulfate of potash magnesia) applied at 300 lbs. per acre would be 107 particles per square foot. Of those particles, 2 would come from Mn sulfate, 6 from Fe sulfate, and 7 from SPM. With such a low particle count per unit area, it is unlikely that any turf response would be observed from the applied micronutrients.

Distribution uniformity is a measurement of how uniform the fertilizer particles are spread across the turf once applied. As fertilizers are blended, shipped, and spread; smaller, heavier particles tend to migrate towards the bottom of the bag. Iron, manganese and magnesium are all metals and tend to be heavier than nitrogen and potassium particles. The lack of uniformity in the bag leads to a reduction in uniformity when the fertilizer is spread and leads to some areas of turf receiving large portions of micronutrients than others.

Iron, Mn and Mg are common components of both granular and foliar agricultural nutrient programs. These elements are commonly applied as soluble salts (sulfates) blended with fertilizer materials. When applied in the sulfate form, Fe and Mn are subject to rapid conversion to insoluble compounds in the soil which reduces plant availability, particularly in high pH soils^{3,4}. In an effort to improve the availability of these nutrients when applied they are often applied in chelated forms that are designed to maintain solubility and improve plant availability, particularly in high pH soils⁵. Glucoheptonate is a relatively new chelate that is available for use in application of nutrients. While glucoheptonate is typically recommended for use as a foliar spray, there is an increasing number of applicators using it for soil applications and soil drenches despite any evidence that it is capable of maintaining metal solubility in alkaline soils.

¹ Rodriguez, I.R., G.L. Miller, and L.B. McCarty. 2001. Bermudagrass establishment on high sand-content soils using various N-P-K rations. *HortScience* 37(1):208-209.

² Snyder, H.H., B.J. Augustin, and J.M. Davidson. 1984. Moisture sensor-controlled irrigation for reducing N leaching in bermudagrass turf. *Agron. J.* 76:964-969.

³ Petrie, S.E., and T.L. Jackson. 1984. Effects of fertilization on soil solution pH and manganese concentration. *Soil Sci. Soc. Am. J.* 48:315-318. doi:10.2136/sssaj1984.03615995004800020018x

⁴ Snyder, G.H., E.O. Burt, and G.J. Gascho. 1979. Correcting pH-induced manganese deficiency in bermudagrass turf. *Agron. J.* 71:603-608. doi:10.2134/agronj1979.00021962007100040020x

⁵ Sommers, L.E., and W.L. Lindsay. 1979. Effect of pH and redox on predicted heavy metal-chelate equilibria in soils. *Soil Sci. Soc. Am. J.* 43:39-47. doi:10.2136/sssaj1979.03615995004300010007x

Information regarding Fe, Mn, and Mg glucoheptonates in soils is limited, and while their use as chelates has been investigated^{6,7}, the stability of glucoheptonates and their influence on metal solubility in alkaline soils would be valuable information that may lead to reduced fertilizer costs. The objective of this research was to determine the solubility of Fe, Mn, and Mg sulfates and glucoheptonates in alkaline soils. In addition, we evaluated turfgrass response to these micronutrients when applied both as granular and foliar formulations.

A Tavares sand (a hyperthermic, uncoated Typic Quartzipsamment) and a Fuquay loamy sand (a loamy, kaolinitic, thermic Arenic Plinthic Kandiudult) were incubated with soluble Fe, Mn, or Mg applied as either the sulfate or the glucoheptonate. At 1 h, 4 h, 1 d, and 1, 2, and 3 wk, soils were extracted with 0.01 mol L⁻¹ CaCl₂ and analyzed for Fe, Mn, or Mg. At 1 h, approximately 98 and 93% of applied Fe was insoluble in the Tavares sand and Fuquay loamy sand, respectively. The greatest differences between soils occurred with Mn solubility, with 54 and 20% rendered insoluble in Tavares sand and Fuquay loamy sand, respectively, at 1 h. Soluble Mg declined at 1 h by 10%, with no further reductions throughout the 3-wk incubation. Soil applications of Fe as sulfate or glucoheptonate should be avoided. Applications of Mn sulfate or glucoheptonate may lead to increased soil solubility immediately following the application but may rapidly decline. Magnesium, however, remains soluble for as much as 3 wks. Glucoheptonate did not increase the solubility of Fe, Mn, or Mg compared with sulfate in either soil.

In addition, we have conducted ten studies to date looking at the response of liquid- and granularly-applied Fe, Mn, and Mg in formulations that have included most common chelated and un-chelated formulations commonly used by the fertilizer industry. Foliar applications of Fe resulted in more consistent response than granular forms. The only granular source of Fe that resulted in a plant response to the application was Fe EDTA and Fe humate. Manganese and Mg applications were less likely to induce a response than Fe. The only form of Mn or Mg that was observed to increase turfgrass quality was a foliar application of Mg sulfate which occurred in only one study.

The primary reason why granular forms of Fe and Mn are not increasing turf quality is due to the rapid oxidation of these metals once they enter the soil. The incubation study clearly found that nearly all Fe was unavailable within one hour of application and most Mn was insoluble after the first day of application. While glucoheptonate formulations of Fe, Mn and Mg may provide other benefits such as foliar absorption and liquid fertilizer stability, they do not provide any advantage or disadvantage with respect to chelation in these soils. To maximize the benefit of micronutrient applications, it is suggested that foliar applications be the primary means of addressing any documented deficiencies.

⁶ Broschat, T.K., and M.L. Elliott. 2005. Effects of iron source on iron chlorosis and *Exserohilum* leaf spot severity in *Wodyetia bifurcata*. *HortScience* 40:218–220.

⁷ Guertal, E.A. 2010. Fertilization of bentgrass with commercial foliar products: Greenhouse evaluations. *Appl. Turfgrass Sci.* 7(1). doi:10.1094/ATS-2010-0914-01-RS

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Introduction

The reduction of water, fertilizer, pesticides and maintenance costs is essential to improving golf course sustainability. As a result, bermudagrass (*Cynodon sp.*) putting greens are being utilized, or considered by more and more golf courses within the southern U.S. Beard and Kenna¹ wrote that water conservation is essential in golf and other turf uses since U.S. population growth is creating a crisis of water availability. The consideration and use of bermudagrass on putting greens is reflecting golf's response to this water crisis, as well as a changing climate in recent years. Bermudagrass' inherent drought and heat tolerance, as well as the development of 'ultradwarf' (i.e. dwarf, very dense types) cultivars that can rival creeping bentgrass in putting green quality and speed, have led many golf courses to consider changing from bentgrass or older bermudagrass greens to new 'ultradwarf' bermudagrass cultivars.

Due to growing interest within the golfing industry to develop on-site testing of turfgrass cultivars, the Golf Course Superintendents Association of America (GCSAA), USGA Green Section (USGA), and the National Turfgrass Evaluation Program (NTEP) cooperated on an initial project² to evaluate new bentgrass and bermudagrass cultivars on golf course putting greens (termed 'on-site testing'). This project was established on USGA specification putting greens at: a) five (5) northern locations for bentgrasses, b) five (5) southern locations for bermudagrass, and c) five (5) transition zone locations for both species. Data was collected in 1998-2001 from thirteen (13) bentgrass locations and eight (8) bermudagrass locations, and was published on the NTEP web site annually and via a final report.

Results from the previous trial were very useful to the golf industry therefore, the United States Golf Association and the National Turfgrass Evaluation Program have partnered to develop a new putting green trial, evaluating twenty-eight (28) total entries including *Cynodon sp.* (15), *Zoysia sp.* (11) and Seashore paspalum (*Paspalum vaginatum*) (2).

Methods

Eleven locations, including seven university research centers and four golf courses from across the southern and middle tier of the United States, were selected to establish and evaluate this trial for five years. University locations were utilized, along with golf courses to evaluate this trial because 1) we found that there was good correlation between university and 'on-site' locations in past bentgrass trials³, and 2) winter injury, disease and other stresses need to be evaluated and while university sites can allow these stresses to occur, golf course cannot. Trial locations varied from east to west within the southern U.S. including: Palm Springs, California; Tucson, Arizona; College Station, Texas; Jay, Florida and Tequesta, Florida (south Florida); southeast and south central U.S.: Griffin, Georgia; Starkville, Mississippi and Fayetteville, Arkansas; and the northern limits of C4 grass use: Lexington, Kentucky; Richmond, Virginia and Bloomington, Indiana. Protective greens covers were utilized in several of the locations to minimize winter damage.

C4 species were chosen to evaluate as they require less water and generally less chemical use than C3 grasses, plus there are many new experimental C4 selections. Zoysiagrass and seashore paspalum selections were included in the trial along with bermudagrass because there is interest in zoysiagrass use on putting greens, and seashore paspalum has limited use now on putting greens in the U.S.

Maintenance parameters were developed to provide an acceptable putting surface with Stimpmeter readings of 2.7 – 3.0 m, while using a higher mowing height (3.5 – 4.2 mm) than typical (2.8 – 3.0 mm) for *Cynodon sp.* cultivars. These parameters were selected because typical maintenance practices for current bermuda putting green cultivars, including vertical mowing and topdressing, are often times too intense and costly for many

¹ Beard, J. B. and M. P. Kenna. 2008. Water Issues facing the turfgrass industry. *USGA Turfgrass and Environmental Research Online* 7(13): 1-14. <http://usgatero.msu.edu/v07/n13.pdf>.

² Morris, K. 2003. Bentgrasses and bermudagrasses for today's putting greens. *USGA Turfgrass and Environmental Research Online* 2(1): 1-7. <http://usgatero.lib.msu.edu/v02/n01.pdf>.

³ Morris, K. N. and G. L. Gao. 2002. Evaluation of new cultivars for putting greens in the U.S. In: Science and Golf IV, Proceedings of the World Scientific Congress of Golf (E. Thain, editor), p. 541-554. World Scientific Congress of Golf Trust, St. Andrews, Scotland.

medium to low budget golf courses. Therefore, an additional trial goal is to identify grasses that provide a quality putting surface with less mechanical inputs.

All entries were established with vegetative plugs in summer 2013, using 4.6 m² plots with three replications. The three different species were planted in separate blocks at each location such that maintenance practices could be varied between species, if needed. Since species were separated in blocks, statistical analysis comparing entries cannot be made between species. Therefore, we are only comparing entries within species and making general observations of species performance.

Results and Discussion

Plots were maintained to maximize establishment during 2013 with data collected on spreading rate and other characteristics. The winter of 2013-2014 was overall the coldest in the last thirty years in the U.S. Therefore, four of the northernmost locations required replanting of some or all entries. Due to the severity of that first winter, all entries were replanted in Fayetteville, Arkansas and 14 entries were replanted in Bloomington, Indiana. Lexington, Kentucky required seven entries to be replanted while one entry was replanted in Richmond, Virginia. All entries were replanted with one-half the number of plugs used in 2013, in the center of each plot in summer 2014. The winter of 2014-2015 was colder than normal as well, but no new replanting was conducted.

Percent establishment data was collected at nine of the eleven locations. Entries 'MSB-264', 'MSB-285' and 'OK-13-78-5' in general established faster than other bermudagrasses. Generally, zoysia entries 'L1F' and 'ZOYSIU' were the fastest to establish. And in general, the two seashore paspalum entries spread as fast as the best bermudas, with the best zoysia entries being considerably slower than both *Cynodon* and *Paspalum*.

Winter kill was noted in the first winter after planting, with the seashore paspalum suffering complete winter injury at Bloomington, Indiana and significant injury at Lexington, Kentucky, despite the protective cover. The bermudas suffered anywhere from 1 – 99% damage in Indiana, with the zoysias generally suffering more damage than the bermudas. We presume the increased winter damage on zoysia was due to the slower establishment and less developed root and rhizome structure, and not necessarily poorer inherent winter tolerance.

Quality ratings collected varied considerably among locations. Since data was analyzed by location and not pooled, no summarized data across all locations is available. However, it can be noted that '08-T-18', 'Sunday', 'MSB-264' and 'MSB-265' bermudas generally performed well in 2015. For zoysia, 'DALZ 1308', 'L1F' and 'FAES 1301' have shown good quality at several locations, thus far.

Ball roll data was collected by several locations in 2014 and 2015. Our goal is a ball roll distance of 275-305 cm, which is considered acceptable at medium to low budget golf courses in the U.S. Ball roll measured in 2014 was significantly influenced by establishment rate and mowing height achieved. Therefore, only one location (Starkville, Mississippi) recorded ball roll distances of at least 275 cm by the end of that growing season for any species. In 2015, several locations recorded ball roll distances of at least 275 cm, with 'Mini-Verde', 'CTF-10', 'Tifeagle' and 'Sunday' bermudagrass entries having some of the highest ratings. Some of the highest zoysia ratings were seen with '10-TZ-74' and 'DALZ 1307', although these ratings were lower than the best bermuda entries. The two seashore paspalum entries performed similar to each other, with ball roll distances comparable to many of the bermudagrasses.

ECONOMIC AND TECHNICAL ANALYSIS OF THE CONSTRUCTION AND MAINTENANCE OF PROFESSIONAL SOCCER FIELDS

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Introduction

The aim of this analysis is to compare and contrast the differences and similarities of the construction and maintenance of soccer fields, from both a technical and economic point of view. We studied two samples: the first with totally modified top soil following the USGA Specifications, all new top soil, as in USGA (United States Golf Association), called the Texas method; the second sample was natural soil mixed with amendants and sand, in order to reduce compaction. This study compares the initial investments, performance during training and finally the cost of maintenance. The drainage system was the same for both samples.

In Italy, the Italian Football Federation (F.I.G.C.) establishes guidelines for soccer field construction. These guidelines pertain to the game, but do not take into consideration turf grass and its basis.

There are many types of soccer fields, depending on its use. Important football clubs have many soccer fields used for training. This rotation of the use of the soccer fields safeguards the quality of turf grass and allows for the maintenance operation. The turf grass in the stadium is used less than training field, thus has more time to regrow.

Soccer fields are more artificial than ornamental turf grass; the combination of soil, plants and climate must be perfect and stable.

Methods

The fields studied are in Veronello, the Chievo Verona Headquarters near Verona, in the north-east part of Italy.

In this study were compared the USGA and the economic soccer field. USGA soccer fields consist of four layers: drainage, gravel, thin gravel, sand and peat. The economic ones are different in top-soil. The differences are minimal but fundamental for system efficiency. In the USGA field, the upper layer is thicker than other systems and guarantees better performance in term of footfall strength. It is made by mixture of silica sand and peat. The sand gives strength and is not a compressed material. Under this layer there is thin-gravel and gravel. The diameter of sand is proportional to the diameter of thin-gravel. The drainage system is surrounded by thin-gravel. A traditional sport field is made on natural soil, with rarely thin sand layer. The porosity and permeability are guaranteed for the first period, but later decrease. Under the soil there is drainage, but it is limited in its functionality due to its depth.

We compared the realization and maintenance costs of different types of solutions.

In the realization cost analysis, we concluded that there are major initial costs for the first method.

The different solution requires a different maintenance operation. This comparison was made analyzing 15 years divided into short, medium and long periods.

Results and Discussion

In a short period of time, the cost of the USGA and the economic method are similar, while the cost of maintenance of first method does not change in medium and long period. However, the cost of second method increases year after year.

A high quality soccer field is efficient if it's healthy. If it suffers from less irrigation, water stagnation, excessive transpiration, the combination of soil, plants, and climate is not in equilibrium and does not support stress.

In Italy there are no regulations for professional soccer field construction. This lack has led to various construction techniques, not always resulting in good performance.

This no-regulation implicate negative aspect such money loss for constant manage. The management operation are very onerous for football clubs and not always guarantee the best performance.

If the high initial cost is spread out over a long period of time, the football can better sustain the costs.

In this study, we tried to demonstrate better performance, in terms of management and economical costs, of the USGA field and the economical one.

Regulations regarding construction methods by the Italian Soccer Federation (F.I.G.C.) would be desirable.

The USGA method requires a high initial cost but, allows for the possibility to save money in the management operation. This type of field is equilibrated and healthy, because the combination between air, plants and water is in equilibrium.

Soccer fields are used heavily in fall, winter and spring, and must be able to manage rainfall without stagnation, avoiding mechanical, physical and agronomical damage.

If the high initial cost is spread out over a long period of time, the football can better sustain the costs.

In this study, we tried to demonstrate better performance, in terms of management and economical costs, of the USGA field and the economical one.

Regulations regarding construction methods by the Italian Soccer Federation (F.I.G.C.) would be desirable.

USING TURF COLORANTS AND PIGMENTS FOR POTENTIAL LONG-TERM COLOR OF DORMANT WARM-SEASON-GRASSES

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Introduction

Bermudagrass (*Cynodon spp.*) is a commonly used warm-season grass for golf courses and sports fields in warmer climatic zones of the world. Turf managers have traditionally overseeded bermudagrass with a cool-season turfgrass during the fall to maintain aesthetically pleasing playing surfaces throughout the winter period of dormancy¹. However, the recent introduction of green colorants has increased interest in these products as an alternative to winter overseeding. Pigments are short-term (~14 day) products that can provide winter color at a fraction of the cost of colorants. The objective of this study was to evaluate the combination of a pigment and colorant for season-long winter color.

Methods

Studies were conducted in central North Carolina in 2014-2015 and Clemson, South Carolina in 2015 to evaluate the combination of a pigment and colorant for season-long winter color. In North Carolina, Transition HC pigment treatments were applied at regular intervals to a MiniVerde bermudagrass green and tees at multiple rates with and without the colorant Green Lawngr. In South Carolina, the pigment PAR was applied to a TifEagle bermudagrass green at multiple rates with and without the colorant Dormant Green.

In the North Carolina study, the design was to apply Transition HC in late fall as the natural turfgrass color began to wane. One treatment was designed to reapply the pigment product on a frequent basis so that green color was maintained. In a second treatment, color was allowed to fade between each application. Both of these treatments were re-applied throughout the fall and winter. The third treatment used the pigment early in the cycle but after non-treated areas had enough cold temperatures to be near total dormancy, Green Lawngr colorant was applied. Since this is a long-lasting product, no further pigment was applied through the winter months. The green in North Carolina was covered with a geotextile cover when temperatures were expected to drop into the mid 20's F (~-2.5 C). The treatment application timings and rates are detailed in the following table. The golf green in this study was covered with a lightweight geotextile cover whenever nighttime temperatures were forecast to be below -4 °C.

The South Carolina study was initiated on 7 January just after the turfgrass on a TifEagle green went fully dormant. A low and high rate of the pigment Par SG was used for two treatments. A third treatment used the colorant Dormant Green followed with a low rate of Par SG 14 days later. The fourth treatment was sequential applications of Dormant Green on 14-day intervals. Greens used in this study were maintained with typical golf course cultural practices.

Results and Discussion

In North Carolina, the low pigment rate did not provide acceptable color when applied at two-week intervals (Fig. 1). The high rate provided acceptable color although there was a tendency for the pigment to turn bluish in color after dormancy. The combination of pigment early in the fall followed by colorant provided the best season-long color. It was noted that the covered green required substantially less product than a turfgrass that is not covered. Nearby trials on taller mown turfgrass that was not covered required at least 40% more product to maintain similar color. In addition, with frequent pigment application, rates needed to be adjusted to avoid an unnatural dark color. Although the treatment with the infrequent pigment application was a lower cumulative amount of product, by the end of the winter it was of the same quality as the frequent application. The colorant application resulted in a more natural color early in the study and was able to maintain that color throughout the winter requiring no further applications on the green.

In South Carolina, the low rate of pigment did not improve the dormant turf color to an acceptable quality following the initial application (Fig 2). The high rate resulted in acceptable quality although it had dropped

¹ Liu, H., L.B. McCarty, C.M. Baldwin, W.G. Sarvis, and S.H. Long. 2007. Painting dormant bermudagrass putting greens. *Golf Course Manage.* 75:86-91.

below an acceptable value within 10 days after application. It continued to have a sharp decline in color through the first

35 days of the study. Pigment reapplication did not result in an increased color. The colorant application followed by the pigment application at 14 days provided acceptable color for the first 14 days but then dropped just below the acceptable value despite the pigment addition. The sequential colorant application treatment provided the best lasting color. Although this area receiving this treatment did have waning color it was not as severe as the other treatments.

In summary, the pigments did not provide acceptable turf color through green-up whereas the colorant had longer lasting, acceptable quality. These studies suggest that pigments applied at regular intervals may be used for acceptable green color in the early season on semi-dormant turfgrasses but may not provide lasting acceptable color on totally dormant turf.

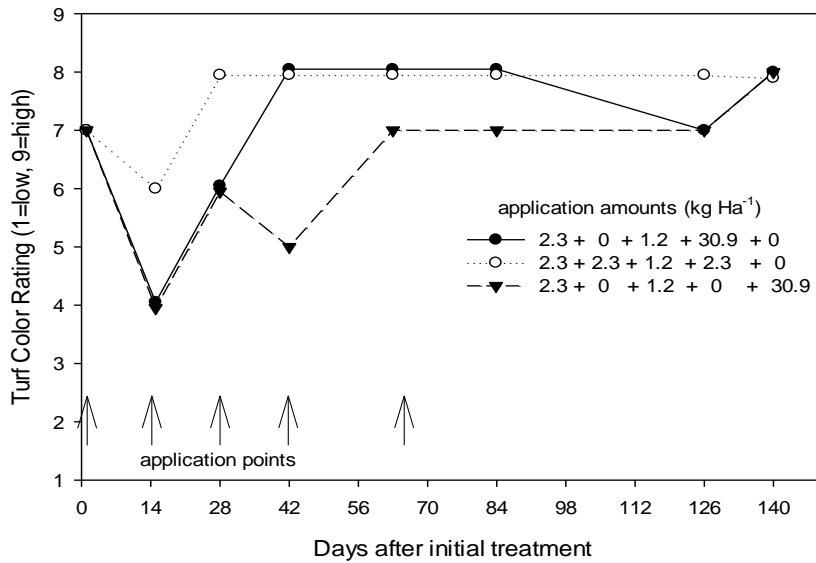


Figure 1. Turf color ratings following application of Transition HC pigment (1.2 or 2.3 L Ha⁻¹) and/or Green Lawngr colorant (30.9 L Ha⁻¹). Initial application was on 1 October 2016 in central NC.

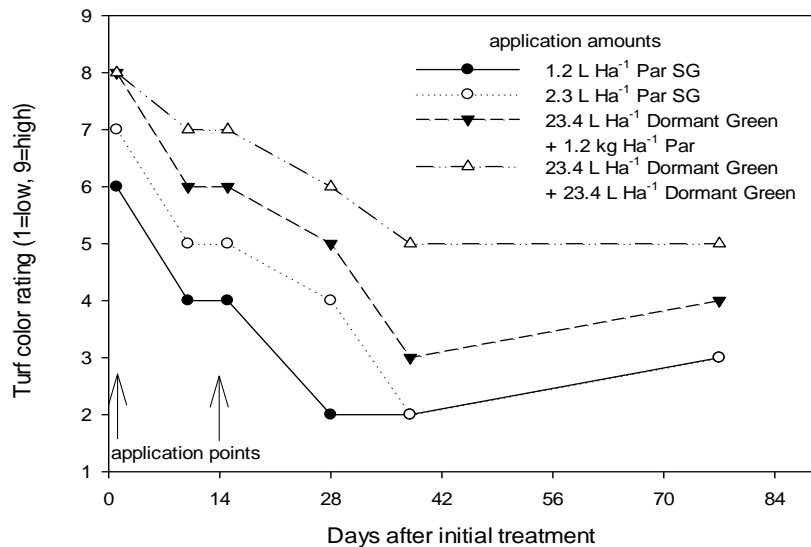


Figure 2. Turf color ratings following applications of Par SG pigment (1.2 or 2.3 L Ha⁻¹) and/or Dormant Green colorant (23.4 L Ha⁻¹). Initial application was on 13 February 2015 in Clemson, SC.

TURFGRASS FOR SPORTS

POSTER PRESENTATIONS

MANAGING GOLF GREENS: ALIGNING GOLF GREEN QUALITY WITH RESOURCE INPUTS

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Introduction

There is a need for golf course managers to manage their facilities more sustainably for both economic and environmental reasons. Golf greens are the most significant area on the golf course for both play and intensity maintenance practices. They are also one of the few areas of the golf course for which there are objective measurements of quality. Quality of greens being one of the (if not the) most important factors by which players judge golf course quality¹. Measuring the quality of golf greens has been described by Windows and Bechelet² who advocate the “Performance Measurement and Development” system developed by the Sports Turf Research Institute (STRI). The gap in knowledge here is that which lies between those who advocate the measurement of golf green performance as a tool for management² and the views of The R&A³ who support the use of reduced inputs. We can measure inputs and the quality of surfaces but there is a research need to consider these two factors together. The development of a performance measurement system for assessing maintenance inputs and their costs together with and against playing quality will potentially allow us to determine where managers are or not achieving quality playing surfaces with prescribed levels of input.

Methods

Four golf courses were selected to collect management data for operational practices and input costs together with field testing for golf greens performance. All four are 18-hole private members courses and can be categorised as parkland. They therefore exhibit the same landscape typology and have similar terrain. They are all located within 60 miles of each other in the North-West of England and have similar relief and climate. Structured interviews have been conducted with the Golf Course Managers as these afforded a suitable option for factual data collection and confirmation of practice⁴. Meetings were held to explain the purpose and nature of the research and establish the parameters for both the information required and access for field testing of greens. The core information required was established from a literature review of golf green management practices. The most significant practices for golf green maintenance were identified as mowing, fertilizer input, irrigation, aeration and topdressing^{5,6,7,8,9}. Interviews with course managers provided operational data for golf green maintenance for the core practices identified from literature including all frequencies and material inputs. Costs for materials and labour were also collected and annualised.

Golf greens on the selected golf courses were assessed for performance quality using recognised tests. These tests included those advocated by the STRI, The R&A and promoted for use to industry^{2,3}. Three golf greens on each course, judged to be the best, worst and medium for playing quality by the course manager, were selected for greens performance testing. Thus sampling was purposive as greens were identified against a criterion⁴. The tests conducted and tools/methods were Green speed (Stimpmeter), Putting consistency (Holing Out), Firmness (Clegg Impact Hammer), Soil moisture (Theta Probe) and Soil organic matter (Loss on ignition test). Field tests were conducted in July of 2013 and again in 2014. All tests were conducted using accepted protocols with replicates over the green. Tests results being aggregated and mean values calculated for analysis. Mean data for each test or factor have been ordered using a bespoke ranking scale specifically designed for on a linear scale from 1 to 10 with 1 representing the highest level of maintenance input and 10 the least. For example fertilizer input inputs of 25g/m² N is ranked as high and ascribed a numeric value of 1 whereas 8 g/m² N is ranked as low input and is 10 on the scale. The parameters for the maintenance scale were derived from the literary sources reviewed. A similar scale has been devised for golf green performance tests where 10 represents the highest level of green surface quality and 1 the lowest. Scales are formulated from

¹ Emmons, R. (1995) *Turfgrass Science and Management*. Delmar. New York.

² Windows, R. & Bechelet, H. (2009). This way. *Turfgrass Bulletin*. Issue 245

³ Isaac, S. (2012) Helping you to help yourself. *Turfgrass Bulletin*. Issue 257.

⁴ Gray, D. E. (2009). *Doing Research in the Real World*. 2nd Edition. Sage Publications Ltd. London.

⁵ Adams, W.A. & Gibbs, R. J. (1994) *Natural Turf for Sport and Amenity: Science and Practice*. CAB International. Oxon, UK.

⁶ Beard, J. B. (1982) *Turf Management for Golf Courses*. Prentice Hall. New Jersey.

⁷ McCarty, L. B. (2001) *Best Golf Course Management Practices*. Prentice-Hall. New Jersey, USA.

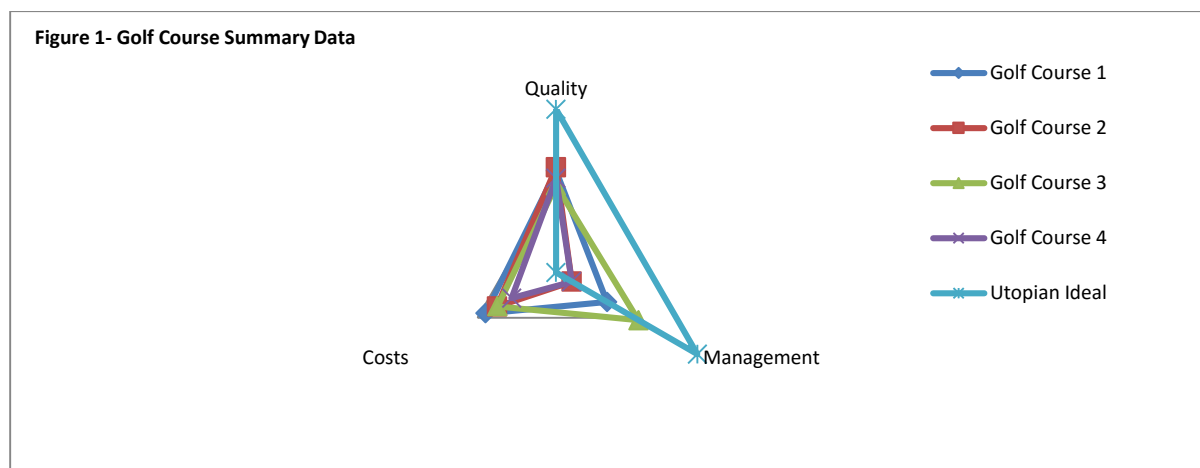
⁸ Ryan, P. (1999) *IN International Turf management Handbook* (Editor D.E Aldous). Butterworth-Heinemann. Melbourne. Australia.

⁹ Turgeon, A. J. (2002) *Turfgrass Management*. (6th Ed). Prentice Hall. New Jersey, USA.

published performance tolerances for tests conducted. For example, green speed as assessed with a Stimpmeter, is done so where the golf ball roll on the surface is measured from 4 -12 ft. On the scale formulated for this research 4ft, recognised as a slow speed for a golf green, is ascribed a value of 1 as quality is low for this metric. Other performance tests adopted, Holing Out, Firmness, Moisture content and Organic matter were also ranked in this way. Data collected from interviews and field tests then for maintenance operations, resource inputs, costs and playing surface standards for golf greens at the four sites have been aggregated. The comparison of maintenance inputs and their costs against playing quality allow objective comparison and determination of management efficacy.

Results and Discussion

Research data indicates where a golf club sits on the proposed performance measurement framework for greens performance above and the quality they are achieving with a known level of resource input. Maintenance inputs for golf greens are compared critically with recommended inputs. Results from green performance assessments can be compared against recommended tolerances for such criteria. Costs indicate expenditure in relation to maintenance practices and quality levels achieved. Similar models for assessing outcomes of works and practices exist in other fields within the built environment domain. In project management a concept known generically as “the iron triangle”¹⁰ (has been used for some time to measure the success of construction projects. Here the criteria used are cost, time and quality, however actual performance measures are not indicated and it is left to the researcher or manager to identify such. Figure 1 below represents the data accumulated from the four case study sites in 2014. This graph illustrates how the case study golf courses compare with a utopian ideal where hypothetical case where costs are low, quality is high and management efficacy is greatest.



This research proposes this performance measurement system for golf course managers to enable them to better manage their golf greens. The stated aim is to arrive at an optimum level of quality with the minimal level of input and thereby reduced economic and environmental costs. In considering existing performance management systems it has been found that none provide a specific tool that could be used to model, control, and monitor and improve the activities at the operational level¹¹. Further in the review of literature it has been found that no such system exists either generally or specifically within this research subject area for use at an operational level. The adopted research methodology and methods have produced valid results and a viable system for Golf Course managers to effectively measure their resource inputs and assess the impact of these on golf green quality. Thus it is possible that mapping the key parameters of quality, costs and inputs in a benchmarking radar chart has been shown to reflect the efficacy of golf green management in a way that allows stakeholders to identify and adjust operational variables. In a survey of 1100 Golf Course Managers in the UK conducted in the summer of 2015 73% of respondents stated that such a system would be beneficial for their work. This performance system satisfies the gap in knowledge that has been identified in literature and in practice.

¹⁰ Atkinson, R (1999) Project Management: Cost, time and quality, two best guesses and a phenomenon, it's time to accept other success criteria. Int. Jnl.Project Management. Elsevier.

¹¹ Ghalayini, A, M & Noble, J, S. (1996). The changing basis of performance measurement. International Journal of Operations and Production Management. Vol 16 Issue 8 pp.63-80.

EVALUATION OF KEY METHODOLOGY FOR DIGITAL IMAGE ANALYSIS OF TURFGRASS COLOR

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Introduction

Color is an important component of overall turfgrass quality, and is traditionally evaluated by visual ratings. However, a common criticism of the visual rating method is its subjectivity. Efforts have been devoted towards developing objective methodologies to quantify turf color differences. Currently widely adopted method combines digital imagery and computerized image analysis. Individual turf areas are first captured in digital images by mounting a digital camera at an appropriate height. The collection of images is then processed via digital image analysis to yield objective color parameters, which are used as indices of turf color. To date, the most commonly used digital image analysis for turfgrass color is one developed within the commercial software SigmaScan Pro¹. This method quantifies the mean hue angle, saturation, and brightness (HSB) values of each image, which are then used to calculate a dark green color index (DGCI) to quantify turfgrass color. While the SigmaScan method has been an effective tool in practice, there are more powerful and flexible image analysis platforms that can offer improvements². This study developed a JAVA plugin for the open source software ImageJ to batch-process turf images for color parameters and DGCI. This new method was first validated with 135 standard colors, and then examined with field plot images of five turfgrass species with each displaying intra-specific color variations. In addition to testing software performance, quantified color parameters from five species of turfgrass are further analyzed for their effectiveness in characterizing color variations, which revealed valuable findings. This work also allowed insight into how camera settings influence color analysis - a potential source of variation that has not been considered before.

Methods

A plugin in ImageJ was developed in Java programming language to batch-quantify turf images for hue angle, saturation, brightness, and calculate DGCI. Output is presented as an image-wise average of these parameters, as well as their standard deviations among image-wise pixels. Digital images (1024 × 685 pixels) were taken with a Sony α330 digital single-lens reflex camera body with a Tamron SP 10-24mm Di-II lens at manually fixed settings (18mm focal length, ISO 800, F-stop 11, calibrated white balance). A custom-built LED light box (2000 lux, 6204 color temperature) was used to provide uniform lighting for all images. Each view was taken at two shutter speed (1/50 sec and 1/20 sec) for evaluation of the impact of exposure settings on color quantification. Function and performance of photography devices as well as the developed plugin were first validated using 135 standard color chips that were of varying hues ranging from yellow to green. Afterwards, images were acquired for further analysis from five research trials at the North Carolina State University Turfgrass Field Laboratory in Raleigh, NC, USA. These included tall fescue (*Festuca arundinacea* Shreb.) of 26 cultivars, Kentucky bluegrass (*Poa pratensis* L.) of 15 cultivars, ryegrass (*Lolium perenne* L. and *Lolium multiflorum* Lam.) of 24 cultivars, 'Tifway' bermudagrass (*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burt-Davy) under 17 different fertilizer treatments, and 'A1'/'A4' blend creeping bentgrass (*Agrostis stolonifera* L.) under 5 levels of nitrogen fertility.). In addition to digital imagery, normalized difference vegetation index (NDVI, Field Scout TCM 500) and visual color assessment (1 to 9 scale, with 1 being yellow or brown) were also determined for each photographed plot. The collection of images was batch-processed by ImageJ method as well as SigmaScan method, comparing processing speed and quantification results. Meanwhile, image analysis acquired color parameters were individually analyzed to examine their effectiveness in characterizing turf color differences and correlation with visual assessment.

Results and Discussion

ImageJ offers a viable and much faster alternative to SigmaScan Pro. Both methods were able to adequately quantify HSB values of standard color chips with nearly identical results (<0.8% difference). However, ImageJ had the distinct advantage of much faster processing speed. Analyzing each 1024×685 pixel image (~400 kilobytes of size) took less than 15 sec in ImageJ but required almost 9 min in SigmaScan Pro. This amounted to considerable time saving when batch-processing a large number of images. ImageJ also offers the capability to

¹ Karcher, D., and M. Richardson. 2003. Quantifying turfgrass color using digital image analysis. *Crop Sci.* 43:943-951.

evaluate each individual pixel within the image. Previous research found that statistical moments (mean, standard deviation, skewness, and kurtosis) of quantified values within an image may be used to improve prediction models². Data analysis on quantified HSB values from field plot images revealed interesting information. First, hue angle was more consistent in identifying color variations than either saturation or brightness (Table 1). Second, hue angle was better correlated with visual assessment of turf color (Table 2). These findings indicated that hue angle was the primary component that rendered DGCI effective in characterizing turfgrass color. In fact, hue angle performed very similarly to the calculated DGCI in analysis of variance and correlation analysis. Therefore, it is proposed that hue angle by itself is an adequate objective color indicator, and can replace the role of DGCI in turf image analysis. Results of this study also demonstrated the importance of manually fixing camera settings for valid comparisons. This applies mainly to aperture and shutter speed, and use of “AUTO” mode should be avoided.

Table 1. Summarized analysis of variance (ANOVA) of visually evaluated color rating, spectral reflectometer measured NDVI, and digital image analysis quantified color parameters for five turfgrass species.

Selected Sources (df)	Visual Rating	Color	NDVI ($\times 10^{-4}$)	Image Analysis Color Parameters (shutter speed = 1/50 sec)			
				Hue Angle	Saturation ($\times 10^{-4}$)	Brightness ($\times 10^{-4}$)	DGCI ($\times 10^{-4}$)
----- <i>Mean squares</i> -----							
Tall fescue							
Genotype (25)	0.64		20.04**	42.71***	6.05*	6.46***	23.18***
Error (50)	0.43		7.73	13.23	2.81	1.99	7.65
F value	1.49		2.59	3.23	2.15	3.25	3.03
CV%	10.21		4.13	4.31	2.60	10.08	5.11
Kentucky bluegrass							
Genotype (14)	1.53***		31.97**	57.81***	5.56	3.80**	24.80***
Error (28)	0.32		8.81	9.49	4.44	1.13	5.13
F value	4.78		3.63	6.09	1.25	3.36	4.83
CV%	9.25		4.42	3.64	3.38	7.80	4.11
Ryegrass							
Genotype (23)	2.74***		35.82***	78.25***	27.33***	20.64***	65.74***
Error (70)	0.21		4.74	2.77	1.06	0.55	1.87
F value	13.05		7.56	28.25	25.78	37.53	35.16
CV%	6.90		3.01	1.68	1.51	5.66	2.23
Bermudagrass							
N treatment (16)	1.23**		17.34**	42.47*	6.45**	5.34***	20.59**
Error (66)	0.48		6.90	22.75	2.57	1.31	7.34
F value	2.56		2.51	1.87	2.51	4.08	2.81
CV%	10.52		3.75	5.72	2.91	6.17	4.92
Creeping bentgrass							
N treatment (4)	5.20***		8.30***	31.10***	0.62	1.05	11.60***
Error (16)	0.19		0.76	1.23	0.25	0.81	0.44
F value	27.37		10.92	25.28	2.48	1.30	26.36
CV%	6.03		1.14	1.26	0.80	4.50	1.21

*, **, ***, significance at the probability level of 0.05, 0.01, and 0.001, respectively.

Table 2. Pearson correlations between visual color rating and objective color parameters in five turfgrass species.

Species	NDVI	shutter speed = 1/50 sec			
		Hue Angle	Saturation	Brightness	DGCI
----- <i>Correlation coefficient with visual color rating</i> -----					
Tall fescue	0.48***	0.78***	-0.54***	-0.82***	0.81***
Kentucky bluegrass	0.54***	0.71***	NS	-0.61***	0.72***
Ryegrass	0.62***	0.77***	-0.66***	-0.72***	0.76***
Bermudagrass	0.53***	0.72***	NS	-0.53***	0.68***
Creeping bentgrass	0.80***	0.82***	NS	NS	0.83***

*, **, ***, significance at the probability level of 0.05, 0.01, and 0.001, respectively.

² Ghali, I.E., G.L. Miller, G.L. Grabow, and R.L. Huffman. 2012. Using variability within digital images to improve tall fescue color characterization. *Crop Sci.* 52:2365-2374.

SITE-SPECIFIC PRECISION MAINTENANCE OF SPORTS TURFGRASS

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Introduction

Greenkeepers of sport lawns are interested in solutions to improve the efficiency of inputs, especially related to the major cultural practices, and also to minimize any potential environmental impact^{1,2}. Plant growth varies significantly within a small area, due to a dynamic interaction between climate, plant and soil properties. The spatial variability results from a complex interaction of factors: biological (pests and microorganisms), edaphic (salinity, organic matter, nutrients, texture), anthropogenic (soil compaction); topography (slope) and climate (relative humidity, temperature, precipitation). The existence of a large spatial variability in sports turf was noted, beginning to be a need to adopt some of the strategies used in precision agriculture (PA). The primary field application in PA is to define SSMUs, where a SSMU is a sub-field area with similar soil and landscape properties that result in similar plant response, input-use efficiency, and environmental impact^{3,4,5}. With the spatial mapping of variables as soil apparent Electrical Conductivity (ECa), Normalized Difference Vegetation Index (NDVI) or Soil Water Content (SWC) and using concepts of PA, several field applications have been suggested⁶ to identify and illustrate specific field cultural practices, such as irrigation, fertilization, cultivation, and salinity management.

In this context the objective of this work was to evaluate if the apparent electrical conductivity of soils (ECa), a vegetation index (NDVI) and soil water content (SWC) may be interesting tools in the management of sports pitches in Mediterranean climates, in order to increase its agronomic, economic and environmental efficiencies.

Methods

Data collection for this study took place on the Ocean Golf Course (37° 3'17.72"N, 8° 3'51.07" W), built in Vale do Lobo in the Algarve and inaugurated in 1968. Turfgrass species used in the golf course are: *Agrostis stolonifera* on greens and a mixture of *Festuca rubra*, *Poa pratensis* and *Lolium perenne* on tees, fairways and roughs. A survey was conducted to identify the spatial variability in holes 5, 8 and 9 through specific mapping applications, including measurements of ECa, NDVI and SWC. The ECa of the soil was measured using the sensor DUALEM-1, which comprises two receivers allowing the measurements at two soil layers: 0.5 m and 1.5 m. It also encompasses a GPS receiver WAAS (Wide Area Augmentation System) procedure, which permits the registration of geographical coordinates for each reading.

Measurements of NDVI were made using the OptRx sensor, model ACS430. The OptRx sensor was synchronized with a Trimble GPS Geoexplorer 6000 series, GeoXH. To each sensor reading was given its geographical reference. Measurements of SWC were performed on the fairway and the green of hole 8. A TDR IMKO TRIME-FM system was used with a probe rod P3-3 (160 mm) to a depth of 10 cm where the roots are mainly. It was established a uniform grid with readings georeferenced 2 by 2 m. ESRI ArcGIS software, version 10 was used. The

¹ Beard, J.B., and M.P. Kenna 2008. Water Quality and Quantity Issues for Turfgrasses in Urban Landscapes. CAST Special Publication 27. Ames, IA: Council for Agricultural Science and Technology.

² Carrow, R.N., J.M. Krum, I. Flitcroft, and V. Cline 2010. Precision turfgrass management: challenges and field applications for mapping turfgrass soil and stress. *Precision agric* 11: 115 – 134. USA.

³ Duffera, M., White, J.G., Weisz, R., 2007. Spatial variability of Southeastern U.S. Coastal Plain soil physical properties: Implications for site-specific management. *Geoderma* 137:327–339.

⁴ Corwin, D.L., Lesch, S.M., Shouse, P.J., Soppe, R., Ayars, J.E., 2008. Delineating site-specific management units using geospatial ECa measurements. p. 247-254. *Em Krum, J. H., Carrow, R. N., Karnok, K. (2010). Spatial mapping of complex turfgrass sites: Site-specific management units and protocols. Crop Science, 50.*

⁵ Yan, L., S. Zhou, L. Feng, L. Hong-Yi., 2007. Delineation of site-specific management zones using fuzzy clustering analysis in a coastal saline land. *Comput. Electron. Agric.* 56:174–186.

⁶ Carrow, R.N., Krum, J.M., Flitcroft, I., Cline, V., 2010. Precision turfgrass management: challenges and field applications for mapping turfgrass soil and stress. *Precision agric* 11: 115 – 134.

extensions Spatial Analyst and Geostatistical Analyst were used to develop, display, analyze and interpret collected data. The topography of the studied holes was sampled considering a 10 m x 10 m regular grid. Using the Voronoy technique (10.0 ArcGIS, ESRI) it was performed a local outliers removal for ECa, NDVI and SWC. ECa, NDVI and SWC surfaces with a "5 m x 5 m" resolution was obtained by means of a spherical semivariogram ordinary kriging interpolation and a geo-statistical analysis.

Results and Discussion

The geo-statistical ECa range was 10.6 m in hole 8 and 8.5 m in holes 5 and 9 which indicates more ECa spatial uniformity on hole 8 when compared to holes 5 and 9. For NDVI the highest geo-statistical range was 14.7 m (hole 5) and the lowest was 4.6 m (hole 9) being this last hole more spatially variable. For SWC the range was 19.2 m being the variable more uniform on space when compared to ECa and NDVI.

NDVI results: On hole 8 the NDVI values ranged between 0.12 to 0.93 and much of its area is in class 0.80-0.85. The highest values were recorded in the central zone of the hole, especially on the fairway and on the green. Roughs had the lowest NDVI values. A considerable area of hole 9 showed lower values of NDVI. In this hole the NDVI ranged between 0.36 and 0.86. The NDVI values are mainly under 0.75. The highest NDVI values were recorded on the green, indicating the presence of a healthier and dense lawn. In every hole the greens were always the areas with the highest NDVI, with values around 0.8. From these results, it appears that the hole 9 has a less healthy lawn.

ECa results: On the hole 8 the ECa values ranged from 1.17 to 29.89 mS m⁻¹ and it lies mostly in the classes under 20 mS m⁻¹. On the hole 9 the ECa ranged between 3.99 and 62.21 mS m⁻¹ and it lies mostly in the class under 10 mS m⁻¹. The green of hole 8 is a push-up type, which is distinct edaphically from an USGA green, such is the one in hole 9. The hole 5 stands out from the other sampling sites by presenting an ECa significantly higher. In this hole the ECa values ranged between 50.34 and 106.19 mS m⁻¹. These ECa level values shows soil salinization problems on hole 5.

SWC results: Values ranged from < 40 % to > 80 % (v/v). Measurements of SWC show that this hole, to a depth of 10 cm, had a greater accumulation of water in the central zone, which showed a better turfgrass visual quality.

An evaluation between these three parameters on hole 8 shows that NDVI values and SWC are those that best correlate.

ENHANCEMENT OF GOLF COURSE MANAGEMENT ONLINE CLASSES THROUGH INSTRUCTOR VIDEOS

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Introduction

As students are increasingly connected and spending more time in the online environment, educators are creating new tools to attract and generate interest in online education. New technology has always been integrated in turfgrass science teaching and learning. As the knowledge base of turfgrass management continues to grow an important means of dissemination is through online education. Since the early 1980s online education has changed little, consisting primarily of recorded lectures, handouts and simple versions of testing. Media content is important in conveying knowledge, and it has been reported that videos improve grades and performances¹. A relatively new method of online education is through e-learning games that are reported to motivate student learning². Puzzles, scavenger hunts, cards, adventures, animations and customization of characters are some of the ideas of games used in online education. Despite technological advances in presenting online material, learners face difficulties because they feel isolated, which negatively influences the learning process. Technological tools that create some interaction between students and professors in an online environment may be the best way to overcome this problem. Students reported that asynchronous video-based communication by their instructor seemed more real, present, and familiar, and that these relationships were similar to face-to-face instruction³. Students with social presence in an online environment were likely to instigate, sustain, and support content-related communication because it becomes more engaging and rewarding, thus knowledge is more effectively transmitted. The objective of this study was to study the impact of weekly instructor videos on student involvement.

Methods

“Turfgrass Management for Golf Course Managers” (HCS3475) is an online course offering at The Ohio State University and was used to compare teaching methods effectiveness. The study comparisons are based on the 2016 course offering. The online HCS3475 had a student enrollment of 156 students. Student demographics represented a diversity of majors across 6 colleges with 4%, 47%, 34%, 17%, 18%, and 3% from Agriculture, Arts and Sciences, Business, Education, Engineering, and Health Sciences, respectively. In addition, 18% of the students were undeclared or in Exploration. Class rank was relatively evenly distributed with freshman accounting for 20% of the student population, while 27%, 26%, and 27% were sophomores, juniors, and seniors, respectively.

The course has 12 modules entitled 1) Turfgrass identification and Morphology, 2) Cool Season Turfgrasses, 3) Warm Season Turfgrasses 4) Soils and Greens Construction, 5) Mowing, 6) Fertility, 7) additional cultural practices, 8) Diseases, 9) Weeds, 10) Insects, and 11) Tournament Course Setup. The course grade was based on 12 required quizzes corresponding to each module. The quizzes consisted of 25 multiple choice questions randomly chosen from a question bank by the computer. Each student could take the quiz twice with the highest score counting. The course duration was 7 weeks.

Course material was presented using lecture recorded presentations and animations. The lecture recorded material consisted of 10 to 15 minutes recorded Microsoft PowerPoint® presentations using the iPad application Explain Everything (<http://explaineverything.com/>). Animations were produced with Tumult Hype (<http://tumult.com/hype/>). These animations consisted of puzzles, scavenger hunts, and practical demonstrations. The third major component for presenting course information was instructor videos. The videos were recorded once weekly to cover any course issues that may arise and to provide some background information on a selected module. The videos run 4 to 6 minutes.

Data collected for each module included the number of views of both lecture presentations and instructor videos. The lecture presentations and instructor videos were self-contained within the course shell (Carmen Courses & MediaSite, The Ohio State University) and only available to students enrolled in the class.

¹ DeCesare, J. A. (2014). The expanding role of online video in teaching, learning, and research. *Library Technology Reports*, 50(2), 5-11.

² Lanzilotti, R., Costabile, M. F., G., Ardito, C., (2013). Integrating Traditional Learning and Games on Large Displays: An Experimental Study. *Educational Technology & Society*, 16 (1). 44-56.

³ Borup, J., West, R. E., Graham, C. R. (2012). Improving online social presence through asynchronous video. *Internet and Higher Education*, 15, 195-203.

Results and Discussion

Based on 35 recorded lectures the average number of student views per lecture was 2.0, which remained relatively constant over the 11 educational modules (Figure 1). The average number of views per student for the instructor videos was 7.8 (Figure 1). The recorded lectures contained material that would appear on the quizzes for each module, while the instructor videos were optional and for the most part did not contain material directly related to information necessary for the quizzes. The instructor videos varied in content and location. The high rates of views of the instructor video showed that asynchronous video communication was appreciated by students and was the favorite engagement in online education⁴, because each instructor video was viewed several times by each student. The results also show that the instructor video motivated students to visit the online educational platform more often. A previous study³ reported that two thirds of the students in an online class mentioned that instructor's videos improved their learning experience. This study agrees with other researches that have shown that increasing students' sense of closeness to a class community develops a positive disposition toward effective technology integration^{5,6}.

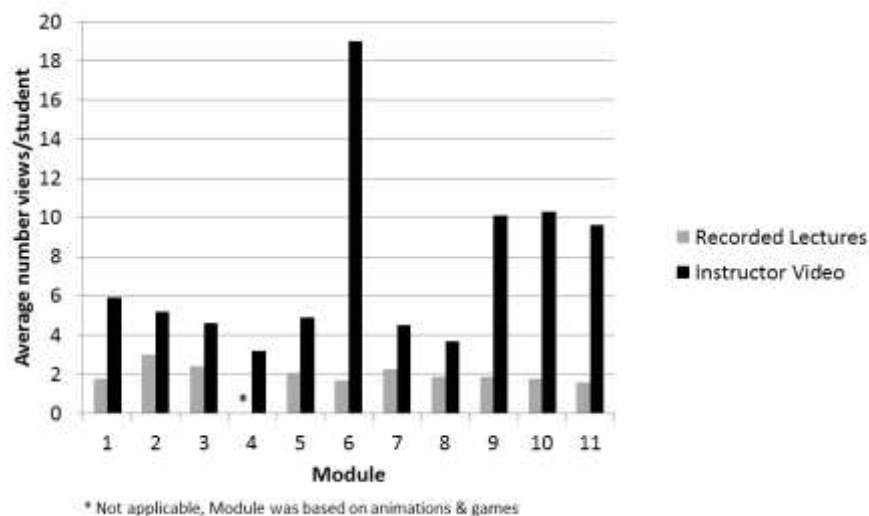


Figure 1. The average number of views per student for lecture presentations and instructor videos for 11 modules in HCS 3475 “Turf Management for Golf Course Managers”. The averages are based on 35 lecture presentations and 9 instructor videos.

⁴ B. Beyerbach, C. Walsh, R. Vannatta, (2001). From teaching technology to using technology to enhance student learning: Preservice teachers' changing perceptions of technology infusion. *Journal of Technology and Teacher Education*, 9 (1) (2001), pp. 105–127

⁵ P. A. Ertmer. (2005). Teacher pedagogical beliefs: The final frontier in our quest for technology integration? *Educational Technology Research and Development*; 53 (4) (2005), pp. 25-39

⁶ Putnam R.T and Borko, H., (2000). What do new views of knowledge and thinking have to say about research on teacher learning? *Educational Research*, 29 (1) (2000), pp. 4–15.

TURFGRASS MAINTENANCE AND MANAGEMENT IN SOCCER FIELDS IN SLOVENIA

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Introduction

Soccer is very popular sport in Slovenia, therefore soccer fields are distributed all around the country. The differences in their appearance are noticeable, as budgets for maintenance and management vary greatly (even up to 100.000 €/year). Our main hypothesis was that turfgrass in the soccer fields owned or used by clubs competing in Slovenian First League is better maintained and managed and more attractive than turfgrass in soccer fields owned by clubs competing in lower ranking competitions.

Methods

In the study, which was performed in 2015, we evaluated the state (soil compaction, sward cover, composition of turfgrass plants [grasses, legumes and herbs], common diseases, pests, and weeds) of different soccer fields in different regions of Slovenia, and recognized the methods used in their maintenance and management. During our study we visited 20 soccer fields in total (4 owned or used by the clubs from the Slovenian First League, 4 by the clubs from the Slovenian Second League, 6 from the clubs from the Slovenian Third League (all leagues are organized by Football Association of Slovenia), and 6 by the clubs from the lower ranks of competition), each one twice – in spring (April – May) and in summer (July). Studied soccer fields are situated between 216 m (FC Zavrč) and 509 m (FC Bled) above the sea level. They are located in the northwestern (3 locations), central (8 locations), southeastern (3 locations) and northeastern (6 locations) regions and which also differ in climate. In the investigation soccer fields with different management budgets are included with the aim to evaluate how this factor influences the state of turfgrass. On every location we interviewed the turfgrass manager and documented the steps taken in maintenance (pests, diseases and weeds control methods, irrigation, mowing, fertilizing, aeration, seeding, daily foot traffic...).

Results and Discussion

Mowing is usually started in March or in April (colder regions). One field (FC Olimpija Ljubljana), which is also equipped with underground heating system, is mowed all year around. Usually turf is mowed twice to three times a week. Half of managers execute aeration and overseeding once a year while the other half does that several times a year. More than half of the visited fields are used on everyday basis, less than half of them are used only a couple times a week. Only two of the examined fields are not fertilized regularly and more than half of them are equipped with automatic underground irrigation system. On 21 spots at each soccer field we measured compaction at 7.5, 15.0 and 22.5 cm of soil depth with soil compaction tester (Spectrum Technologies) and at the same time we also measured water content, temperature and electrical conductivity with HD2-Mobile Moisture Meter and Trime®-Pico64/32 sensors. Sward cover and composition of turfgrass were evaluated using our own method. We placed a 1 m² wooden frame on 5 random spots while diagonally crossing the field. Using this method, we were able to get comparable results for all locations we visited. On each of the 5 spots we evaluated both parameters. Sward cover was documented by estimating how dense the turf is – if no disproportionate bare spots were visible the spot was marked with 100 % cover – and if there was a bare spot we estimated what is the percentage of it and subtracted that from 100 %. The other parameter was composition of plant cover – to estimate what the percentage of grasses, legumes and other herbs was. If there were no legumes or herbs we marked the spot with 100 % grass cover. The fields were also inspected for weeds occurrence. While inspecting the field in order to find weeds we were also looking for signs of fungal infections – brown spots of dry or dying grass, purple coloured grass leaves etc. In this study we didn't focus on identifying different species but rather getting the overall impression of occurrence. We classified each field into one of three categories depending on occurrence of soil fungi – 'occurrence on less than 1 % of the whole field surface', 'occurrence on 1-5 %' and 'occurrence on more than 5 %'. Soil samples were taken from every soccer field, and sent into analysis to Agricultural Institute of Slovenia. Phosphorus and potassium content was analyzed with AL-method along with the pH value and organic matter. Results were then compared with turfgrass specific values found in publication¹. Soil test results showed that soccer fields had pH from 5.3 (FC

¹ Bundesinstitut für Sportwissenschaft (1993). Grundsätze zur funktions- und umweltgerechten Pflege von Rasensportflächen. Koeln: 27 p.

Radomlje) to 7.5 (FC Stojnci), P_2O_5 values from 9 (NK Stojnci) to 160 mg 100 g⁻¹ (FC Ivančna Gorica), K_2O values from 10 (FC Maribor and FC Dobrovce) to 36 mg per 100 g of soil (FC Bled) and percentage of soil organic matter ranged from 2.0 (FC Olimpija Ljubljana) to 9.3 (FC Bled). Ground cover was in average lower at first (spring) inspection and accounted from 86.8 % (SLO 2nd League clubs) to 97.3 % (SLO 1st League clubs), meanwhile at summer inspection (with one field exception) ground cover always exceeded 95 %. An average percentage of grasses at first inspection amounted from 94.78 % (SLO 3rd League clubs) to 100 % (SLO 1st League clubs), while at second inspection it ranged from 89.3 % (SLO 3rd League clubs) to 100 % (SLO 1st League clubs). The highest percentage of legumes (*Trifolium repens*) at first inspection was 15.4 % and at the second one even 53.0 % (both at FC Komenda). On the same soccer field, we also determined the highest portion of herbs (4.4 and 3.0 %, respectively), with most abundant *Plantago major* species. During our first examination, no soil fungi infestation was recorded on turfgrass, while during the second examination we recorded the soil fungi on all soccer fields. Although some places in the fields showed severe symptoms, only the soccer field of FC Dob fit into category 'occurrence on 1 to 5 % of the whole surface', all other fields were in the category 'occurrence on under 1 % of the surface'. The third category, 'occurrence on over 5 % of the surface' was empty. On inspected soccer fields no major problems with pests were reported, in some cases only earthworm casts were present on the playground. We also found out that several managers suffer a lack of knowledge in proper turfgrass management and that application of mineral fertilizers is not professional and the usage of plant protection products on soccer field is almost negligible. We conclude that in Slovenia turfgrass maintenance and management of soccer fields differ greatly and that the budget the owners intend for the fields is not the most important factor of determining the appearance of the soccer fields.



Figure 1: Perfectly managed turfgrass in goal and partly penalty area on soccer field FC Stojnci, May 20 2015 (photo: S. Trdan)



Figure 2: Assessment of ground cover and floristical composition at soccer field Stožice in Ljubljana, July 16 2015 (photo: S. Trdan)



Figure 3: Heavily weeded turf on soccer field FC Komenda, July 28 2015 (photo: S. Trdan)



Figure 4: Heavy areas of fungal infestation on soccer field FC Dob, July 21 2015 (photo: S. Trdan)

WATER MANAGEMENT

ORAL PRESENTATIONS

LOW-COST URBAN ET MEASUREMENTS AND ESTIMATES

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Introduction

Projected increases in drought occurrence and water shortages due to rising demand for other uses has led to more interest in the use of evapotranspiration (ET) based irrigation scheduling in urban environments. ET based irrigation scheduling is, however, somewhat hard to apply to urban landscapes because of the difficulty to locate representative weather stations for the estimation of regional reference evapotranspiration (ET_o), the need to correct the ET_o values for local microclimate, and the lack of landscape coefficients for small areas and mixed vegetation. Accurate estimates of landscape water use are needed as input information to manage the timing and amount of water to apply to maintain high-quality landscapes while minimizing non-recoverable losses of water due to inefficient application. Improving efficiency is generally achieved by uniform water application at a rate that minimizes or eliminates runoff and by applying the correct minimum amount of water to maintain the landscape health and appearance. Most water losses from well-managed, irrigated landscapes result from ET. Thus, having accurate ET information between irrigation events, the correct application rate, and knowing the distribution uniformity, provides sufficient information to efficiently irrigate with minimal non-recoverable water losses. While it is possible to measure soil water to estimate local landscape ET (ET_L), using the product of ET_o and correction coefficients to estimate ET_L is generally less expensive and more likely to be adopted by urban irrigators. The critical need is to have accurate estimates of regional ET_o , a local climate coefficient (K_{LC}) that estimates the local ET_o as: $ET_{oL}=ET_o \times K_{LC}$, and a vegetation coefficient (K_v) that estimates the landscape ET_L as: $ET_L=ET_{oL} \times K_v$. It is assumed that the K_v is the same in all microclimates. In this presentation, a relatively new and inexpensive method to directly measure ET_L for use in irrigation scheduling and for determination of K_v values is presented.

Methods

The surface renewal (SR) method for estimating sensible heat flux density (H) was first presented by Paw U and Brunet¹ (1991), and it has developed into a relatively simple, low-cost alternative to using a sonic anemometer to determine H . The SR method uses a fine wire thermocouple to measure high frequency temperature, and traces of the temperature data show a repeating ramp like structure with the ramp amplitude and duration depending on the sensible heat flux density. A structure function is used to calculate the 2nd, 3rd, and 5th statistical moments of the temperature differences for a selected time lag between the data points. The moments are then used to calculate a 3rd order function of the ramp amplitude following van Atta (1977). The equation is solved for the mean ramp amplitude and ramp duration during a half hour period. The ratio of the ramp amplitude (a) to the duration (d) provides an estimate of the change in air temperature with time ($dT/dt=a/d$). If the volumetric heat capacity of the air $C_v=\rho C_p$ for air density (ρ) and specific heat at constant pressure (C_p) are known, conservation of energy is used to estimate H to or from the surface using the equation: $H \approx \rho C_p \cdot (dT/dt) \cdot z$. This equation assumes that the dT/dt is equally distributed within the volume below the measurement height ($z=V/A$), where A is a unit area. Normally, the dT/dt is not distributed equally within the air volume, so a calibration factor (α) is included to adjust for the non-uniform heating within the volume. Thus, an estimate of H is given by: $H=\alpha \cdot \rho C_p \cdot (dT/dt) \cdot z$. For about two decades, researchers used a sonic anemometer to simultaneously measure H and calculated the least squares regression of H versus H' through the origin to determine the α calibration factor. For an unstable atmosphere, the ramp amplitude, H' , and H are positive, and they are negative for stable conditions. When data are collected over taller canopies, recent research has shown that separate α calibration factors are needed for stable (H' is negative) and unstable (H' is positive) atmospheric conditions. For short term (half-hourly) measurements, the latent heat flux (LE) is calculated as the residual of the energy balance: $LE=R_n-G-H$, where R_n is the net radiation and G is the ground heat flux. Since G is approximately equal to zero on a daily basis, one can also estimate $LE=R_n-H$ using daily total R_n and H . Then, actual daily ET is estimated as: $ET_o=LE/L$, where ET_o is in mm d^{-1} , LE is in $\text{MJ m}^{-2}\text{d}^{-1}$, and

¹ Paw U, K.T. and Brunet, Y., 1991. A surface renewal measure of sensible heat flux density. In: 20th Conf. on Agricultural and Forest Meteorology, IO-13 Sept. 1991, Salt Lake City, UT, Am. Meteorol. Soc., Boston, MA

$L=2.45 \text{ MJ Kg}^{-1}$. Thus, one can easily determine ET_o by measuring R_n and $H=\alpha H'$ or by estimating R_n from remote sensing and using $H=\alpha H'$.

Results and Discussion

To illustrate the advantages from using SR, figure 1 shows the results from an experiment to measure ET_o from a winegrape vineyard grown on a 13% south facing slope. The graph shows daily ET_o and precipitation from a nearby weather station, and it also shows plots of daily ET calculated using half-hourly R_n and G with H (from EC), $\alpha H'$ (from SR), and $\alpha H'$ with $G=0$. There was clearly little or no difference in ET estimated using H , $\alpha H'$, or $\alpha H'$ with $G=0$. For agricultural research, we still recommend using both a sonic anemometer for H and a thermocouple for $\alpha H'$ because the combination method provides two estimates of H and helps to validate the results. Complete documentation on how to program and set up a combination EC and SR flux station is give in Shapland et al.². Recently, a method to determine α by compensating for thermocouple size was reported in Shapland et al.³. Using the thermocouple compensation method, it is unnecessary to calibrate the SR method with a sonic anemometer.

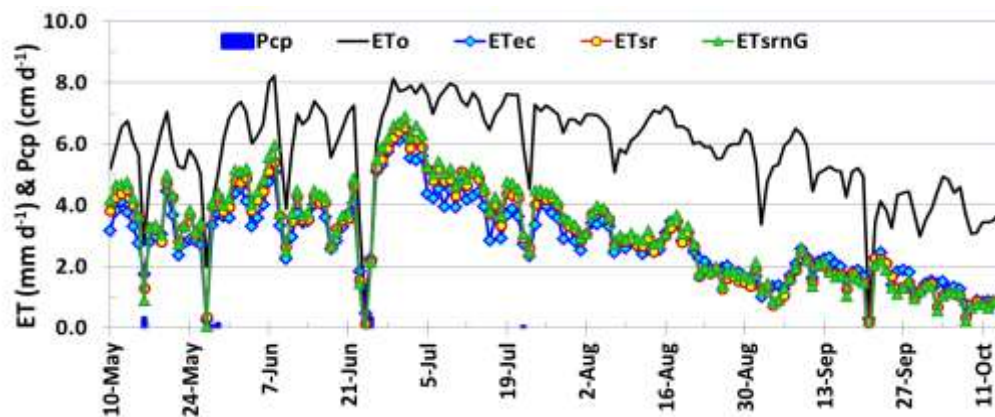


Figure 1. Evapotranspiration using H from eddy covariance (ET_{ec}), H from surface renewal (ET_{sr}), H from SR with daily $G=0$ (ET_{srnG}), reference evapotranspiration (ET_o), and precipitation (Pcp) from data collected over wine grapevines near Diamond Springs, California on a 13% south facing slope.

Conclusions

The SR method has been used to measure $\alpha H'$ and to estimate LE of landscape vegetation with good success⁴. The biggest advantage in using the SR method to estimate LE as the residual of the energy balance comes from the lower sensor cost. If R_n is accurately estimated using remote sensing, then one can estimate LE from the R_n and $\alpha H'$ (from SR). Sonic anemometers are generally mounted within a fully adjusted boundary layer but above the roughness layer, whereas one can estimate H' with thermocouples mounted near the canopy top, which reduces the fetch requirement considerably. In mixed landscape plantings, an EC system likely requires even more fetch to compensate for the surface roughness. Since SR is based on conservation of energy, a higher roughness layer should have little or no impact on the fetch requirement for SR measurements. Thus, the SR method should perform well over small areas of mixed plants where EC is inadequate because of the bigger fetch requirement.

² Shapland, T.M., McElrone, A.J., Paw U, K.T. and Snyder, R.L. 2013. A turnkey data logger program for field-scale energy flux density measurements using eddy covariance and surface renewal. *Italian J. of Agrometeorology*, 1/2013.

³ Shapland, T.M., Snyder, R.L., Paw U, K.T., and McElrone, A.J. 2014. Thermocouple frequency response compensation leads to convergence of the surface renewal alpha calibration. *Agricultural and Forest Meteorology*, 189-190: 36-47.

⁴ Snyder, R.L., Pedras, C., Montazar, A., Henry, J.M., and Ackley, D. 2014. Advances in ET-based landscape irrigation. *Agricultural Water Management* (147): 187–197.

MODELLING IRRIGATION DEMAND AND VALUE OF WATER FOR GOLF

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Introduction

Whilst most freshwater abstracted in Europe is used to support agriculture, golf represents a relatively small but economically valuable sector dependent on irrigation to maintain fine turf surfaces (to optimise playability including bounce and speed). There is, however, a growing perception that golf represents a profligate and unjustified use of water, contributing to environmental damage and impacting on other sectors. Although the demand for irrigation in golf is believed to be significant and rising there is in fact very limited evidence to substantiate any underlying trend. In south-east Spain, tourism (including golf) grew by 50% between 1998 and 2003¹, increasing pressure on already limited supplies and creating conflict with irrigated agriculture in that region. Evidence from other countries confirms that the golf sector is growing, driven by societal changes in leisure time and increases in disposable income although no studies have quantified the volumes abstracted or linked these to water resource impacts. Given that golf is reported to be the most valuable and largest global leisure pursuit with an estimated 57 million players (27.4 million in USA; 5.5 million in Europe, 14 million in Japan, and 5.5 million in Canada)² and generating €15.1 billion for the European economy³, from an environmental policy perspective it is important to consider its water resource needs objectively and put these in context with competing demands from other sectors from an economic perspective. This research aimed to provide the first quantitative geospatial assessment of irrigation demand and value of water for golf in Europe. The outputs are intended to provide an important contribution to the limited scientific evidence on water abstraction for golf⁴ and to provide an equivalent scale of analysis often presented for agriculture⁵ that can inform water regulation and policy in Europe. A brief outline of the methodology developed is given below.

Methods

A GIS combination methodology was developed, involving (i) an inventory of golf course characteristics and water management practices at Member State level, (ii) an assessment of the impacts of climate variability on golf irrigation based on a linear regression analysis of reported abstractions against an aridity index using gridded rainfall and evapotranspiration (ET) climatology, (iii) an economic assessment of water use and value for golf based on geospatial modelling of blue (irrigation) and green (rainfall) components of water use, and (iv) a comparison of modelled golf irrigation demand against projected changes in EU water resource availability to identify 'hotspot' river basins where future water demand for golf might be at risk. In contrast to the extensive range of datasets published in the scientific and grey literature on irrigated agriculture in Europe (e.g. FAO AQUASTAT), there exists no equivalent publically accessible yet comprehensive database for golf. Datasets created by the industry are available for particular countries, but commercially confidential and limited in scope. A list of all relevant data sources was collated using industry literature sources and targeted internet searches. This included identifying all golf and greenkeeper federations, golf associations and golf unions known to exist across Europe. Using a GIS, the spatial distribution of golf courses was mapped. The GIS data were then combined with a gridded agroclimate dataset, containing a variable known as 'potential soil moisture deficit' or PSMDmax. This was derived from a global dataset containing rainfall and reference evapotranspiration (ET) data at a grid resolution of 10' latitude/longitude. This agroclimate index provides a good visual indication of the gradient in aridity that exists across Europe. The spatial variability in agroclimate

¹ World Wildlife Fund (2003) Development in the drought. The incompatibility of the Ebro water transfer with sustainable development in the Southeast region of Spain. WWF, April 2003.

² RCGA (2006). Royal Canadian Golf Association Canadian Golf Survey (RCGA)(2006) Association Canadian Golf, Canada

³ SMS (2013). The economic impact of golf on the economy of Europe. Sports Marketing Surveys Inc. pp 39.

⁴ Rodriguez Diaz, J. A., Knox, J. W. and Weatherhead, E. K. (2007) Competing demands for irrigation water: Golf and agriculture in Spain *Irrigation and Drainage* 56(5): 541-549.

⁵ Siebert, S., Burke, J., Faures, J.M., Frenken, K., Hoogeveen, J., Döll, P., and Portmann, F.T (2010). Groundwater use for irrigation – a global inventory *Hydrological Earth System Sciences* 14: 1863-1880.

reflects differences in the monthly balance between rainfall and ETo; generally, the higher the PSMDmax, the greater the aridity, and hence greater dependence on irrigation. The PSMDmax values range from 0 to 1100 mm, with the highest (600-1000 mm) in southern Europe (Spain, Italy, Portugal) and the lowest (200-400 mm) across northern Europe. The greatest concentration of golf courses is actually in northern and central Europe; in southern Europe, most courses are located along the Mediterranean coastline, in close proximity to major urban areas or tourist developments.

Previous research has demonstrated a strong correlation between irrigation need and agroclimate (PSMDmax) and that the relationship is statistically robust over a wide range of environmental conditions. Most golf courses pay for the volume of water used, so good records are available on the volumes of water abstracted. Data on metered abstractions from selected golf courses in the UK ($n = 12$) and Spain ($n = 14$) were used to derive a linear regression correlation between irrigation need (mm) and PSMDmax, taking care to adjust the volumes abstracted (m^3) to the area irrigated (ha) in each Member State. By combining the regression equation with the European gridded PSMDmax data, with information on the location and irrigated area for each golf course, the theoretical volumetric irrigation demand (m^3) by Member State in Europe was estimated.

We quantified the value of water in golf using the residual method. This involved estimating the value of water expressed as the average profit attributable to water, taking into account the price and quantity of other inputs and the total volume of water used. Given the wide climatic differences in the countries studied, rainfall meets most of the turf water requirements for some of them (notably in humid or temperate northern latitudes), whilst in more arid countries irrigation is an essential component of course management to maintain turf quality and playability. This has implications for the application of the residual valuation method to assess the economic value of water for irrigating golf courses that are overcome by distinguishing between blue (irrigation) and green (effective rainfall) water in the analysis. Finally, to examine 'water risks', data relating to the ratio of water withdrawals to water availability for Europe were extracted⁶ based on the WaterGAP model⁷ and combined with GIS data on the location of golf courses in Europe to identify 'hotspots'.

Results and Discussion

The total irrigated area for golf was estimated to be 205,000 ha with half this concentrated in northern Europe (UK and Germany). By comparison, the total area equipped for agricultural irrigation in the EU27 in 2003 was 10 Mha, with the majority in the Mediterranean region. For example, France, Greece, Italy, Portugal and Spain account for 75% of the total area equipped for irrigation in the EU27⁸. Golf thus constitutes only a very small component of the total irrigated area in Europe. The total theoretical irrigation demand for golf was calculated to be $172 \times 10^6 m^3$ per year, ranging from 17,900 m^3 per course in northern Europe to 172,780 m^3 in southern Europe, respectively. Overall, this figure represents only 0.065% of total water freshwater abstraction in Europe. Of course, the large agroclimatic gradient has a major influence on water demand. For example, over half (61%) of total demand is concentrated in Spain, despite having only 6% of courses in Europe. Collectively, 85% of all golf water use is concentrated in southern Europe (Spain, Italy, Portugal, and Greece). Compared to agricultural abstractions, golf water use is negligible; for example, in Spain it is estimated to represent only 0.6% of current agricultural water use⁹. By combining data on the location of golf courses with outputs on water stress from the WaterGap model, the number of courses and proportion of irrigation demand considered to be 'at risk' was assessed. The GIS analysis suggests that nearly a fifth (19%) of golf clubs and a third (29%) of the total volume of water demanded by the golf sector is at 'high risk' due to being located in river basins likely to experience 'severe' water stress. The analyses confirm that the volumetric demand for golf is small compared to agricultural abstraction, but significant in some catchments in dry summers, notably where tourist developments are concentrated and where water supplies are constrained. Irrigating golf is an economically rational use of water delivering very high benefits ($\text{€}/m^3$) far in excess of those accrued in high value agriculture, even though the transfer of resources away from sectors such as agriculture would inevitably be controversial. The outputs provide a valuable contribution to limited evidence on water for golf and offer an equivalent scale of analysis often presented for agriculture that can inform EU policies regarding water regulation.

⁶ Flörque, M., Alcamo, J. (2004). European Outlook on Water Use. Center for Environmental Systems Research. University of Kassel. Germany.

⁷ Alcamo, J., P. Döll, T. Henrichs, F. Kaspar, B. Lehner, T. Rösch, and S. Siebert (2003). Development and testing of the WaterGAP 2 global model of water use and availability. *Hydrological Sciences*. 48 (3), 317-337

⁸ Wriedt, G., Van der Velde, M., Aloe, A., and Bouraoui, F. (2009). Estimating irrigation water requirements in Europe. *Journal of Hydrology* 373(3-4): 527-544.

⁹ INE. (2012). Encuesta sobre el uso del agua en el sector agrario. Año 2010. National Institute of Statistics. Spain (in Spanish).

IRRIGATION REQUIREMENTS FOR PERENNIAL RYEGRASS (LOLIUM PERENNE L.) SALINITY MANAGEMENT

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Introduction

Extended drought and increasing urbanization in California and other arid and semi-arid regions of southwestern USA continue to put pressure on already diminishing potable water resources, especially for landscape and turfgrass irrigation. Since January 2010, all municipalities in California have been required to adopt a water efficient landscape ordinance in an effort to conserve water¹ (California Model Water Official Landscape Ordinance, 2009). Using alternative sources of water for irrigation is one solution to limit the strain on fresh water resources. Recycled water, also known as effluent, reuse, reclaimed, or wastewater has become an increasingly common and necessary resource for irrigating larger turf areas. One limiting factor for the application of reduced water, especially under salt-affected conditions, is the omnipresence of cool-season turfgrasses on golf courses, athletic fields, public green space, and residential lawns in California. Plant responses to heat, drought and salinity are complex and thus research is limited, especially for turfgrass and perennial ryegrass in particular. The objectives of this study were to evaluate the interactions of irrigation water quality, quantity, and soil salinity on perennial ryegrass turf quality, growth, and cover to predict more accurately leaching requirements for turfgrass salinity management.

Methods

A study was conducted from 2011-2012 at the University of California, Riverside (UCR) Turfgrass Research Facility in Riverside, CA. Soil was a Hanford fine sandy loam (coarse-loamy, mixed, superactive, nonacid, thermic Typic Xerothents). A modified line-source experiment was constructed on a 972-m² area. Four irrigation lines spaced 9 m apart alternated between distribution of potable and saline water to establish an irrigation salinity gradient (EC ~ 0.6 to 4.2 dS m⁻¹) in between lines. Potable water originated from the San Bernardino and Riverside Basins, while saline water was made by mixing salts in potable water within two 19000-L storage tanks (Snyder Industries, Inc., Lincoln, NE) containing submersible pumps for mixing and agitation (Table 1). Saline water ion composition was based on Colorado River water (personal communication, D.L. Suarez) and contained elevated concentrations of salts including Na⁺, Cl⁻, and SO₄²⁻ but not HCO₃⁻ and CO₃²⁻. The study area was divided into four separate irrigation zones perpendicular to the irrigation lines, each controlled by a separate valve interfaced to a central irrigation controller. Each zone was irrigated independently by the four alternating irrigation line sources, further dividing the study area into twelve 81-m² plots (three plots per irrigation zone). Irrigation amounts or Kc values of 80, 100, 120, and 140% reference evapotranspiration (ET_o) were randomly assigned to the areas. Irrigation was applied based on the previous 7-d cumulative ET_o based on a modified Penman equation with a wind function². Irrigation water volume was collected using catch cans from locations within every other subplot and analyzed for salinity to establish water quality levels (EC_w) of 0.6, 1.7, 3.0, 3.5, and 4.2 dS m⁻¹. The area was seeded with perennial ryegrass 'SR 4550' (Seed Research of Oregon, Corvallis, Oregon) on 18 April 2011 at a rate of 4.5 kg ha⁻¹ and irrigated with potable water only during establishment. Saline water and irrigation treatments were initiated on 21 July 2011. Visual assessments of turfgrass quality and cover were evaluated at the start of the experiment and bi-weekly thereafter. Quality was evaluated by texture, color, uniformity, and density on a 1 to 9 rating scale (1 = dead turf, 6 = minimally acceptable, light green, thin and 9 = dark green, dense, uniform turf). Turfgrass cover was estimated on a percentage scale (0% = no turf cover, and 100% = complete turf cover). Clippings were collected bi-weekly for each subplot using a walk-behind rotary mower. Composite soil samples were collected before irrigation treatments were initiated, in October 2011 prior to the rainfall season and cooler temperatures in Riverside, CA, and in October 2012. Soil solutions were extracted using distilled water to determine electrical conductivity from the soil saturation extract (EC_e), sodium absorption ratio (SAR), sodium concentration [Na], and other chemical constituents. Stepwise and multiple linear regression were used to determine the

¹California Department of Water Resources. 2009. Model water efficient landscape ordinance. <http://www.water.ca.gov/wateruseefficiency/docs/MWEL009-10-09.pdf> (accessed 3 Mar. 2016).

² Doorenbos, J., and W.O. Pruitt. 1984. Guidelines for predicting crop water requirements. Environ. Assessment Office, Irrigation and Drainage Pap. 24. United Nations, Rome.

relationship between irrigation quantity and water quality on soil salinity and turf response (SAS, Ver. 9.3, 2010, Cary, NC). Proc Corr was used to correlate turf response variables (quality, cover and dry weight) with irrigation quantity (% ET_o), water quality (EC_w) and soil salinity.

Results and Discussion

Results from stepwise linear regression revealed that changes in turf quality and cover during the 2-yr study were best described by irrigation amount, water quality, and time. Regression equations were subsequently used to calculate the number of days for perennial ryegrass quality to fall below a minimally acceptable quality rating of 6 (1 to 9 scale, 9 = best) and turf cover to drop below 90% for each EC_w treatment during the experiment. At 80% ET_o, the equation predicted that turf quality (Quality = 8.31 - 0.39EC_w - 0.01Days; R² = 0.54^{***}) and cover (Cover = 123.2 - 3.6EC_w - 0.15Days; R² = 0.46^{***}) could not be maintained above minimally acceptable levels for one year regardless of water quality. Similarly, at 100% ET_o, turf quality and cover could not be maintained above minimally acceptable levels for one year, reaching quality (Quality = 8.81 - 0.43EC_w - 0.008Days; R² = 0.42^{***}) and cover (Cover = 122.2 - 5.2 EC_w - 0.09Days, R² = 0.36^{***}) thresholds at 318 d and 323 d, respectively (June 2012) at low EC_w (0.6 dS m⁻¹). Even under non-limiting irrigation conditions (120% ET_o), the equation predicted that turf quality (Quality = 8.43 - 0.17EC_w - 0.007Days; R² = 0.33^{***}) and cover (Cover = 107 - 0.96EC_w - 0.05Days; R² = 0.21^{*}) could not be maintained above minimally acceptable levels for one year, reaching thresholds of 332 d and 328 d (June 2012) at low EC_w (0.6 dS m⁻¹). Only the highest irrigation amount (140% ET_o) was predicted to sustain turf quality (Quality = 8.8 - 0.25EC_w - 0.006Days, R² = 0.34^{***}) and cover (Cover = 110.5 - 2.08EC_w - 0.04Days; R² = 0.23^{***}) above minimally acceptable standards for 441 d and 481 d, respectively when irrigated with potable water (0.6 dS m⁻¹). Given the soil and environmental conditions in Riverside, CA, these data indicated that perennial ryegrass quality and cover could be sustained with irrigation water quality (EC_w) up to ~ 1.7 dS m⁻¹ applied at 140% ET_o. Overall, the performance of perennial ryegrass 'SR 4550' in this experiment suggests that a sufficient amount of irrigation water (120 – 140% ET_o) above reference evapotranspiration (ET_o) must be applied to maintain acceptable quality and cover in Riverside, CA, especially when using recycled water for irrigation.

Table 1. Properties of saline and potable irrigation water used in the line-source gradient study in Riverside, CA

Properties	Potable	Saline
pH	7.8	7.6
EC, dS m ⁻¹	0.6	4.4
TSS, mg L ⁻¹	390	2835
SAR, meq L ⁻¹	3.2	18.3
Na ⁺ , mg L ⁻¹	53	524
K ⁺ , mg L ⁻¹	4	130
Ca ²⁺ , mg L ⁻¹	66	126
Mg ²⁺ , mg L ⁻¹	12	152
Cl ⁻ , mg L ⁻¹	31	996
NO ₃ ⁻ -N, mg L ⁻¹	5.2	5.1
HCO ₃ ⁻ , mg L ⁻¹	215	210
CO ₃ ²⁻ , mg L ⁻¹	0.01	0.01
SO ₄ ²⁻ , mg L ⁻¹	78	708
B, mg L ⁻¹	0.08	0.11

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Introduction

European golf courses are increasingly facing restrictions regarding the use of irrigation water¹. This is especially the case in southern Mediterranean countries such as Spain and Portugal², but even Danish authorities do not allow many golf courses to abstract more than 5-7000 m³ water per season. In some counties with humid or temperate climates where there are reduced pressures on water abstraction, the environmental and agronomic impacts caused by over-irrigation on turf quality are equally challenging. Both water scarcity and excess highlight the critical importance of sound irrigation and drainage management in turf.

As for other crops, the irrigation water requirements for turf can be estimated by the difference between natural precipitation and actual evapotranspiration (ET_a) which is the product of a crop coefficient (K_c) and the reference evapotranspiration (ET_o) calculated by Penman-Monteith or similar combination equations³. The K_c for cool-season turfgrasses has been reported to be in the range 0.7-1.0^{4,5} but this is an over-simplification as the actual K_c varies depending on soil water content (SWC). We have earlier documented that the K_c for green and fairway turf typically ranges between 1.5-2.5 on the first day after irrigation to field capacity (FC) and that values of 0.7-1.0 are not attained until the third day after irrigation to FC⁶. The K_c during an irrigation cycle typically follows a hyperbolic function, and for creeping bentgrass (*Agrostis stolonifera*) growing on a sand-based, USGA-specification green, we determined this function to be $K_c = 2.15 \times d^{-0.74}$, where d = day number after irrigation to FC⁶.

The management implication of this hyperbolic K_c function is that frequent irrigation to FC results in excessive water use. Water savings can either be achieved by deep and infrequent 'wilt-based' irrigation or by deficit irrigation strategies which can be conducted at various frequencies but which always imply that the soil's water reservoir is not refilled, or – in other words – that the ET_a is not fully replenished⁷. Therefore, a highly relevant question for turfgrass managers is which of these methods will produce the best turf quality with the least use of water. Managing sand based turf at lower SWC will increase the risk of localized dry spots and a second question is therefore the implication of various water-saving irrigation strategies on the need for soil surfactants.

Methods

Here we report on an irrigation trial carried out on an experimental green at NIBIO Turfgrass Research Centre Landvik, SE Norway, in 2010 and 2011. The USGA-spec. rootzone had an ignition loss of 1.0 %, the SWC at FC was 20 % (v/v), and the turfgrass species was creeping bentgrass 'Independence'. The trial was established under a mobile rainout shelter with a split-block design and four replicates. There were six irrigation treatments on main plots in one direction and the use/non-use of the soil surfactant Revolution™ (19 l ha⁻¹ preventatively before start of trial followed by 9.5 l ha⁻¹ every other week during the trial period) in the other direction. The six irrigation treatments were: (1) Irrigation to FC six times per week, (2) Irrigation to FC twice per week, (3) Irrigation to FC once per week, (4) Deficit irrigation 6x per week, (5) Deficit irrigation 2x per week and (6) Deficit irrigation 1x per week.

¹ Strandberg, M., K. Blombäck, K., A.M.D. Jensen and J.W. Knox (2012). The future of turfgrass management – challenges and opportunities. Acta Agriculturae Scandinavica Section B – Soil and Plant Science 1: 3-9.

² Rodriguez Diaz, J. A., J.W. Knox and E.K. Weatherhead (2007). Competing demands for irrigation water: Golf and agriculture in Spain Irrigation and Drainage 56: 541-549.

³ Allen, R.G., M.E. Jensen, J.L. Wright and R.D. Burman (1989). Operational estimates for evapotranspiration. Agronomy Journal 81: 650-662.

⁴ Brown, P.W., C.F. Mancino, M.H. Young, T.L. Thompson, P.J. Wierenga and D.M. Kopec, 2001: Penman Monteith crop coefficients for use with desert turf systems. Crop Sci. 41: 1197-1206.

⁵ Ervin, E.H. and A.J. Koski 1998. Drought avoidance aspects and crop coefficients of Kentucky bluegrass and tall fescue turfs in the semiarid West. Crop Science 38: 788-795

⁶ Aamlid, T. S., Knox, J.W., Riley, H., Kvalbein, A. and Pettersen, T. (2016). Crop coefficients, growth rates and quality of cool-season turfgrasses. Journal of Agronomy and Crop Science 202: 69-80.

⁷ Fry, J., and B. Huang, 2004: Applied Turfgrass Science and Physiology. John Wiley & Sons, Hoboken, NJ, USA. 310 pp.

Plot irrigation was conducted either by hand watering or using an irrigation trolley designed for experimental trials. The amount of water added in the various treatments was calculated from ET_0 and the K_c function for creeping bentgrass greens. In addition to weekly assessments of turf grass quality, localized dry spots, green speed and surface firmness, the volumetric water content in the 0-12 cm topsoil was always recorded before irrigation using a hand-held TDR instrument (Hydrosense, Campbell Scientific Ltd., Australia).

Results and Discussion

Table 1 summaries selected key results. During the experimental period in 2011, the total water use in treatment 4 (light and frequent deficit irrigation) was 66 % lower than in treatment 1 (light and frequent irrigation to FC) and 29 % lower than treatment 3 (deep and infrequent irrigation). Despite this, there were no differences in turf quality until 28 July when the maximum temperatures rose to 25 °C and above. During the following weeks, only treatments 1, 2 and 4 produced acceptable (>5.0) turf quality, partly because they had less localized dry sports than the other treatments. Green speed was not affected by irrigation treatments (data not shown), but surfaces became significantly softer with more frequent irrigation and with increasing irrigation rates. Root development was favored by infrequent irrigation.

Table 1. Turfgrass water use, visual quality, soil water content (TDR) at irrigation, visual turf quality, localized dry spots, surface firmness, and root dry weight as affected by various irrigation treatments. Data are from the experimental period 20 June – 22 Aug. 2011, except root dry weight which was determined after the treatment period in 2010.

Irrigation Treatment	Water per irrigation, mm	Total water use, mm	Soil water content at irrigation, %	Visual turf quality in August (1-9)	Localized dry spots, % of plot area	Surface firmness (gravities) ³	Root dry weight below 5 cm, g m ⁻²
1. FC 6x per wk	6.6	356	12.3 a ⁴	6.0	6	71 c ⁴	102 b ⁴
2. FC 2x per wk	12.9	233	10.9 ab	5.6	6	74 bc	110 b
3. FC 1x per wk	19.2	173	8.9 bc	4.6	13	79 b	150 a
4. DEF 6x per wk	2.4	123	11.2 ab	5.5	8	76 bc	110 b
5. DEF 2x per wk	7.1	127	9.8 abc	4.9	11	75 bc	98 b
6. DEF 1x per wk	11.8	106	7.5 c	4.0	27	85 a	142 a
P-value	-	-	0.011	0.06	0.08	<0.0023	0.005

¹ FC = irrigation to field capacity ² DEF = deficit irrigation ³ Clegg hammer, weight 2.25 kg.

On average for irrigation treatments, Revolution resulted in an insignificant improvement in turf quality from 4.8 to 5.4 on average for observations in August 2010. This improvement was accompanied by a significant reduction in localized dry spots from 24 to 4 % of plot area. Effects of Revolution on surface hardness or root development were not significant and there were no significant interactions between irrigation treatments and the soil surfactant (data not shown).

In conclusion, this research has shown strong

benefits of light and frequent deficit irrigation on a creeping bentgrass green. Issues that need further clarification are to what extent current irrigation systems are sufficiently uniform to provide only 2-4 mm per irrigation application, or if this requires manual intervention in the form of hand-watering. Possible implications of irrigation scheduling on the competition from *Poa annua* should also be considered.

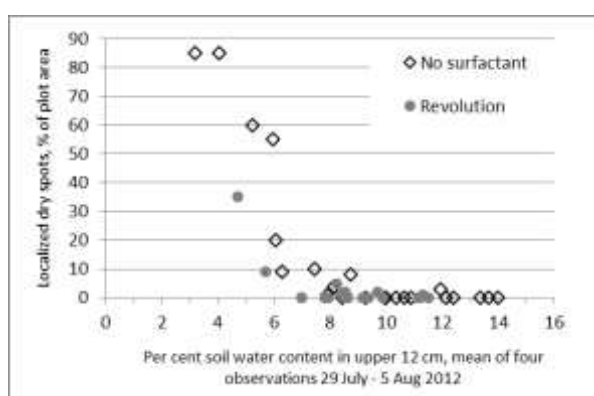


Fig.1. Relationship between soil water content during a week with severe heat stress and the development of localized dry spots.

ASSESSING EVIDENCE ON THE AGRONOMIC AND ENVIRONMENTAL IMPACTS OF TURFGRASS IRRIGATION MANAGEMENT

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Introduction

In recent years, rising competition for water coupled with new environmental regulations have exerted pressure on water allocations for golf¹. Promoting more efficient water use has become a major driver for change in the European golf sector. Improving irrigation management to enhance fine turf quality whilst reducing the environmental impacts of irrigation has thus been the focus of recent research. In this paper, we reviewed published scientific and industry evidence on the agronomic and environmental impacts of irrigation on turfgrass using a systematic review (SR) methodology developed by the Centre for Evidence Based Conservation (CEBC)². This methodology follows a series of well-defined and rigorous steps, including a detailed description of the methodology including the data extraction strategy, data collection, analyses and synthesis of the findings.

Methods

The SR focused on three topics, namely: (i) irrigation management; (ii) turfgrass agronomy (turf quality, growth and rooting), and (iii) environmental impacts, notably nitrogen leaching. The SR question was “*turfgrass irrigation management: what are the agronomic benefits and environmental impacts?*” Bibliographic searches identified 650 relevant documents, of which 86 then met the inclusion criteria. Quantitative and qualitative data were extracted from 79 peer-reviewed papers and 7 documents from the grey literature. To assess irrigation management strategies, we distinguished between irrigation amount (IA) and irrigation frequency (IF). The IA was based on the soil water depletion since the last irrigation, and the amount of water applied was expressed as percentage of either water evaporated in a US Class A evaporation pan (% E_{pan}), reference evapotranspiration (% ET_0), or crop evapotranspiration (% ET_c). Selected findings from the SR analysis are summarised below.

Results and Discussion

Irrigation management and turf quality

Turf Quality (TQ) scores were based on a scale from 1 to 9, with 1 representing uneven and poor; 5-6 was the minimal acceptable quality and 9 constituted an even, uniform and very high quality turf. SR evidence from 32 papers showed a direct relationship between increases in both IA and IF and higher TQ scores. The strength of the relationship was higher for IA than for IF reported treatments. In general, the higher TQ scores were achieved when the turf was irrigated to replace transpired water (100% ET_c). When compared to irrigation at 100% ET_c , the TQ scores were not significantly different within studies when irrigated to replace 75-80% ET_c . Below this, average TQ decreased gradually. The IA required to maintain an acceptable TQ was, on average, between 50 and 60% ET_c , but did vary markedly between studies. Based on the SR data, a correlation between TQ and IA showed a high degree of variability between different studies (for E_{pan} , ET_0 and ET_c scheduling methods, reported R^2 values of 0.33, 0.32 and 0.26, respectively). Variability could be explained by other factors including study location, turf species/cultivars, fertilization treatments and soil type. Within studies, variations between treatments, for example, comparing different species at different IA, were more noticeable when turfgrass was subjected to deficit irrigation than when was irrigated with no deficit. In this case, turfgrass maintained a higher TQ score when drought tolerant species were used, when mowing height was increased, surfactants were applied and/or when high temperatures were avoided. Nine studies compared the effects of different IF on TQ. The data suggested that, at least in sand-based root-zones, short irrigation intervals (1 to 4 days) do not dramatically change TQ. In general, when the irrigation interval increases, the TQ score decreases gradually. In opposition, in treatments where irrigation intervals were greater, plants seemed able to maintain

¹ Strandberg, M., Blombäck, K., Dahl Jensen, A.M., and Knox, J.W. (2012) The future of turfgrass management – challenges and opportunities. *Acta Agriculturae Scandinavica Section B – Soil and Plant Science* 1: 3-9.

² CEBC, 2010. *Guidelines for Systematic Reviews in Environmental Management*. Bangor, UK.

TQ values even when subjected to drought stress. It was reported to be linked to greater root development at depth.

Irrigation management and turf rooting

A significant relationship between IF and root distribution and depth was identified. In general, the longer the irrigation interval, the greater the root development. With frequent irrigation, turfgrass showed higher rates of roots at shallower soil layers, due to the soil being close to field capacity. With regard to IA effects on root development, no clear evidence was identified. No substantial differences were identified when root development for IA from 60 % to 100% ET_c were compared. For lower IA of 20%, 25%, 50% ET_c, two studies reported deeper root development^{3,4}, but with detrimental effects on TQ. In many cases, no difference in TQ was reported between species or cultivars under well-watered conditions. However, under water stressed conditions, those species or cultivars that were able to develop deeper, more extensive root systems performed better, and had a quicker recovery from stress. Finally, it was noted that root development is related to other factors, not just irrigation. Root development increases at higher mowing, decreases at high temperatures and varies between turf species and soil type. In addition to enhancing TQ under drought conditions, deep, extensive root systems led to reductions in nitrate leaching, provided water application did not exceed field capacity and did not cause water movement beyond the rootzone^{5,6}. Conversely, no clear evidence was identified regarding the relationship between root system development and nitrogen uptake, which seems to be to be more closely related to turf growth rate.

Irrigation management and dry matter production (DMP) from clippings

Evidence from 13 papers suggested DMP in clippings follows a similar trend to TQ in response to IA treatments. When TQ and DMP (g m⁻² day⁻¹) were compared within studies, the correlation based on seven papers fluctuated between R² 0.32 and 0.80. With reference to the effect of IA on DMP, no observed changes in DMP for irrigations above 60% E_{pan} were reported. For lower IA treatments, the DMP diminished. DMP was also affected by other factors such as season, turf species and N fertilization. Four studies reported a linear correlation between CMP in clippings and N uptake. Under normal turf growth conditions, there was no apparent relationship between N uptake and N leaching. However, it was reported that decreasing turf growth rates caused by the end of growing season or environmental stresses, reduced water and N uptake, which then triggered an increase in N leaching.

Irrigation management and environmental impacts (nitrate leaching)

Evidence showed that over-irrigated turfgrass led to increased N leaching losses. Based on results from four studies, the influence of fertilization rates on nutrient leaching was much higher when turf was over-irrigated. A delay in watering after fertilizer application also substantially reduced the nitrogen losses in leaching⁷. This was caused by an increase in residence time of NO₃⁻-N in the root zone, which led to greater absorption. In addition to fertilizer rate and type, other factors that increased the quantity of nitrate leaching when turfgrass was over-irrigated included (i) sand-based root zones, (ii) low organic matter in soil, (iii) young turfgrass, and (iv) not having a dense, actively growing and healthy canopy.

³ Fu, J., Fry, B., and Huang, B. 2007. Tall fescue rooting as affected by deficit irrigation. HortScience, 42(3):688–691.

⁴ Shahba, M., Abbas, M., and Alshammary, S. 2014. Drought resistance strategies of seashore paspalum cultivars at different mowing heights. HortScience, 49:221–229.

⁵ Barton, L. and Colmer, T.D. 2006. Irrigation and fertiliser strategies for minimising nitrogen leaching from turfgrass. Agricultural Water Management, 80:160–175.

⁶ Barton, L., Wan, G., and Colmer, T. 2006 Turfgrass (*Cynodon dactylon* L.) sod production on sandy soils: II. Effects of irrigation and fertiliser regimes on N leaching. Plant and Soil, 284:147–164.

⁷ Bowman, D., Devitt, D., Engelke, M., and Ruffy, T W. 1998. Root architecture affects nitrate leaching from bentgrass turf. Crop Science, 38:1633–1639.

IMPROVING WATER USE EFFICIENCY WITH SOIL INCORPORATION OF ORGANIC MATTER

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Introduction

Healthy soil that is rich in organic material absorbs rainwater, helps prevent flooding and soil erosion, and filters water pollutants. Healthy soil also stores water and nutrients for plant use in drought, promoting healthy plants that require less irrigation, pesticides, and other resources. It has long been known that relatively small increases in organic matter can double the available water capacity of the soil¹. In general, soils across the Southeastern U.S. are low in organic matter. This is function of soil forming factors and the climatic conditions of a subtropical environment. To gain the benefits of organic matter it has to be added to the soil. For establishment of perennial turfgrasses, the sole opportunity is during site preparation prior to laying, or seeding, the grass. Often soil preparation, tilling or amending with organic matter, is overlooked and not valued as a practice that can make a lawn “sustainable”.

Compost can be a viable source of organic matter. Locally derived composts (e.g. sod byproducts, organic peat, yard/wood trimmings, etc.) have been previously researched². These studies incorporated compost into the turfgrass canopy to investigate the potential for fertilization and disease suppression. Some compost provided improved turfgrass color and quality and reduced incidences of the disease dollar spot (*Sclerotinia homoeocarpa*). While compost may be a disease suppression option, the use of these materials as soil amendments for the purpose of long-term water conservation needs further investigation. The objective of this study was to determine the impacts of proper site preparation, which included tilling and soil incorporation of compost, on turfgrass water use efficiency.

Materials and Methods

In June of 2012 a field study was established on a Cecil sandy loam (fine, kaolinitic, thermic Typic Kanhapludults)¹. From previous research² two promising organic amendments were selected (sod by-products and the yard/wood trimmings composts) as amendments to be tilled into the soil prior to sodding. The two types of organic matter were incorporated into the upper 10 cm of the test plots at 2.5, 5.0, and 10.0 kg m⁻². A control treatment that was tilled but had no organic matter incorporated was also included. These whole plot treatments were replicated four times and arranged in a randomized complete block design with three grass species [bermudagrass (*Cynodon dactylon* (L) Pers. x *C. transvaalensis* Burtt-Davy) – TifGrand, centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.) – TifBlair, and zoysiagrass (*Zoysia japonica* Steud.)– JaMur] arranged as split plot factors within whole plots for a total of 84 test plots. To ensure suitable grass for subsequent years, supplemental irrigation was applied at a 30% deficit (not exceeding 1.8 cm per week) assuring drought stress. Despite the three species having individual management recommendations, for ease of plot maintenance all were mowed at the same height (3.8 cm) and fertilized at the same annual nitrogen rate (98 kg ha⁻¹).

On a 1 to 9 scale, with 1 = brown, dead grass, 6 = minimally acceptable turf, and 9 = healthy, green grass, visual turfgrass quality and color was assessed during drought conditions. Using a Spectrum Technologies FieldScout TDR 300 Soil Moisture Meter² with 3-inch tines, volumetric water content (VWC) was measured daily during drought periods. The 2013 growing season was abnormally wet, allowing for three dry-down cycles (DDC). On the same plots, there was opportunity for four DDC during the 2014 growing season. There were a total of seven DDC over the two growing seasons where a DDC ranged from 4 to 31 days following a rainfall event that exceeded 8 mm. Data were analyzed using SAS JMP with means separated by least significant difference with an $\alpha=0.05$.

Results and Discussion

For VWC and TQ, there was no difference between years but individual DDC were different (data not presented). When averaged for both years, the soil below centipedegrass had the highest VWC, followed by bermudagrass, and zoysiagrass the lowest (Table 1). The difference in VWC for centipedegrass and

¹ U.S. Dept. of Ag. 2014. Soil Survey of Spalding County, Georgia. Web Soil Survey, Version 7.

² Spectrum Technologies, Inc., Plainfield, IL.

bermudagrass was only 1.5%. The VWC for zoysiagrass was 7.3% and 5.7% lower than centipedegrass and bermudagrass, respectively. Considering bermudagrass and zoysiagrass are both rhizomatous and stoloniferous, it would have been expected the VWC of these two species would have been more similar. Zoysiagrass consistently had lower VWC when compared to the other species regardless of length to the DDC or amount of rainfall preceding the DDC (data not presented). These data indicate the zoysiagrass cultivar JaMur used more water than TifGrand bermudagrass or TifBlair centipedegrass. All three species retained acceptable TQ.

There was no difference among compost sources when the VWC or TQ was analyzed across years (Table 2). However, the amount of incorporated compost did affect soil VWC. The rate of 10.0 kg m⁻² had a greater VWC than either of the two lower rates (Table 3). There may be a point where the incorporation of organic matter may increase VWC to a level that the root zone becomes too wet, limiting root growth and compromising the grass' ability to persist through periodic droughts. In this study TQ was not adversely affected by incorporation of compost at the highest rate.

This study indicates that the incorporation of compost into the upper 10-cm of soil prior to establishment of warm-season grasses can improve water holding capacity, providing a soil reservoir of water. This increased capacity can aid in reducing the need for supplemental irrigations between rainfall events, especially in subtropical environments where rainfall is plentiful but variable in timing.

Table 1. Average volumetric water content (VWC) and turfgrass quality (TQ) of three turfgrass species.

Species	VWC (%)	TQ (1-9)
Bermudagrass	30.1 b	6.5 a
Centipedegrass	31.7 a	6.3 a
Zoysiagrass	24.4 c	6.1 a

Means averaged over two consecutive years and not connected by the same letter are significantly different.

Table 2. Average volumetric water content (VWC) and turfgrass quality (TQ) of two, soil-incorporated composts.

Compost Source	VWC (%)	TQ (1-9)
Sod	28.1 a	6.3 a
Trimblings	29.4 a	6.3 a

Means averaged over two consecutive years and not connected by the same letter are significantly different.

Table 3. Average volumetric water content (VWC) and turfgrass quality (TQ) of three rates of composts incorporated into the upper 10-cm of soil.

Compost Rate (kg m ⁻²)	VWC (%)	TQ (1-9)
0.0	27.3 b	6.3 a
2.5	28.0 b	6.3 a
5.0	28.6 b	6.3 a
10.0	29.7 a	6.3 a

Means averaged over two consecutive years and not connected by the same letter are significantly different.

SOIL SURFACTANT AND TRINEXAPAC-ETHYL IMPROVE TURF QUALITY AND ROOTING CHARACTERISTICS OF BERMUDAGRASS AND SEASHORE PASPALUM UNDER DEFICIT IRRIGATION

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Introduction

Warm-season grasses, such as bermudagrass [*Cynodon dactylon* (L.) Pers.] and seashore paspalum (*Paspalum vaginatum* Swartz) are commonly used for turfgrass areas in the warmer and drier parts of the world where heat, drought and salinity can cause problems. In order to conserve irrigation water, turfgrass areas are frequently irrigated below evapotranspiration replacement levels. Strategies to alleviate the damaging effects of deficit irrigation and to improve turfgrass quality include the application of soil surfactants and plant growth regulators¹. This study investigated the effect of both chemicals on turfgrass quality and root architecture of bermudagrass and seashore paspalum under deficit irrigation at two water qualities from differing irrigation systems.

Methods

A two-year study (2012-2013) was conducted at New Mexico State University to investigate the effects of soil surfactants 'Revolution' (modified methyl capped block copolymer) and 'Dispatch' (alkyl polyglucoside blended with a straight block copolymer) and the plant growth regulator 'PrimoMaxx' {A.I. Trinexapac-ethyl (TE)[4-(cyclopropylhydroxymethylene)-3,5-dioxocyclohexanecarboxylic acid]} on turf quality and root growth under reduced irrigation. The study was conducted on Princess 77 bermudagrass and Sea Spray seashore paspalum mowed at a height of 1.25cm and irrigated at 50% reference evapotranspiration for short grass (ET₀)² with either an overhead sprinkler or a subsurface drip irrigation system. Irrigation was applied with either potable [electrical conductivity (EC) = 0.6 dSm⁻¹] or saline ground water (EC= 2.3 dSm⁻¹). For a detailed description of the irrigation systems, water quality, soil characteristics, fertilization and general maintenance please refer to Schiavon et al¹. Surfactants and TE were applied monthly from May until October at the rate of 20 L ha⁻¹ mo⁻¹ for Revolution, 2.3 L ha⁻¹ mo⁻¹ for Dispatch and 1.6 L ha⁻¹ mo⁻¹ for TE. Visual quality was determined monthly on a scale of 1 (worst) to 9 (best). Roots collection followed the procedure described by Serena et al³. Soil cores were collected at the end of each growing season (mid-November) and analyzed for rooting characteristics using WinRhizo software. The parameters of interest were root length density (cm cm⁻³), root diameter (mm), and root weight density (g cm⁻³). The experimental design was a completely randomized split-plot, with a combination of irrigation system and water quality as whole plot, and grass species, chemicals and sampling date as subplot treatment. All treatments were replicated three times. Data were subjected to ANOVA followed by multiple comparison of means using Fisher's protected least significant difference test at the 0.05 probability level.

Results and discussion

Turfgrass quality: Statistical analysis revealed a significant interaction between water quality, irrigation system grass species and chemicals. Means are presented in Table 1. Overall, subsurface drip-irrigated plots had greater quality than sprinkler-irrigated plots (Table 1). Bermudagrass exhibited greater quality when irrigated from sprinklers and seashore paspalum had greater quality when irrigated from below ground. The surfactant Revolution provided higher turf quality on bermudagrass only when plots were irrigated from a sprinkler system (Table 1). Trinexapac-ethyl provided greater quality for both grasses when irrigated with the subsurface drip system (Table 1). Similar results were documented by Schiavon et al¹, indicating that subsurface-drip distributed water more efficiently when deficit irrigation is used. Moreover, chemical treatments such as a soil surfactant or a plant growth regulator improves turfgrass quality under deficit irrigation¹.

¹ Schiavon, M., B. Leinauer, M. Serena, B. Maier, and R. Sallenave. 2014. Plant Growth regulator and soil surfactants' effects on saline and deficit irrigated warm-season grasses: I. Turf quality and soil moisture. *Crop Sci.*54:1-12. doi:10.2135/cropsci2014.10.0707

² Snyder, R.L., and S. Eching. 2007. PMDay.xls spreadsheet software for estimating daily or hourly reference evapotranspiration using the Penman-Monteith equation. University of California, Davis. <http://biomet.ucdavis.edu/Evapotranspiration/PMdayXLS/PMday.xls>.

³ Serena, M., B. Leinauer, M. Schiavon, B. Maier, and R. Sallenave. 2014. Establishment and rooting responses of bermudagrass propagated with saline water and subsurface irrigation. *Crop Sci.* 54:827-836. doi: 10.2135/cropsci2013.07.0512

Roots: Applications of TE increased root weight density (2.7133 g cm^{-3}) at 0-5 cm depths compared to Revolution (2.0679 g cm^{-3}) and Dispatch (2.0532 g cm^{-3}). Control plots (1.6424 g cm^{-3}) had the lowest root weight density. At the depth of 0-40 cm, TE increased root weight density under drip irrigated plots for both water qualities compared to the control treatment (Table 2). Turfgrasses irrigated with potable water from the subsurface and treated with Revolution had also greater root weight density than control plots. Applications of TE increased root length density (cm cm^{-3}) at the depth of 0-5 cm depth for both grasses compared to controls (6.5 cm cm^{-3} vs. 3.7 cm cm^{-3} in 2012 and 14.3 cm cm^{-3} vs 6.9 cm cm^{-3} in 2013). Both Revolution and TE also resulted in higher root length density compared to the control plots for the entire sampling depth under both irrigation systems (Table 3). Moreover, treating both bermudagrass and seashore paspalum with either Revolution, Dispatch or TE also resulted in smaller diameter roots at the 0-5 cm depth (data not shown). No information is available on the effects of TE on turfgrass roots of deficit irrigated warm-season grasses as all previous research was conducted on cool-season grasses under sufficient irrigation⁴. Similarly, no reports have been published on the effects of soil surfactants on root architecture of warm-season turfgrasses and data are only available for creeping bentgrass (*Agrostis palustris* Huds.)⁵. Our results suggested a positive relationship between turfgrass quality and root parameters. Applications of surfactants resulted in greater root length density, greater root weight density, and thinner roots, all indicators of a healthier turf stand. In summary, TE and soil surfactants have been shown to improve turf quality and rooting characteristics of grasses grown under deficit irrigation and should be included in turfgrass water conservation strategies. More research is needed to investigate if combining both chemistries has an even more synergistic effect on turfgrass quality and rooting characteristics than applying these chemicals alone.

Table 1. Turf quality for Princess 77 bermudagrass [*Cynodon dactylon* (L.) Pers.]] and Sea Spray seashore paspalum (*Paspalum vaginatum* Swartz) treated with Dispatch, Revolution or Trinexapac-ethyl (TE) and compared to an untreated Control. Grasses were irrigated with either potable (0.6 dSm^{-1}) or saline water (2.3 dSm^{-1}) from either a sprinkler or a subsurface drip-system. Quality ratings were taken on a scale from 1 (worst) to 9 (best). Values represent an average of 30 data points and are averaged over 2 years (2012 and 2013), 5 months (June to September) and 3 replications.

		Potable		Saline	
		Sprinkler	Drip	Sprinkler	Drip
Bermudagrass	Control	6.1	6.3	6.6	6.2
	Dispatch	5.3	6.2	6.3	6.3
	Revolution	6.9	6.5	7.0	6.3
	TE	5.0	6.6	6.5	6.0
Seashore paspalum	Control	4.8	7.0	6.3	7.0
	Dispatch	5.3	6.9	6.4	7.0
	Revolution	5.2	7.0	6.2	6.6
	TE	5.5	7.2	6.2	7.0

LSD = 0.614

Table 2. Root weight density (g cm^{-3}) at a depth of 0 to 40 cm for plots treated with Dispatch, Revolution or TE (Trinexapac-ethyl) and compared to an untreated Control. Grasses were irrigated with either potable (0.6 dSm^{-1}) or saline water (2.3 dSm^{-1}) from either a sprinkler or a subsurface drip-system. Values represent an average of 12 data points and are pooled over 2 years (2012 and 2013), 2 grasses {Princess 77 bermudagrass [*Cynodon dactylon* (L.) Pers.]] and Sea Spray seashore paspalum (*Paspalum vaginatum* Swartz)} and 3 replications.

	Potable		Saline	
	Sprinkler	Drip	Sprinkler	Drip
Control	1.0170 ef [‡]	1.2139 cdef	1.0456 ef	1.3262 bcde
Dispatch	1.3679 abcd	1.2075 cdef	1.1768 cdef	1.4023 abcd
Revolution	1.1398 def	1.4874 abc	1.1586 def	1.0100 f
TE	1.2253 cdef	1.6400 a	1.3233 bcde	1.6010 ab

[‡] Values followed by the same letter are not significantly different according to LSD (0.05).

Table 3. Root length density (cm cm^{-3}) at a depth of 0 to 40 cm for plots treated with Dispatch, Revolution or TE (Trinexapac-ethyl) and compared to an untreated Control after the growing periods of 2012 and 2013. Grasses were irrigated from either a sprinkler or a subsurface drip-system. Values represent an average of 12 data points and are pooled over 2 water qualities [potable (0.6 dSm^{-1}) or saline (2.3 dSm^{-1})], 2 grasses {Princess 77 bermudagrass [*Cynodon dactylon* (L.) Pers.]] and Sea Spray seashore paspalum (*Paspalum vaginatum* Swartz)} and 3 replications.

	2012		2013	
	Sprinkler	Drip	Sprinkler	Drip
Control	17.58 i [‡]	16.60 i	26.54 fgh	44.56 bc
Dispatch	25.05 fgh	20.70 hi	39.69 cd	44.25 bc
Revolution	24.94fgh	24.18 gh	40.29 c	33.75 de
TE	28.21 efg	31.06 ef	47.05 b	55.64 a

[‡] Values followed by the same letter are not significantly different according to LSD (0.05).

⁴ Beasley, J.S., B.E. Branham, and L.M. Ortiz-Ribbing. 2005. Trinexapac-ethyl affects Kentucky bluegrass root architecture. Hort Sci. 40:1539-1542.

⁵ Karnok, K.J., and K.A. Tucker. 2001. Wetting agent treated hydrophobic soil and its effect on color, quality and root growth of creeping bentgrass. Int. Turfgrass. Soc. 9:537-541.

WATER MANAGEMENT

POSTER PRESENTATIONS

RECLAIMED WATER USE FOR TURFGRASS IRRIGATION

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Introduction

Some regions of Portugal have excellent conditions for the practice of golf. The development and practice of this sport may cause problems for the region's water resources. The use of treated wastewater is a fundamental solution. The APA⁶ refers a typical consumption of 288,000 m³ of irrigation water for a 18 holes golf course. In areas such as the Algarve, where water is a scarce natural resource, typically from dams and lesser from aquifers, the use of alternatives water sources to irrigate golf courses has a strong interest and protects the water resource. The wastewater reuse is an issue of established interest⁷. The agronomic and economic benefits of using wastewater include the high levels of nutrients such as nitrogen and phosphorus that can stimulate plant growth and crop yield and reduce the use of chemical fertilizer. Microbiological and chemical characterization is needed for safety use of wastewater. Microbiological characterization is done for the evaluation of the presence of few fecal bacteria, and these are very important for the users (growers, farmers, and in the case of golf courses for the local workers and players). Alonso et al.⁸ refers that current water reclamation technology can ensure > 99.99% of pathogen removal. Also Mancino and Pepper⁹ mention that the probability to occur human diseases due to the use of wastewater for irrigation is low. However direct human contact may occur and health hazard should happen¹⁰. Several authors refer that fecal bacteria may be eliminated from soils, by photodegradation, by soil conditions (pH, texture, moisture, among others) and the presence of other microorganisms in few days after its application^{11,12,13}. Since the use of wastewater may require expensive treatment processes to guarantee its safe use the objective of this study was to evaluate the inactivation, under different climatic conditions, of fecal indicators and a pathogenic bacteria pulverized on surface of turfgrass, simulating irrigation procedures on golf courses. This study aimed to evaluate the survival of indicator and pathogenic bacteria on turfgrass under different climatic conditions (clear sky, partly cloudy and heavy cloudy), with and without wind and air temperature between 18 and 30 °C.

Methods

Three different plots of *Lolium perenne* lawn with an area of 0.6m x 0.6m each one were inoculated with known concentrations of *Enterococcus faecalis* NTCC 775, *Escherichia coli* NTCC 9001 and *Salmonella typhimurium*. ATCC 14028 The experiment was carried out in the experimental field of the Gambelas Campus at the University of Algarve, Portugal. A total of nine plots were used. Each prepared microbial suspensions were applied in three individual plots at 1 L plot⁻¹ using sterile hand-spreaders, in three different days with different climatic conditions. Samples were collected in three different moments: M0, just after application; M4, four hours after the application, and M6, six hours after the application.

The bacterial suspensions of *E. coli*, *E. faecalis* and *S. typhimurium* used in the field experiments were prepared using Ringer solution at a microbial concentration of 10⁸ cells mL⁻¹. From each sprayed plot, and for each sampling time (M0, M4, and M6), thirty grams of turfgrass were taken aseptically and homogenized with 270

⁶ APA (2009). Manual de Boas Práticas Ambientais para Campos de Golfe. Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional. Pp. 333. ISBN 978-972-8577-45-2.

⁷ Salgot, M., Huertas, E., Weber, S., Dott, W., and Hollender, J.. (2006). Wastewater reuse and risk: definition of key objectives. Desalination, 187: 29–40.

⁸ Alonso M.C., L.P.C. Dionísio, A. Bosch, B.S. Pereira de Moura, E. Garcia-Rosado and J.J. Borrego (2006). Microbiological quality of reclaimed water used for golf courses irrigation. Water Science & Technology. Vol. 54 Nº 3, pp 109–117.

⁹ Mancino, C.F. and Pepper, I.L. (1997). Irrigation of turfgrass with wastewater. In: Wastewater Reuse for Golf Course Irrigation, US Golf Association (ed.), Lewis Publishers, Boca Raton, pp. 173–191.

¹⁰ McLain J.E.T. and C.F. Williams (2012). Assessing environmental impacts of treated wastewater through monitoring of fecal indicator bacteria and salinity in irrigated soils. Environ Monit Assess, 184: 1559–1572.

¹¹ O'Toole, J., Sinclair, M., Diaper, C., & Leder, K. (2008). Comparative survival of enteric indicators, *E. coli* and somatic and F-RNA bacteriophages on turf-grass irrigated with recycled water. Water Science and Technology, 58(3), 513–518.

¹² Sidhu, J.P.S., J. Hanna and S.G. Toze (2008). Survival of enteric microorganisms on grass surfaces irrigated with treated effluent. Journal of Water and Health, 06.2: 255-262.

¹³ Higgins J. and C. Hohn (2008). Effects of prevalent freshwater chemical contaminants on in vitro growth of *Escherichia coli* and *Klebsiella pneumoniae*. Environmental Pollution 152: 259-266.

mL of sterile Ringer's solution (Merck, Darmstadt, Germany) for 90 sec in a sterile plastic bag using lab blender (Masticator, IUL Instruments, Barcelona, Spain). Serial decimal dilutions, were prepared, in sterile Ringer's solution. Samples collected from the plot were *E. coli* suspension was sprayed were tested for this bacteria and the same procedure was done for *E. faecalis* and *S. typhimurium*. Enumerations were done in selective media using the membrane filtration technique¹⁴. Appropriate volumes of the dilutions were filtrated through sterile cellulosic membranes (HA, Millipore, USA) with 0.45µm pore diameter. The quantitative analyses were done in triplicate. Culture media and incubation conditions used were: (a) m-FC agar (Difco) for *E. coli* (44.5 ± 0.5 °C, 24 h) with confirmation of the β-glucuronidase activity using the MUG technique proposed by Gauthier et al. (1991)¹⁵. (b) m-Enterococcus agar (Difco) for *Enterococcus faecalis* (36 ± 1 °C , 48 h); (c) Xylose lysine desoxicolate selective agar XLD (Oxoid) for *Salmonella* (36 ± 1 °C , 24 h).

The enumerated bacteria values had been submitted to a variance analysis (ANOVA); differences were considered significant when p<0.05. Normality of sample distribution and homogeneity of variances were verified before ANOVA¹⁶. The comparative analysis of the treatment averages was realized through the New Multiple-Range Duncan Test¹⁷. For the statistical analysis it was used the SPSS ver. 19.0 (SPSS Incorporation, 1989-2010, U.S.A.) and the Microsoft Excel (Office 2013).

Results and discussion

The results indicated population reductions of 4 orders of magnitude for *E. coli* and 2 orders for *E. faecalis* and only 1 order for *Salmonella*. In a partially cloudy day there were significant reductions in both *E. coli* (p <0.001), *E. faecalis* (p <0.001) and *S.typhimurium* (p <0.001), with reductions of two orders of magnitude, after 4 hours for *E. coli*, and after 6 hours, for *E. faecalis* . For *Salmonella* a reduction after 6 hours was only of one order of magnitude. After 4 hours the survival rate was 2.5% for *E. coli*, 16.1% for *E. faecalis* and 22.2% for *S.typhimurium*. Air temperature were around 18 °C at all sampling moments. In a day with a clear sky and air temperatures around 30 °C it was observed also significant reductions in *E. coli* (P <0.05), *E. faecalis* (p <0.001) and *Salmonella* (p <0.001). After 4 hours there were three orders of magnitude reduction for *E. coli* and 1 order of magnitude for *Salmonella*. After 6 hours the reduction was of four orders of magnitude for *E. coli*, two orders for *E. faecalis* and one for *S.typhimurium*. After 4 hours the survival rate was 0.1% for *E. coli*, 64.7% for *E. faecalis* and 8.0% for *S.typhimurium*. Two hours later the survival rate was, respectively, 0.01, 1.4 and 5.3%. In a day, with heavy cloudy sky, samples were taken immediately after inoculation and 6 hours after. No significant reduction was found for *E. faecalis* (p = 0.453), but significant reductions were found for *E. coli* (p <0.01) and for *S. typhimurium* (p <0.001). After 6 hours the survival rate was 2.2% for *E. coli*, 62.4% for *E. faecalis* and 3.2% for *S. typhimurium*. *E. coli* and *E. faecalis* showed different degrees of inactivation. This result is in agreement with several authors¹⁸. Sidhu et al. (2008)⁷ refer lower *Salmonella* spp inactivation compared with *E. coli* in turfgrass studies. O'Toole et al. (2008)⁶ results for *E. coli* survival suggests that a 4 hour withholding period should confer a reduction in the health risk associated with bacterial enteric pathogens. It was shown that sunny and even cloudy days, may reduce the pathogenic microorganism's populations in a few hours, and above all because these populations are strongly reduced when using treated wastewater. An efficient wastewater treatment associated with the inactivation effects of the climatic conditions (sunlight and temperature) will allow the use of treated wastewater for golf course irrigation without health risks for both players and users.

¹⁴ APHA (1998). Standard Methods for the Examination of Water and Wastewater, 20th edn, American Public Health Association, Washington, DC.

¹⁵ Gauthier M.J., V.M. Torregrossa, M.C. Balebona, R. Cornax, and J.J. Borrego (1991). An intercalibration study of the use of 4-methylumbelliperyl-B-D-glucuronide for the specific enumeration of *Escherichia coli* in seawater and marine sediments. Syst. Appl. Microbiol., 14, 183–189.

¹⁶ Zar J.H. (1999). Biostatistical Analysis. 3rd Ed. Prentice-Hall International, Inc., New Jersey, U.S.A.

¹⁷ Duncan D.B. (1995). Multiple range and multiple F tests. Biometrika, vol. 11, pp. 1- 42.

¹⁸ Sinton, L.W., R.J. Davies-Colley and R.G. Bell (1994). Inactivation of Enterococci and Fecal Coliforms from Sewage and Meatworks Effluents in Seawater Chambers. Applied and Environmental Microbiology, 60(6): 2040-2048.

ESTABLISHMENT, ROOT DEVELOPMENT, AND NITRATE LEACHING OF NATIVE WARM-SEASON-GRASSES IRRIGATED WITH TAILORED WATER

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Introduction

As a response to shrinking supplies of potable water, treated effluent has become an important water source used for turfgrass irrigation. Decentralized water treatment systems can produce water with adjusted levels of nitrate in the effluent water based on the plant's growing requirements. The levels of essential plant nutrients such as nitrogen found in effluent water could potentially enhance establishment and growth¹. However, if application rates of nitrogen are greater than what can be utilized by the plants, excess nitrate could leach below the rootzone and contaminate groundwater. Rooting characteristics² and N uptake by turf³ are among factors affecting nitrate leaching from turfgrasses. Therefore, optimizing both irrigation and fertilizer application in accordance with root development during the growth of a turfgrass stand is particularly important for reducing nitrate leaching⁴. Little is known about the feasibility of establishing warm-season turfgrasses with recycled water or what if any impacts there would be on nitrate leaching or salt accumulation in soils. It was the objective of this study to investigate the establishment rate of three warm-season grasses propagated from seed and irrigated with tailored water (treated effluent adjusted to 15 ppm of NO₃-N) and to measure the impact of such irrigation water on nitrate (NO₃-N) leaching, electrical conductivity (EC), and root development.

Materials and Methods

A greenhouse experiment was conducted at New Mexico State University in Las Cruces, NM, USA during summer of 2015 using tailored water to establish bermudagrass [*Cynodon dactylon* (L.)] cv. Princess77', buffalograss [*Buchloe dactyloides* (Natt.) Eng.] cv. 'SWI 2000', and inland saltgrass [*Distichlis spicata* (L.)] from seeds in a local loamy sand. Control containers were irrigated with potable water and received amounts of granular Ca(NO₃)₂ fertilizer to match the total N applied to containers irrigated with tailored water. Irrigation was applied daily at 100% of reference evapotranspiration. Leachate was collected weekly at a depth of 10 cm using soil solution access tubes (SSAT), (IRROMETER Company Inc., Riverside, CA) and drainage water was collected in trays placed below each container. Leachate and drainage were analyzed for NO₃-N and EC. At the end of the study, soil samples were collected and analyzed for total N, NO₃-N, and EC. Root samples were collected three times during the research period at a depth of 0-5, 5-15, and 15-50 cm to calculate the root length density (RLD) and the root weight density (RWD) using WinRhizo software (Regent Instrument Inc.) and an Epson Perfection V700 Scanner. Turf cover was assessed weekly by means of digital image analysis. The experimental design was completely randomized with three grasses, two irrigation treatments, and three replicates.

Results and Discussion

Nitrate-N in the leachate of bermudagrass and buffalograss did not exceed 10 mg L⁻¹ at both depths after June 30 [21 days after seeding (DAS)]. Nitrate-N in the drainage water of inland saltgrass irrigated with tailored water remained below 10 mg L⁻¹ after July 7 (28 DAS) (Fig. 1).

¹ Ozturk, M., S. Gucel, S. Serdal, and A. Guvensen. 2011. An overview of the possibilities for wastewater utilization for agriculture in Turkey. *Israel J. of Plant Sci.* 59.2-4: 223-234.

² Geron, C.A., K. Danneberger, S.J. Traina, T.J. Logan, and J.R. Street. 1993. The effect of establishment methods and fertilization practices on nitrate leaching from turfgrass. *J. Environ. Qual.* 22:119-125.

³ Miltner, E.D., B.E. Branham, E.A. Paul, and P.E. Rieke. 1996. Leaching and mass balance of ¹⁵N-labeled urea applied to a Kentucky bluegrass turf. *Crop Sci.* 36:1427-1433.

⁴ Bowman, D.C., and T.D. Colmar. 2006. Irrigation and fertilizer strategies for minimizing nitrogen leaching from turfgrass. *Agric. Water Manage* 80:160-175.

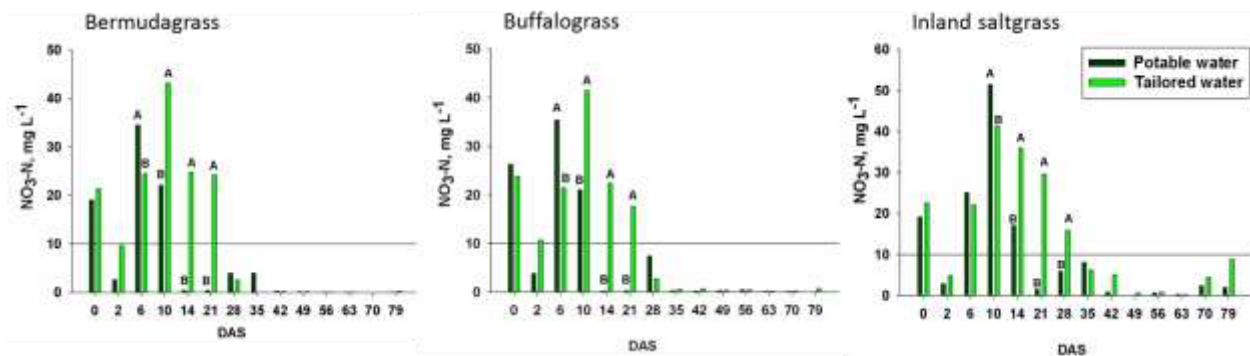


Fig. 1. Changes in NO₃-N content in the drainage water of bermudagrass, buffalograss, and inland saltgrass at days after seeding (DAS) irrigated with either potable or tailored (treated effluent with 15 ppm of NO₃⁻N) water. Bars with the same letters (separately for each sampling date) are not significantly different from one another (Fisher’s protected LSD at α = 0.05).

Bermudagrass established with tailored water was the fastest (24 DAS) to reach 50% cover (Table 1) However, water quality did not affect DAS to reach 75% cover for bermudagrass and buffalograss. Saltgrass irrigated with tailored water did not reach 75% coverage by the end of the research period.

Table 1. Days after seeding (DAS) to reach 50% and 75% coverage.

Grass	DAS 50%	DAS 75%
Bermudagrass granular	35B [†]	46BC
Bermudagrass tailored	24C	37C
Buffalograss granular	33B	54ABC
Buffalograss tailored	32B	69AB
Saltgrass granular	48A	82A
Saltgrass tailored	55A	d.n.r.

[†] Values in a column followed by the same letter are not significantly different from one another (Fisher’s protected LSD, α = 0.05). d.n.r. did not reach

At the end of study, grasses irrigated with tailored water had higher total N concentration in the leaf tissue, however, values did not differ between grasses. Also, irrigation with tailored water resulted in a greater clipping yield for all grasses than irrigation with potable water. Biomass was highest for inland saltgrass and lowest for bermudagrass. Root Length Density and RWD were not affected by grass species or water quality in July and August, and differed only between depths. At the end of the research period in September, inland saltgrass had lower RLD and RWD at 0 to 5 cm than bermudagrass and buffalograss (Table 2). Irrigation with tailored water resulted in greater RLD at 0 to 5 cm than irrigation with potable water (Table 3).

Table 2. Root length density (cm cm⁻³) of three grasses in September at three depths. Each value represent an average of two water qualities (potable and tailored) and three replicates.

Grass	0-5	5-15	15-35
Bermudagrass	7.82a [†]	2.19b	0.9a
Buffalograss	7.21a	4.48a	0.75a
Inland saltgrass	4.13b	1.97b	0.66a

[†] Values in a column followed by the same letter are not significantly different from one another (Fisher’s protected LSD, α = 0.05).

Table 3. Root length density (cm cm⁻³) in September at three depths irrigated either potable or tailored water. Each value represent an average of three turfgrasses and three replicates.

Water	0-5	5-15	15-35
Potable	5.38b [†]	3.14a	0.74a
Tailored	7.4a	2.62a	0.8a

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SURFACTANT SEED COATING TECHNOLOGY: A METHOD TO INCREASE SOIL MOISTURE AND IMPROVE REWETTABILITY OF SANDS

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Introduction

Significant water restrictions have been implemented for turfgrass managed areas, particularly on homelawns and in drought prone areas such as California, USA¹. As water resources decrease and restrictions increase, any crop designated as non-essential, such as turfgrass, will receive limited water rights. These limitations are particularly critical in coarse textured soils with low water holding capacity and during seedling establishment. Water availability affects the number of seeds that will germinate² and reduces cover. Soil surfactants have been applied in turfgrass systems to not only ameliorate water repellency but to also increase volumetric water content (VWC) and improve turfgrass quality under deficit irrigation³. On golf courses, surfactant applications are routinely made weekly to monthly via irrigation systems or sprayers. A novel surfactant delivery method, surfactant seed coating technology (SC), was initially developed to improve germination of native species plants in wildfire affected locations and in areas where irrigation is only via rainfall⁴. Further research determined SC increased VWC in water repellent soils⁴. The objective of this research was to determine if SC would improve seed germination in a hydrophilic 100% sand soil.

Methods

Two greenhouse trials were conducted in 2014 and 2015 at the University of Florida's Institute of Food and Agricultural Sciences (IFAS), Fort Lauderdale Research and Education Center. Plastic (6.35 cm x 6.35 cm) pots were filled to approximately 1.25 cm below the top of pot with 100% hydrophilic sand (SAND). Particle size analyses showed that the majority (>75%) of the sand was categorized as coarse (0.5mm) with a saturated hydraulic conductivity 129.3 cm/hr. Perennial ryegrass seed was treated with an alkyl terminated block copolymer surfactant at 10% (w/w) (SC) (Aquatrols Corp of America, Paulsboro, NJ) or left untreated (CHK). Surfactant coating doubled the weight of the seed, therefore, the untreated seed treatments were sown with approximately twice the number of seeds as the SC treatment. After seeds were hand sown, seeds were covered with approximately 0.65 cm of sand and watered immediately with approximately 6.5 mm of water to facilitate movement of surfactant off the seeds. Thereafter, a deficit irrigation regime was imposed with misters applying 2.5 mm of water every other day during the establishment phase. Percent establishment was visually rated using a scale of 0-100%, where 0%= no seedlings emerged. Volumetric water content (VWC) readings were collected once seeds were established using a HH2 data collector and ML2 moisture probe (Delta-T Devices, Houston, TX). Plants were brought to field capacity then allowed to dry down until wilt was observed to determine treatment separation and rewettability of soils. Four and two dry down periods were initiated in trial 1 and trial 2, respectively. Treatments were arranged in a completely randomized block design with 4 replications and all data were subjected to analysis of variance using PROC GLIMMIX with Tukey's multiple comparison procedure to determine significant differences ($P < 0.05$).

Results and Conclusions

In trial 1, VWC was significantly greater in SC treated sand when compared to CHK on 6 of 10 dates. In trial 2, VWC was significantly greater in SC treated when compared to CHK on 2 of 7 dates (Table 1). Final percent cover was similar in both trials despite twice the number of untreated seeds planted when compared to the SC

¹ Rogers, P. 2015. California water conservation weakening as drought worsens. Tougher rules on the way? Mercury News. Online at: http://www.mercurynews.com/drought/ci_27729990/california-drought-state-passes-mandatory-new-water-conservation?source=infinite-up

² Bewley, J.D., J.B Kent, H.W.M. Hilhorst and H. Nonogaki. Seeds. Physiology of Development, Germination and Dormancy. 3rd Ed. Springer. 2013.

³ Mitra S. J. Kissinger, A. Chavez, R. V. Plumb and M. N. Awady. 2003a. Reducing irrigation water on turfgrasses by application of surfactants. *Misr Journal of Agricultural Engineering*. 20(4):43-52.

⁴ Madsen, M.D. 2009. Influence of soil water repellency on post-fire revegetation success and management techniques to improve establishment of desired species. Ph.D. diss. Brigham Young University. Provo, UT.

treatment. These results indicate that SC improved seed germination as well as increased VWC in a hydrophilic sand. SC technology may improve turfgrass seedling establishment when irrigation is limited due to water restrictions.

Table 1. Volumetric water content (%) as affected by seed coating within sand. Trial 1 and Trial 2.

Trial 1										
Trt	Dec31	Jan4	Jan6*	Jan7	Jan8*	Jan9	Jan10	Jan11*	Jan12	Jan13
SC	9.70 a	7.68 a	2.70 a	15.30 a	9.13 a	18.28 a	16.53 a	8.95 a	13.40 a	8.50 a
CHK	8.35 a	6.50 a	2.30 b	5.65 b	3.47 a	11.55 b	9.55 b	5.13 b	6.58 b	3.38 b
Trial 2										
Trt	Mar4	Mar6	Mar9	Mar11*	Mar13	Mar16*	Mar18			
SC	12.80 a	7.15 a	5.00 a	3.47 a	18.05 a	2.43 a	9.20 a			
CHK	10.62 a	5.88 a	4.65 a	3.85 a	11.85 b	0.80 b	8.00 a			

*After VWC was collected on these dates, plants were irrigated to field capacity.

Means with different letters are statistically different at the 0.05 probability level based on the Tukey multiple comparison method.

**TECHNOLOGY ADVANCES AND TURFGRASS MAINTENANCE
(MOWING, FERTILIZATION, ENVIRONMENT, CERTIFICATION)**

ORAL PRESENTATIONS

PRECISION FARMING PRACTICES IN SPORTS TURF MANAGEMENT

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Introduction

Precision farming is the application of geospatial techniques and sensors to identify spatial and temporal variations in the soil, weather conditions, plant adaptability, irrigation and chemical control requirements and to deal with them using alternative strategies. To foster crops management in agriculture, precision farming has gained ground in the past 20 years¹ and aims to obtain detailed site-specific information, in order to apply water, fertilizers and pesticides² only where, when and in the amount needed by plant. It can also provide information on both light and temperature requirement, through sensors that return accurate measurements. Precision farming was developed to make the best management decisions to preserve the environment and sustain agriculture, but recently it became a precious tool also for the management of turfgrasses for sport field surfaces, with the aim to achieve the best quality with the least impact on the environment and players. Several parameters can be measured and monitored and the related information are elaborated to facilitate the turf manager decisions, including pitch playing quality parameters concerning ball-surface and athlete-surface interactions³. As in several top football venues worldwide, in many “Ligue 1” football pitches precision farming, on top of the above aspects, takes on a further meaning of advanced environment conditioning. A suite of artificial conditioning devices (under floor heating and ventilation, above ground ventilation, artificial lighting, incubator greenhouses for penalty areas, etc.) are commonly used to modify turfgrass growing conditions for the benefit of pitch aesthetics, performance and health.

Methods

Monitoring parameters for precision farming in sport fields:

Water and irrigation

Rain and humidity sensors are on-field climatic tools used to prevent the execution of the sprinklers during or after a rainfall and to monitor soil water content. Evapotranspiration measurement has also become a tool for decision support, by supplying an indication of real-time and integrated plant water consumption. Furthermore, all these data can be combined through the use of artificial intelligence systems that predict the water stress and indicate the amount of water to be reintegrated, resulting in an energy and environmental saving.

Fertility

Electrical and electromagnetic sensors measure electrical resistivity/conductivity, capacitance or inductance affected by the composition of soil. Rapid response, low cost and high durability have made electrical and electromagnetic sensors an attainable technique for the soil mapping. Obtained maps have been correlated to: soil texture, salinity, organic matter, moisture content, and other soil attributes⁴. Nitrogen sensors can detect nitrate levels in fields, helping the greenkeeper to reduce fertilizer use. In addition to the potential economic savings, N-sensors can also help to quantify and reduce groundwater pollution caused by excess nitrates that flow from fields into streams, lakes and oceans.

The analysis of radiation reflected by turf plants can supply precious information on species quality and colour, Leaf Area Index (LAI), chlorophyll, biomass, drought stress and the nutritional status. Therefore, it could be gathered via remote sensing, both from satellite or drone imagery, as a diagnostic tool for detecting variations in all these parameters. For spectral reflectance, the normalized difference vegetation index (NDVI) has been the most commonly used for plant performance or stress indicator⁵. Recently, the use of handheld crop sensors

¹ Hatfield J. L. 2000. Precision agriculture and environmental quality: Challenges for research and education. *USDA National Resources Conservation Service*.

² Pons G., Compta M., Berjaga X., Lulli F., Lopez J.M. 2014. Intelligent System for Optimisation in Golf Course Maintenance. *Frontiers in Artificial Intelligence and Applications: Artificial Intelligence Research and Development*. 269: 67-76.

³ Fédération Internationale des Football Associations. 2012. FIFA quality concept for artificial turf—Handbook of test methods. FIFA, Zurich, Switzerland.

⁴ Adamchuk V. I., Hummel J. W., Morgan M. T., and Upadhyaya S. K. 2004. On-the-go soil sensors for precision agriculture. *Computers and electronics in agriculture*. 44: 71-91.

⁵ Carlson T. N. and Ripley D. A. 1997. On the relation between NDVI, fractional vegetation cover, and leaf area index. *Remote sensing of Environment*. 62: 241-252.

is increasing as they are affordable and easy-to-use measurement devices that can be used to assess the health of a turf in order to make better nutrient management decisions (e.g. GreenSeeker). The sensor emits brief bursts of red and infrared light, and then measures the amount of each type of light that is reflected back from the plant, then it displays the measured value in terms of an NDVI reading (ranging from 0.00 to 0.99). The strength of the detected light is a direct indicator of the health of the crop: the higher the reading, the healthier the plant.

Diseases and pesticides

Much can be achieved by early forecast of the onset of turf diseases. Estimation of tendencies for disease and pest conditions (pest aetiology) are measures that can effectively reduce the need for chemicals. The processing of index correlations between turf grass status (water content, NPK content, presence of weeds, presence of fungal diseases, presence of abiotic stresses, colour, cover, etc.) and on-site sensor data (temperature, humidity, radiation, leaf wetness, evapotranspiration, etc.) can lead to intelligent recommendations that can be applied to the specific environment for adequate decision.

Other parameters

Modern stadiums are often provided with ventilation and heating systems and in order to optimize their use, the market offers a wide range of devices with different features simultaneously, like monitoring moisture levels and daily soil temperatures, tracking salt build-up, collecting the data and compiling tables, graphs and dashboards wireless. This information can then be accessed from any internet-connected computer and allow to make the correct adjustment of the ventilation/heating system (e.g. Toro Turf Guard). Moreover, sport fields frequently require artificial lighting especially during winter, when direct natural radiation is very limited. The greenkeeper decision on light supply requirements is supported by dedicated devices and applications that are able to visualize sunlight and shade throughout the year for any site (e.g. Sun Surveyor), and that can measure the photosynthetically active radiation (PAR) through quantum sensors (e.g. Apogee quantum Sensor).

Conclusions

The basic objectives of site-specific management are to improve turf quality and playability and to protect the environment. Information about the variability of different parameters within a field is essential for the decision-making process. The possibility to obtain different data rapidly and inexpensively remains one of the major aim of precision farming, and this is a particularly welcome opportunity in the management of high-end natural turf football venues.

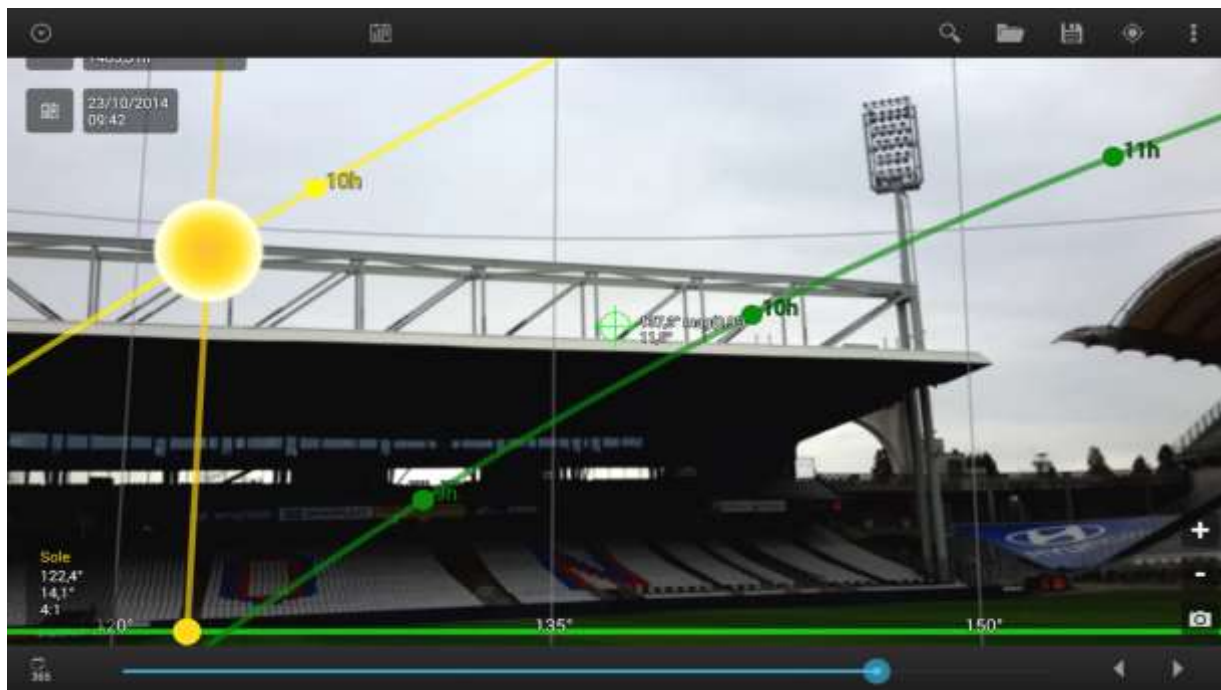


Figure 1. Example of Summer solstice (yellow) and Winter solstice (green) sun trajectories as captured through the Sun Surveyor app in augmented reality mode (“Gerland” Stadium, Lyon, FRANCE. 15th December 2014).

FERTILIZER USE RESTRICTIONS AND ORGANIC LAWN CARE: EVALUATION OF COMPOST TEA, COMPOST TOPDRESSING AND CULTIVATION ON TURF COLOUR, QUALITY AND WEED ENCROACHMENT

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Introduction

In response to total daily maximum nutrient and sediment load limits placed on watersheds throughout the United States, several states within this country have passed legislation restricting the amount of nitrogen and phosphorus that can be applied to turfgrass. In states such as Maryland restrictions placed on mineral and synthetic fertilizers apply to compost as well. This has resulted in the loss of the use of P containing compost on lawns having optimal to excessive soil test P and has placed an annual cap of 146 kg N ha⁻¹ on the application of compost to mature tall fescue (*Festuca arundinacea* Schreb.) lawns when P is not a factor constraining its use¹. Recommended rates of compost application have traditionally been much higher than 146 kg N ha⁻¹ yr⁻¹ to account for that fact that most of the N in compost is not available for plant uptake in the year it is applied². When compost is applied to turfgrass it is frequently done in combination with hollow tine cultivation to insure a layer of organic matter does not form on the soil surface. In recent years the use of compost tea on turf has become a popular component of organic lawn care programs. Its use as a liquid spray has been promoted as a way to provide nutrient and biological benefits to plants and soil however, only a few studies involving the use of compost tea on turfgrass appear to exist in the literature³. The continued inclusion of compost and compost derived materials as integral components of an organic lawn care program depend on turfgrass performance. In this study we evaluated the effect of compost topdressing and the use of a slowly available synthetic fertilizer on turf colour, quality and weed encroachment when both are applied at the same annual N rate. The effect of compost tea and hollow tine cultivation on same three response variables was also examined as was the effect of compost when applied at a rate commonly used by turfgrass practitioners.

Methods

A sewage sludge based biosolids compost, municipal yard trimmings compost, and a polymer coated urea fertilizer (Signature 35-0-0, Loveland Products, Inc. Greeley CO) were applied at 156 kg N ha⁻¹ once per year for 3 years to a tall fescue and Kentucky bluegrass (*Poa pratensis* L.) lawn in College Park MD and to a tall fescue lawn in Potomac Maryland. The College Park and Potomac lawns were 4 and 5 years old, respectively, when the study began and were mowed at 6.4 to 7.6 cm and 10 cm with the clippings returned. Each of three N source treatments was subjected to 0, 1 or 2 passes of Ryan GA 30 equipped with 1.9 cm by 12.7 cm tines that were spaced 6 cm apart from one another. The cultivation treatments were imposed once per year, in the fall, immediately prior to applying the 3 N-source treatments. The cores were broken apart and left in place following cultivation. Three additional non cultivated treatments included in study were an untreated control and the two compost sources applied to a depth of 1 cm. Compost tea, brewed from the yard trimming compost, was applied monthly in years 2 and 3 to one half of each whole plot at a rate of 1630 L ha⁻¹ throughout the growing season. The fertilizer and cultivation treatments were arranged as a randomized complete block design with each treatment being replicated three times within 3 × 3 m whole plots. When compost tea was added as a third treatment in the second year of the study, the structure of the treatments was a randomized complete block split plot design. Data analysis consisted of analysis of variance (ANOVA) of the factorial arrangement of treatments present within the experiment design, and mean contrasts that compared the control treatment with the five other non-cultivated treatments.

¹Turner, T. 2013. Fertilizer recommendations for commercially maintained lawns and turfgrass in Maryland. University of Maryland Turfgrass Technical Update. TT 115, 9 pp.

²Johnson, G.A., Y.L. Quan and J.G. Davis. 2006. Effects of compost topdressing on turf quality and growth of Kentucky bluegrass. Applied Turfgrass Science. doi:10.1094.ATS-2006-0113-01-RS

³Miller N. A. and J. J. Henderson. 2012. Organic management practices on athletic fields: part 1: the effects on color, quality, cover, and weed populations. Crop Science, 52:890-903.

Results and Discussion

Cultivation in the fall resulted in quicker early spring green up in all three years of study at College Park with interactions between nitrogen source and cultivation treatment occurring in study year 2 and 3 (data not shown). In these two cases, increasing intensity of cultivation had a positive effect on turf color in plots receiving either the synthetic fertilizer or biosolids compost. The effect of cultivation on color was limited to early spring with no consistent color response being seen with the use of this practice after the month of March in all three years of the study. With the exception of one date, cultivation had no effect on turf quality at either site. Similarly, with exception of one date at College Park, compost tea had no effect on turf color or quality at either site.

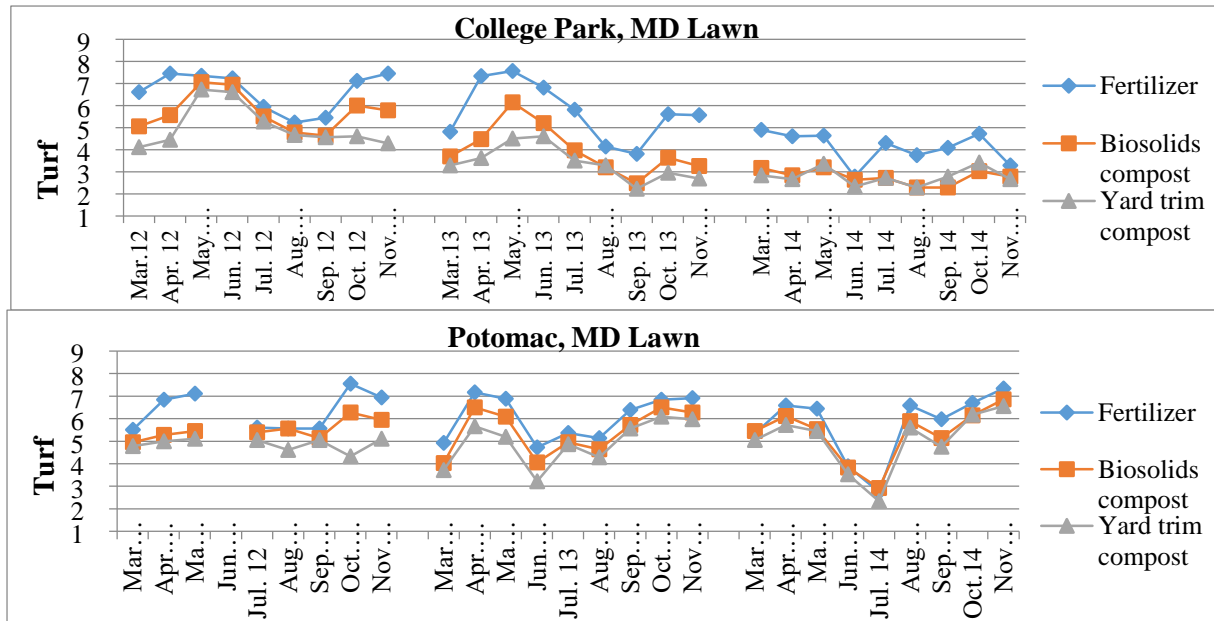


Figure 1. Turf quality in response to polymer coated urea (i.e., synthetic) fertilizer, biosolids compost and yard trimmings compost applied at $156 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. † No data was collected in this month. Turf quality 1-9 scale, 6 = commercial acceptance.

Nitrogen source and compost amount affected turf quality, colour and weed encroachment. With the exception of the months of May and June 2012, the use of the synthetic fertilizer consistently resulted in the higher quality ratings when compared to yard trimming compost when both were applied at the same N rate at College Park (Fig. 1). Similarly, plots receiving the biosolids compost had lower turf quality than turf receiving the synthetic fertilizer on most dates at College Park when both were applied at same N rate. The biosolids compost when applied to a depth 1.0 cm at College Park had higher turf quality on all rating dates than the synthetic fertilizer applied at $156 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (data not shown). Conversely the plots receiving either rate of the yard trimmings compost at Potomac site had quality that was no better than non-fertilized control on all but two evaluation dates (data not shown). The use of the synthetic fertilizer promoted earlier spring (i.e., March) greenup and longer late fall color (i.e., Nov.) that did the two composts at College Park, but at all other times and locations there was little different in turf color for the three materials when all were applied at the same N rate (data not shown). The encroachment of broadleaf and annual grass weeds was more severe at College Park than at Potomac, with significantly higher levels of the two weed types being observed in plots receiving either of the two compost types compared to the use of the synthetic fertilizer applied at the same N rate (data not shown). The higher mowing height and greater native soil organic matter contents measured at Potomacsite (3.0 versus 4.5%) are likely factors responsible for the smaller differences seen in turf quality and weed encroachment at this site. The results of this study indicate that should bagged fertilizer N application restrictions be extended to include compost materials, a likely consequence of this action would be a decline in turfgrass quality.

EFFECTS OF NITROGEN, PHOSPHORUS, MOWING HEIGHT AND MYCORRHIZA INOCULATION ON PURE RED FESCUE AND MIXED FESCUES / BENTGRASS GOLF GREENS

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Introduction

Red fescue (RF; *Festuca rubra*) is used on golf greens in northern Europe because of the excellent putting quality and low requirements for nitrogen (N) and water and its high resistance to diseases. However, because RF is less dense than most alternative species, RF greens can be invaded by annual bluegrass (AB; *Poa annua*). Mixed greens with fescue and colonial bentgrass (CB; *Agrostis capillaris* L.) are common in UK and Scandinavia, and may be more competitive to this weed. Only few golf courses grow RF + velvet bentgrass (VB; *A. canina*), but this combination is interesting because VB is drought tolerant¹ and has a high playing quality². Invasion of AB into fescue/bentgrass was exacerbated by increased N input³, but Arbuscular-mycorrhizal fungi (AMF) has been reported to reduce the biomass of AB in mixed AB / bentgrass greens⁴.

Lower mowing may give preference to bentgrass over RF, but there is also a risk that mixed greens will lose the typical playing characteristics of fescue greens⁵. The objective of this research was to determine the effects of mowing heights, N and Phosphorus (P) rates, and AMF inoculation on visual quality, botanical composition, competition from AB and playability on a USGA putting green seeded with pure RF, RF+CB or RF+VB.

Methods

The field experiment was conducted as a split-split block experiment at Landvik, Norway, from Aug. 2010 to Oct. 2012. The root zone consisted of sand/peat 90/10 % (v/v) and the soil P content was 1.5 mg 100 g⁻¹ (P-AL). The experiment had 4 factors: (1) *seed mixture*: 100% RF, 90/10% RF+CB or 90/10% RF+VB; (2) *mowing heights*: 4.0 or 5.5 mm; (3) *N-rates*: 5, 10 or 15 g m⁻² yr⁻¹ and (4) *P-rates/mycorrhiza*: 0 and 1.8 g P m⁻² yr⁻¹ without inoculation and 0 g P m⁻² yr⁻¹ with SYMBIVIT^{®6}. SYMBIVIT[®] was applied at a rate of 150 g m⁻² on June 2nd 2011 and May 15th 2012. All fertilizers were applied in liquid form; N and P at 2-wk intervals, and K and micronutrients at 4-wk intervals. The green was mown three times a week, regularly top dressed with a total of 9 mm pure sand per season, groomed once a week and subjected to wear 1-3 times a week. The trial was irrigated to field capacity each time the soil moisture at 0-12 cm depth was 8 % (v/v) or less.

The turf density and overall impression were recorded monthly from June 2011 to October in 2012 using a scale from 1-9. The number of tillers of fescue and bentgrass was counted in 11 mm soil cores extracted from the plots. An AB plug, 56 mm diameter, was inserted in all plots on Aug. 18th 2011, and the diameter was measured 14 months later. The surface hardness was recorded monthly during both growing seasons using a Clegg hammer (2.25 Kg). The ball distance was recorded in September 2011, and monthly from July to September 2012 with a short stimpmeter⁷. A cylindrical sample for determination of root weight, 56 mm in diameter and 25 cm deep, was taken from each plot in October 2012.

For the mycorrhiza assay, 2 soil cylinders of 21 mm diameter and 20 cm deep were taken randomly in Sept.-Oct. 2012 from plots mowed at 5.5 mm. Roots were prepared according to Koske and Gemma⁸. The percentage of roots colonized by mycorrhiza was quantified according to the 'grid-line intersect' method⁹. Thatch/mat thickness and the organic matter content were measured from two soil cores of 21 mm in diameter, taken on

¹Aamlid, T. S., J. W. Knox, H. Riley, A. Kvalbein and T. Pettersen. 2016. Crop coefficients, growth rates and quality of cool-season turfgrasses. *J. Agr. Crop Sci.* 202: 69-80.

²Espevig, T. 2011. Winter hardiness and management of Velvet bentgrass (*Agrostiscanina*) on golf greens in the Nordic climate. Philosophia Doctor Thesis 2011:14: Norwegian University of Life Sciences.

³Lodge, T. A. and D. M. Lawson. 1993. The construction, irrigation and fertiliser nutrition on golf greens. Botanical and soil chemical measurements over 3 years of differential treatment. *J. Sports Turf Res. Inst.*, 69: 59-73.

⁴Gange A. C. 1998. A potential microbiological method for the reduction of *Poa annualis* L. in golf greens. *J. of Turfgrass Sci.*, 74: 40-45.

⁵Kvalbein, A., A. M. D. Jensen, P. Rasmussen and T.S. Aamlid. Red Fescue Management: Guidelines Based on Greenkeepers' Experiences. 2013. Danderyd, Sweden: Scandinavian Turfgrass and Environment Research Foundation. 25 pp.

⁶Symbiom. 2012. Products: Symbivit. Available at: <http://www.symbiom.cz/index.php?p=symbivit&site=en> (accessed 29 Mar.2016).

⁷Gaussoin, R.E., J.L. Nus, and L. Leuthold. 1995. A modified Stimpmeter for small plot turf research. *HortScience* 30:547-548.

⁸Koske, R. E. and J. N. Gemma. 1989. A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research*, 92: 486-505.

⁹Giovannetti M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist*, 84 (3): 489-500.

Oct. 15th 2012. Soil pH (H₂O) and content of plant available phosphorus¹⁰ were calculated from 2 soil samples taken to 20 cm depth on Oct. 2012 and Apr. 2013 from RF plots mowed at 5.5 mm. The data were analysed using SAS PROC MIXED procedure with random block effects (SAS version 9.2; SAS institute, Cary, NC, USA)¹¹. Significant differences among treatments were identified by Tukey-Kramer LSD at the 0.05 probability level.

Results and Discussion

Mixed fescue / bentgrass plots, especially RF+VB, gave better visual turf quality and density, and less development of AB than plots with pure RF. Mixed swards also had higher number of tillers. The densest RF+VB combination gave softer surfaces, but together with pure RF, the best ball roll. Higher roots dry weight and organic matter content in the thatch/mat layer were also found for RF+VB.

Increasing N rate from 5 to 15 g N m⁻² yr⁻¹ improved visual turfgrass quality, tiller density and turfgrass colour and increased the percentage of bentgrass tillers from 53% to 64% on RF+CB greens and from 86% to 92% on RF+VB greens (Fig. 1). Surface hardness increased in the order RF+VB < RF+CB < RF. Ball roll distance decreased with increasing N rates and was better with RF and RF+VB than with RF+CB.

Increasing mowing height gave better visual turfgrass quality, turfgrass colour and density, shorter ball roll distance and more organic matter in the thatch/mat.

The main effects of N or mowing height on AB were not significant, but the combination of close mowing and more N increased AB competition on pure RF greens.

Applications of AMF (SYMBIVIT®) did not increase mycorrhiza colonization of grass roots, and neither P rate nor SYMBIVIT® influenced the competition against AB. By the end of the trial, the content of plant available P (P-AL) in the soil was, however, 10% lower after inoculation with SYMBIVIT®.

In conclusion, green characteristics were more influenced by species composition and N level than by mowing height and P-rate. Our results showed that the inoculation with AMF to the established green through top dressing after hollow coring had no effect. Regarding seed mixture, the control of AB will be easier on RF+bentgrasses than pure RF greens, but a mixture of RF+VG is likely to become dominated by VB even under low input management. This leaves RF+CB and low N input as the best choice regarding visual quality and competition against AB. The backside of this mixture is that CB will reduce the green speed compared to pure red fescue.

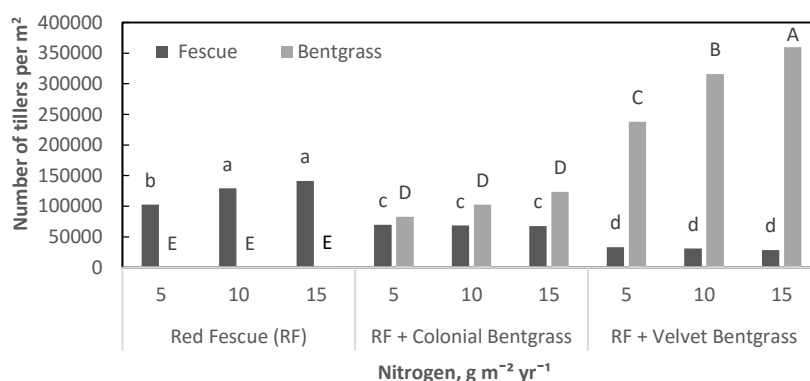


Figure 1. Combined effect of N levels and species combination on number of red fescue and bentgrass tillers in October 2012. Same letter above bars indicate no significant differences according to LSD ($P \leq 0.05$). ANOVA was performed separately for fescue and bentgrass tillers.

¹⁰Murphy, J and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta, 27: 31-36.

¹¹SAS Institute. 2008. SAS/STAT 9.2 user's guide. SAS Inst., Cary, NC.

SATELLITE MONITORING OF TURFGRASS SOD PRODUCTION

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Introduction

Plants reflect radiation and this supplies precious information on the nutritional and water status of many plant species, including turfgrass and hence can be used via remote sensing as a diagnostic tool for managing fertilization and irrigation. Like all modern agriculture sectors, turfgrass production and management is headed towards cost reduction, resource optimization and reduction of the environmental impact. In recent years the development of new technologies has provided new tools for monitoring agricultural crops. In particular, the combined adoption of GIS, GPS, multispectral lenses on satellites and cartographic techniques allow large scale management in agriculture. However, while turfgrass status evaluation by proximity-sensed spectral reflectance is by now an established and reliable practice¹, more could be achieved in terms of monitoring of large turfgrass areas. (i.e. golf, sports fields, parks and sod farms), by gathering similar spectral reflectance information through remote sensing and in particular through satellite imagery. The analysis of satellite-sourced imagery carrying spectral reflectance data had until recently three major limitations for effective use on turfgrass areas: (1) high costs of images, (2) relatively low resolution resulting in large pixel size, thus little suited to the smaller and very variable areas associated with turfgrass and (3) few spectral bands that restricted the calculations of turfgrass-specific vegetation indices. However, a new generation of satellites has been launched, which can supply high-resolution images with a wide array of spectral bands. To investigate satellite imagery application to sod farming, we tested the results and data supplied by three satellites on three locations at two acquisition dates on naturally-occurring variation in sod farms. Results show that traditional and new indices and bands can be very useful to improve sod farming monitoring.

Methods

The following tests were carried out: 3 acquisition sites sod farms (Plantec, Ginosa, IT; Richter, Zàvod, SK; Ostfoldgress, Dilling, NO); 2 dates (Summer 2015 and Autumn 2015); 3 different satellites: WorldView-2 (WV2), GeoEye-1 (GO1), Pleiades-1A (P1A); response on areas with naturally-occurring variability due to variety, soil and agronomic practices. On these, vegetational indices (Tab. 1) were calculated and analyzed to detect the ones that supplied better discrimination between different sod farm areas, both between and within plots. As far as naturally-occurring variability we monitored the following in a randomized block with 3 replicates: (1) Harvested vs. non harvested areas; (2) European vs. American varieties of the same species; (3) Areas subject to natural *P. annua* or *C. dactylon* infestation. All satellite imagery was acquired through Satellite Imaging Corporation (SIC), 18911 Manor Spring Court, Tomball, TX, USA. All imagery was processed via a ENVI software (RSI Inc., Boulder, CO – USA). All data was subject to ANOVA with Tukey's HSD used to detect differences between means. Furthermore, as a measure of sensitivity to variation, the absolute value percent variation between treatments response for the bands and VI was calculated. For brevity reasons, only the average response to EU\US varieties' at Plantec shown.

Results and Discussion

All treatment means were found to be significantly different at $P \leq 0.05$ at all bands and most Vi's (Tab. 2). The single wide bands that could not discern differences between means were: Coastal and Blue. This was true for all locations, for all treatments and for all sensor types. This would indicate that the small differences in pixel size (definition) do not prevent satellite imagery from detecting differences in any plot bigger than the 2 m level. This is a very welcome and encouraging finding, as it rids this specific crop from the need of ultra-high definition requirements for day-to-day interpretation of the agronomic situation in a sod farm. No significant difference was found between dates, so for all (Ostfoldgress, Plantec and Richter), only the summer data is shown. As can be seen from the data in Table 3, the yellow band available in WV2 multispectral satellite imagery can be very useful for devising and calculating some new vegetational indices that show a great sensitivity to turfgrass variability. In particular, NDVI-NIR2-Y, NDVI-NIR1-Y, NIR2/Y, NIR1/Y and NDVI-F1 = Sum

¹ Caturegli, L., F. Lulli, L. Foschi, L. Guglielminetti, E. Bonari, and M. Volterrani. 2014. Monitoring Turfgrass Species and Cultivars by Spectral Reflectance. European Journal of Horticultural Science 79: 97–107.

NIR Sum Y-Red all show an absolute value percent variation that is higher than 100% between the values obtained on EU and US varieties.

Table 1. List of bands and vegetational indices compared. * Vegetational indices devised and used for the first time in this study.

Vegetational Index	GO1-P1A	WV2	Equation
Atmospherically Resistant Vegetation Index	ARVI	ARVI	$((\text{NIR}-(\text{R}-(\text{B}-\text{R}))) / (\text{NIR}+(\text{R}-(\text{B}-\text{R}))))$
Enhanced Vegetation Index	EVI	EVI	$(2,5((\text{NIR}-\text{R}) / (\text{NIR}+6\text{R}-7,5\text{B}+1)))$
Green Atmospherically Resistant Index	GARI	GARI	$((\text{NIR}-(\text{G}-1,7(\text{B}-\text{R})) / (\text{NIR}+(\text{G}-1,7(\text{B}-\text{R}))))$
Green Difference Vegetation Index	GDVI	GDVI	$(\text{NIR}-\text{G})$
Green NDVI	GNDVI	GNDVI	$((\text{NIR}-\text{G}) / (\text{NIR}+\text{G}))$
Normalized Difference Vegetation Index	NDVI	NDVI	$((\text{NIR}-\text{R}) / (\text{NIR}+\text{R}))$ Note: NIR1 for WV2
NDVI (WV2)	Na	NDVIb	$((\text{NIR}2-\text{G}) / (\text{NIR}2+\text{G}))$
Soil Adjusted Vegetation Index	SAVI	SAVI	$((1,5(\text{NIR}-\text{R}) / (\text{NIR}+\text{R}+0,5)))$
Simple Ratio	SR	SR	(NIR/R)
WorldView Improved Vegetative Index	Na	WV-VI	$((\text{NIR}2-\text{R}) / (\text{NIR}2+\text{R}))$
NDVI-NIR1-Y*	Na	NDVI-NIR1-Y	$((\text{NIR}1-\text{Y}) / (\text{NIR}1+\text{Y}))$
NDVI-NIR2-Y*	Na	NDVI-NIR2-Y	$((\text{NIR}2-\text{Y}) / (\text{NIR}2+\text{Y}))$
NDVI-F1*	Na	NDVI-F1	$((\text{NIR}1+\text{NIR}2)-(\text{R}+\text{Y})) / ((\text{NIR}1+\text{NIR}2)+(\text{R}+\text{Y}))$
NDVI-F2*	Na	NDVI-F2	$((\text{NIR}1+\text{NIR}2)-\text{Y}) / ((\text{NIR}1+\text{NIR}2)+\text{Y})$
NIR1/Y*	Na	NIR1/Y	$(\text{NIR}1/\text{Y})$
NIR2/Y*	Na	NIR2/Y	$(\text{NIR}2/\text{Y})$
Y/R*	Na	Y/R	(Y/R)
YR NDVI*	Na	YR NDVI	$(\text{Y}+\text{R}) / (\text{Y}-\text{R})$

Table 2. Treatments of naturally occurring variation: NDVI response. Means followed by the same letter do not differ statistically for $P \leq 0.05$

	PLANTEC	RICHTER	OSTFOLDGRESS
Harvested vs. non harvested areas			
Harvested	0.09 a	0.08 a	0.09 a
Non harvested	0.89 b	0.85 b	0.91 b
European vs. American varieties of the same species			
European	0.65 a	0.79 a	0.75 a
American	0.90 b	0.92 b	0.92 b
<i>Poa annua</i> infestation			
Infested (>5%)	0.75 a	0.76 a	0.68 a
Non infested (<5%)	0.90 b	0.92 b	0.92 b

Table 3. Bands and VI (Plantec WV2 only) absolute value percent difference between lowest and highest between values for EU and US varieties. Bands and indices with the highest value are most sensitive to variation. Best 15 are shown.

Bands or VI values	[%]	Bands or VI values	[%]	Bands or VI values	[%]
NDVI-NIR2-Y	265,0	WV-VI	92,1	G	50,3
NDVI-NIR1-Y	168,6	NDVI	72,4	$(\text{Y}+\text{R}) / (\text{Y}-\text{R})$	22,6
NIR2/Y	157,8	R	60,0	NIR2	21,0
NIR1/Y	155,9	NDVI-F2 = Sum NIR Y	55,6	NIR1	20,1
NDVI-F1 = Sum NIR Sum Y-Red	125,7	Y	53,1	Y/R	17,4

Conclusions

While this confirms the exceptional technical, agronomical and scientific value of WV2 satellite imagery, traditional bands and indices (R and NDVI) calculable from 4-band multispectral imagery of satellites such as GO1 and P1A, still obtain values above 60% variation between EU and US species' pixels. As such, GO1 and P1A could be the lighter (and less expensive) alternative for adequately monitor sod farming turfgrass production, while WV2 can be considered a more "upmarket" and sensitive alternative for more technical and scientific work.

Acknowledgments

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**TECHNOLOGY ADVANCES AND TURFGRASS MAINTENANCE
(MOWING, FERTILIZATION, ENVIRONMENT, CERTIFICATION)**

POSTER PRESENTATIONS

CAN ROBOTIC IMPROVE THE QUALITY OF THE LAWN?

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¹Department of Agriculture, Food and Environment, University of Pisa, Italy.

Introduction

Robotic mowers are electric lawn mowers designed by several manufacturers and can autonomously care for turf maintenance. Robotic mowers can be programmed by the user to perform optimal turf maintenance and usually operate every day. Robotic mowers are designed to simulate the action of “animal grazing” on the turf. Since robotic mowers cut every day, clippings are so small (fractions of a mm) that they are dropped on the turf for a further supply of nitrogen and other nutrients¹. Robotic mowers provide many advantages like: performing boring and tiring tasks for humans, avoid humans from exposure to dust, pollen and allergens, remove humans from danger of mowing blades or roll over in case of lawn tractors, reduce pollutants^{2,3}. Robotic mowers operate within a boundary wire (usually shallow buried into the soil) which creates an electromagnetic fence³. Robotic mowers generally move randomly following linear trajectories. In other words, robotic mowers follow straight lines until they find the boundary wire and then change direction. This kind of pattern can be very effective for green areas with many obstacles but leads to mowing overlapping³. The most recent robotic mowers designed for large areas can be equipped with a GPS for a “random assisted” pattern⁴ or with a differential GPS for systematic trajectories⁵. All robotic mowers can automatically reach the charging station when their batteries start to run out². Some robotic mowers may be equipped with one or two extra wires, usually named guide wires, which help the robotic mower to pass through narrow passages in complex areas and to reduce the time required to reach the charging station⁴.

The aim of this trial was to compare robotic mowing with traditional rotary mowing on a tall fescue turf fertilized with different nitrogen rates and to determine: i) mowing effects on turf quality, texture and density ii) operative performances of the two mowing systems.

Methods

The experimental trial was carried out in S. Piero a Grado, Pisa (Italy) from September 2014 to July 2015 on a stand of *Festuca arundinacea* cv 'Grande'. The experimental turf was established on a soil characterized by the following physical-chemical properties: 91% sand, 5% silt, 4% clay, pH 6.5, 1.3 g kg⁻¹ of organic matter; EC 0.46 dS m⁻¹, water availability 3.45 % w/w.

On April 21st 2015, a two-way factor experimental design (AxB) with 3 replications was adopted.

Treatment (A) consisted in 4 levels of nitrogen fertilization: 0, 50, 100 and 150 kg ha⁻¹ N (ammonium sulphate 21-0-0). Treatment (B) consisted in 2 mowing systems: robotic mowing with Husqvarna robotic mower mod. Automower® 330X (Fig. 1), rotary mowing with John Deere gasoline walk-behind rotary mulching mower mod. JS63. Mowing height of all mowers was set at 3.5 cm. Irrigation was applied as necessary to avoid wilt to turf.

Every week, from April 21st to July 14th (12 WAT= Weeks After Treatment), the following parameters were assessed: turf quality, mowing quality, weed cover, disease.

At 12 WAT, a 50 cm² core sample per plot was collected and the following parameters were assessed: leaf texture and shoot density.

Statistical analysis of biometric data was carried out with COSTAT 6.400 software. All data were analyzed by two-ways ANOVA, and an all pairwise Fisher's Least Significant Difference (LSD) test at the probability level of 0.05.

The operative characteristics of the two different mowing systems were assessed during June and July.

¹Starr, J.L. and H.C. DeRoo. 1981. The fate of nitrogen fertilizer applied to turfgrass. *Crop Science* 21:531-536.

²Hicks R.W., Hall E.L., 2000. A Survey of Robot Lawn Mowers. *Society of Photo-Optical Instrumentation Engineers*. (online article). pp. 262-269.

³Ragonese A., Marx J., 2015. The applications of sensor technology in the design of the autonomous robotic lawn mower. 15th Annual Freshman Engineering Conference, April 11, Pittsburgh, USA, Paper 5094.

⁴Husqvarna 2015, Husqvarna automower 105/310/315/420/430X/450X operator's manual (available online).

⁵Zucchetti Centro Sistemi Spa, 2016a. L400 Elite (online). <http://www.ambrogiorobot.com/en/ambrogiorobot>.

Results and Discussion

As expected, nitrogen fertilization increased turf quality. There was no interaction between nitrogen fertilization and mowing systems. Twelve weeks after treatment, turf density was significantly increased by robotic mower (3.2 shoots cm^{-2}) compared to rotary mower (2.1 shoots cm^{-2}). Leaf texture was finer for the robotic mower (2.1 mm) compared to the rotary mower (2.7 mm). Turf quality resulted higher for robotic mower (7.3) compared to rotary mower (6.4). Weed incidence resulted lower for robotic mower if compared to rotary mower (6 and 9 % respectively).

Robotic mower operational time was set at 10 h per day, including mowing time and charging time. Overall mowing time was 7.8 h. The electric energy consumption was 2.21 kWh (power required about 30 W).

The gasoline walk behind mulching mower, which cut the grass once per week, covered the same area in 1.02 h, with an average working speed of 3 km h^{-1} and a working width of 53 cm. Turning time did not considerably affect the total operative time (only 0.06 h week^{-1}). The maximum engine power was 5 kW. Gasoline consumption was about 1.36 L week^{-1} .



Figure 1. Husqvarna robotic mower mod. Automower® 330X

In this trial, average turf height was lower where robotic mower was employed because turfgrass was cut every day while rotary mower operated only once a week. The lower average turf height may have improved shoot density, turf quality and reduced leaf texture. The lower power requirement of the robotic mower could be mainly due to the lower power needed for mowing (the maximum power requirement of the machine is only 40 W) as the turfgrass is daily mowed and the machine only cuts small clippings. The overall efficiency of the robotic mower is higher than the efficiency of the gasoline mower. The robotic mower does not require labour time and the total number of operating hours expected for a robotic mower is much higher than the number of operating hours of a rotary mower. Moreover, the two different mowing systems are not equal from a turf quality point of view. Cutting everyday leads to a better turf quality. Based on turf quality aspects and energy consumption, the use of the robotic mower could be a promising alternative to traditional mowers. Further research is needed to test the effects of robotic mowing on weed control with a specific trial on several selected weed species.

Acknowledgments

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RADIATION EXTINCTION WITHIN A CAMOPY OF HYBRID BERMUDAGRASS

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Introduction

Within a canopy leaves act as a primary organ for the interception of solar radiation¹. Radiation reaching every points throughout canopy layers derives from unfiltered radiation (direct and diffuse) and filtered radiation which is modified and attenuated as it passes through the leaves². The canopy has a complex structure, due to the spatial distribution arrangement not only of the leaves, but also of all the other organs forming the aerial portion of the plant³. Such additional characteristics include number of leaves, leaf inclination and leaf thickness⁴. Green Leaf Area Index (LAI) is an important indicator of photosynthetic and transpiration capacity also in turfgrass canopies. Additionally, aboveground biomass is an indicator of ecosystem productivity and is strongly related to LAI⁵. Many studies have been carried out on penetration of radiation into field crops^{6,7} but to date no results are available on turfgrasses, mainly due to the difficulties of introducing sensors into the turfgrass canopy without disturbing the natural position of leaves^{8,9}. In the present research hybrid bermudagrass (*Cynodon dactylon x transvaalensis*) 'Patriot' was studied in order to explore the influence of the architecture of canopy on transmittance at different levels of the profile.

Methods

The trial was carried out in S. Piero a Grado, Pisa (Italy), at the Department of Agriculture, Food and Environment (DAFE) of the University of Pisa (43°40' N, 10° 19' E, 6 m. a.s.l.), on mature stand of *Cynodon dactylon x transvaalensis* 'Patriot'. The sward was established in 2010 on a calcareo fluvisoil with pH 7.6 and 2.1 % of organic matter. In February 2015 a scalping at 1.0 cm was carried out to remove winter brown leaves. No fertilizer was applied to the turf before the trial started. On April 15th and June 16th 2015, a fertilization with 100 kg ha⁻¹ of N (ammonium sulfate) was carried out. From June 16th to August 31st 2015, the turf was mowed weekly at 12 cm with a rotary mower. Spectroradiometric measurements and phytometric determinations were carried out on August 31st, September 1st and 2nd at 8 different levels into the canopy: 0, 1.5, 3, 4.5, 6, 7.5, 9, 10.5 cm and at the top. Data collected in each day represented a replication.

Spectroradiometric measurements

Reflectance (R) was measured using a spectroradiometer LI-COR1800 (LI-COR Inc., Lincoln, NE, USA) with a fiber-optic wire and LI-COR 1800-06 telescope. The telescope was mounted on a purpose-built trolley 130 cm above the ground and with a vision angle of 15°. The surface area monitored corresponded at ground level to approximately 2000 cm² (diameter \varnothing = 50 cm). Measurements were taken between 11.30 am and 1.30 pm (local time) of each day, in the complete absence of cloud cover. The radiation reflected by a white panel made from barium sulphate was measured as reference in order to detect any possible variation in irradiance. Reflectance readings were carried out in the 390–1100 nm region at 5 nm intervals. Transmittance (T) within

¹Gates, D.M., H.J. Keegan, J.C. Schleter, and V.R. Weidner. 1965. Spectral properties of plants. *Applied Optics*, 4:11-20.

²Sinoquet, H., X. Le Roux, B. Adam, T.Ameglio, and F.A. Daudet. 2001. RATP: a model for simulating the spatial distribution of radiation absorption, transpiration and photosynthesis within canopies: application to an isolated tree crown. *Plant, Cell & Environment*, 24(4):395-406.

³Caturegli, L., F. Lulli, L. Foschi, L. Guglielminetti, E. Bonari, and M. Volterrani. 2014. Monitoring turfgrass species and cultivars by spectral reflectance. *European Journal Horticultural Science*, 79:97–107.

⁴Stewart, D.W., C. Costa, L.M. Dwyer, D.L. Smith, R.I. Hamilton, and B.L. Ma. 2003. Canopy structure, light interception, and photosynthesis in maize. *Agronomy Journal*, 95(6):1465-1474.

⁵Lee, H. 2008 Estimation of visual quality and canopy characteristics of turfgrass using spectral reflectance and digital imagery. Kansas State University, Manhattan, Kansas, USA.

⁶Tsubo, M., and S.Walker. 2002. A model of radiation interception and use by a maize–bean intercrop canopy. *Agricultural and Forest Meteorology*, 110(3):203-215.

⁷Sinclair T.R., and E.R. Lemon. 1974. Penetration of photosynthetically active radiation in corn canopies. *Agronomy Journal*, 66:201-205.

⁸Kopec, D.M., J.M. Norman, R.C. Shearman, and M.P. Peterson. 1987. An indirect method for estimating turfgrass leaf area index. *Crop Science*, 27:1298–1301.

⁹White, R., K. Steinke, C. Fontanier, and J. Thomas. 2010. Developing a Device to Quantify Light Penetration in Turfgrass Canopies. *Crop Science*, 50:1066-1069.

the canopy was measured with the same spectroradiometer with a cosine corrected sensor (\emptyset 7 mm). The sensor was fixed to the tip of an optical-fiber cable that transmitted the signal to the spectroradiometer. Transmittance was determined at 8 different levels within the canopy, from ground level (0 cm) to 10.5 cm. In order to carry out the numerous measurements with the sensor at the required heights, a purpose-designed apparatus was setup. Absorbance (A) was calculated as: $A=1-(R+T)$. The average solar radiation intensities (Photosynthetic Photon Flux Density-PPFD) between 11.30 am and 1.30 pm were similar in the 3 days of measurement: August 31st 1710 $\mu\text{mol m}^{-2}\text{s}^{-1}$; September 1st 1825 $\mu\text{mol m}^{-2}\text{s}^{-1}$; September 2nd 1802 $\mu\text{mol m}^{-2}\text{s}^{-1}$.

Leaf Area Index (LAI)

After the spectroradiometric measurements 8 layers of phytomass were sampled using the stratifying clipping method suggested by¹⁰ and also applied in¹¹. For each layer ΔLAI was calculated as the leaf area/ground area ($\text{m}^2 \text{m}^{-2}$)¹².

Statistical analysis

The relationship among cumulative LAI and the transmittance in PAR (%) was studied using CoStat software (CoHort, Monterey, CA, USA) and Pearson's correlation coefficient (r) was calculated. Exponential function proved to be the type most suited to mathematical representations of the transmittance and cumulative LAI.

Results and Discussion

In this paper only data of transmittance within the canopy are reported. In Figure 1 Transmittance curves at each level of measurement are shown, in the PAR region and in Near Infrared. In PAR, particularly at the peak of chlorophyll absorption (680 nm), the closer you get to the ground as the values approach zero.

In Figure 2, which represents PAR transmittance in relation with downward cumulative LAI, we can observe the high Pearson's correlation coefficient ($r=0.96$) and that in the PAR region transmittance decrease exponentially with the increase of downward cumulative LAI. In this preliminary research carried out on a turfgrass mowed at 12 cm (however a real situation in horse race tracks), we observed how the available radiation is lower in the layers closer to the soil, mainly at the peak of chlorophyll absorption.

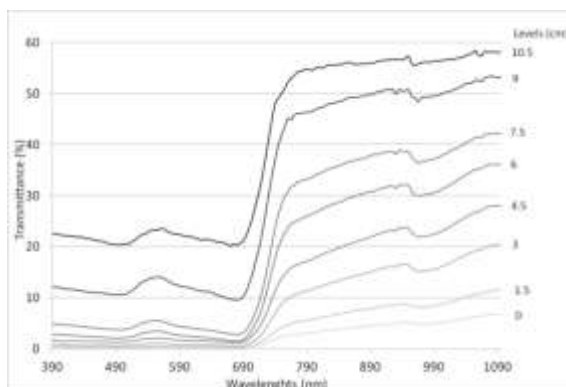


Figure 1. Transmittance curves (390-1090 nm) studied at each level (0, 1.5, 3, 4.5, 6, 7.5, 9, 10.5 cm) of the canopy of *Cynodondactylon x transvaalensis* 'Patriot'

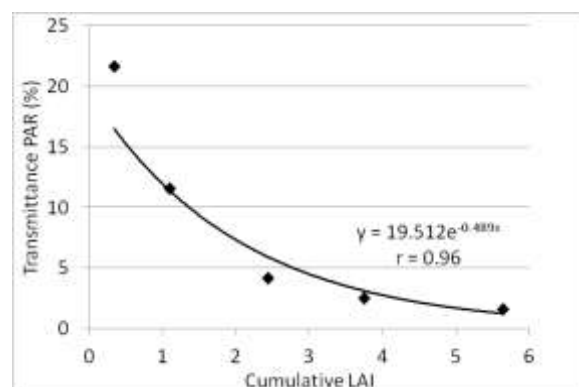


Figure 2. Regression of Transmittance in the PAR region on downward cumulative LAI

¹⁰Awal, M.A., H. Koshi, and T. Ikeda. 2006. Radiation interception and use by maize/peanut intercrop canopy. *Agricultural and Forest Meteorology*, 139(1):74-83.

¹¹Volterrani M., N. Grossi, G. Pardini, S. Miele, and M. Gaetani. 1995. Preliminary study on light penetration within canopies of fodder crops. *AgricolturaMediterranea*, 25:40-50.

¹²Monsi, M., and T. Saeki. 2005. On the factor light in plant communities and its importance for matter production. *Annals of Botany*, 95(3):549-567.

USING DIGITAL IMAGE ANALYSIS AND COLOUR SCALE TO DETERMINE TURFGRASS COLOUR

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Introduction

Turfgrass colour is one of the most important aspects to determine turfgrass quality, because it is a good indicator of general condition of turf which is related to nutrition, disease, insect nematode, water or other environmental stress problems¹. In general, darker green colour is preferred by consumers. Therefore, turfgrass researchers use visual rating scale of 1 (lighter) to 9 (darker) for turfgrass colour evaluation. However, this rating is a subjective measurement which is often inconsistent among raters when evaluating the same turf plots. Because of that, recently, digital image analysis (DIA) is used to measure turfgrass colour in order to determine it objectively. Using DIA, turf colour can be converted to numerical numbers for objective measurement; however, it is difficult to imagine the colour from the value obtained from the digital imagery. The objectives of the following studies were (i) to determine if a digital image could calibrate with a colour scale, and (ii) if digital image analysis was capable of quantifying ryegrass cultivar's genetic colour differences.

Methods

Develop Shizuoka Turf Colour Measuring System (SSCSS)

Research was conducted at Shizuoka Prefectural Research Institute of Agriculture and Forestry located in Iwata, Japan. A digital image was taken with 1 to 7 green colour scale (Fujihira, Bunkyo-Ku, Tokyo) which is used for identifying rice leaf colour. In order to analyze numerous images automatically, a stand for a digital camera attached with the colour scale was made (Figure 1). All images for analysis were taken using the stand. Afterwards, the object for the analysis and images from each plate were cropped from a colour scale and analyzed with SigmaScan Pro software² for dark green colour index (DGCI) using macro created by Karcher and Richardson³. Then, DGCI from colour scale were used to make calibration curves. Six linear regression lines using two colour plates which were colour plates of 1 and 2, 2 and 3, 3 and 4, 4 and 5, 5 and 6, and 6 and 7 were used for calibration curves. Using the calibration curves, DGCI from the object for the colour analysis was converted to the colour based on the green scale.

A Green Colour Scale Calibration

Using a SSCSS, images of two colour scales were taken under three light levels, sunny ($\text{PAR} > 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$), overcast ($\text{PAR} 100\text{-}1000 \mu\text{mol m}^{-2} \text{s}^{-1}$), and under shaded ($\text{PAR} < 100 \mu\text{mol m}^{-2} \text{s}^{-1}$) using a digital camera (Nikon S8200, Minato-ku, Tokyo) with a camera setting of auto (Figure 2). Camera stand was positioned for not casting the own shade to the image. Light levels were measured with quantum light meters (Spectrum Technologies, Inc., Plainfield, IL) before taking images. Each colour plates were cropped from two colour scales. And then, each image of colour plates was analyzed by the method described above if the colour plate can be analyzed the designated scale value under various light conditions.



Figure 1. SSCSS setup



Figure 2. Image of two colour scales were taken using SSCSS for calibration

¹Beard, J.B. 1973. Turfgrass Science and culture. Prentice-Hall, Englewood Cliffs, NJ. p12.

²SPSS Inc. 1998. Sigma Scan Pro 5.0. SPSS Science Marketing Department, Chicago, IL.

³Karcher, E., and M.D. Richardson. 2005. Batch Analysis of Digital Images to Evaluate Turfgrass Characteristics. Crop Sci 45:4:1536-1539.

Ryegrass Cultivar Colour Differences

Plots from a ryegrass cultivar trial were used to assess the ability of digital image analysis to quantify visual colour differences among cultivars. The trial was established in spring 2013 at Shizuoka Prefectural Research Institute of Agriculture and Forestry. Individual plots were 5.74 m² and maintained at a 5.0 cm mowing height. The study was replicated three times in a completely randomized block design. Digital images were taken using SSCSS as described previously on each replication of ryegrass plots. The plots were photographed in April 2014. The data from same cultivars were compared with ryegrass genetic colour from National Turfgrass Evaluation Program (NTEP) 2005 to 2009 perennial ryegrass final report. NTEP genetic colour were evaluated 1 being light green and 9 being dark green and are collected when the turf is actively growing and is not under stress⁴. Data were analyzed using JMP. A one-way analysis of variance (ANOVA) was used for analysis and Fisher's Protected LSD test was used to separate main effect means.

Results and Discussion

A green colour scale calibration worked well except under shaded conditions (Table 1). When images were taken under sunny and overcast conditions, SSCSS values were close to an actual colour plate which is on average ± 0.32 , and standard deviations were less than 0.47. When there was not enough light, SSCSS calculated colours darker than actual, and standard deviations were over 1.00 for darker colour plate. Because of that, images should be taken on sunny or overcast days. However, if there is too much sunlight, camera stand will create shade depending on time and direction of stand toward sun. A partly shaded image will affect the analysis. Thus, time of the day and direction of stand will be critical when taking an image. The digital imagery successfully differentiated the ryegrass colour (Table 2). The SSCSS ranked ryegrass colour similar to NTEP genetic colour. Converting the values obtained from DIA using 1 to 7 colour scales worked satisfactory. If the turfgrass colours were too dark, converted values exceeded maximum scale colour of 7. Currently, SSCSS can analyze limited area from a single image. If the analysis of area increased, more reliable readings would be obtained. Further research is needed in order to determine turfgrass colour faster and more accurately.

Table 1. A Green Colour Scale Calibration

Color plate	PAR <100		100-1000		>1000	
	SSCSS					
	Mean	Std Dev†	Mean	Std Dev	Mean	Std Dev
1	1.46	0.45	0.45	0.47	1.15	0.27
2	2.32	0.39	1.39	0.27	1.87	0.22
3	3.80	0.57	2.68	0.24	3.21	0.19
4	4.83	0.40	3.84	0.20	4.09	0.09
5	6.21	0.68	5.04	0.10	5.38	0.12
6	7.23	1.02	6.21	0.06	6.17	0.18
7	8.61	1.22	7.37	0.04	7.83	0.63

†Std Dev = Standard deviation

Table 2. Comparing ryegrass colour with SSCSS and NTEP genetic colour

Cultivar	SSCSS		Cultivar	NTEP	Genetic Color
AMAZING GS	5.6	a b	AMAZING GS	6.9	a
Zoom	5.5	a b c	Zoom	6.7	a b
HARRIER	5.4	a b c d	HOMERUN	6.7	a b
HAWKEYE 2	5.4	a b c d e	SR4600	6.7	a b
HOMERUN	5.2	b c d e f	SILVER DOLLAR	6.6	a b
SR4600	5.0	c d e f	HAWKEYE 2	6.5	b
SILVER DOLLAR	5.0	c d e f	HARRIER	6.5	b
PALMER III	5.0	d e f	PANTHER GLS	6.4	b c
BARLENNIUM	4.9	e f	MANHATTAN 5 GLR	6.1	c
MANHATTAN 5 GLR	4.8	f	BARLENNIUM	5.8	c d
PANTHER GLS	4.7	f	PALMER III	5.7	d

Value within columns follow by the same letter are not significantly different from another (LSD, $\alpha=0.05$).

⁴Morris, N.K. 2016. A Guide to NTEP Turfgrass Ratings. Retrieved from <http://www.ntep.org/reports/ratings.htm#tap>

ASSESSMENT THE VISUAL TURFGRASS QUALITY BY REMOTE SENSING TECHNIQUES

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⁴MedtiBio, Centre for Mediterranean Bioresources and Food, Faro, Portugal

⁵LEAF, Linking Landscape, Environment, Agriculture and Food, ISA, Lisboa, Portugal

Introduction

The maintenance of a golf course represents an agronomic and environmental challenge: wear, disease and pests, climatic stresses, nutritional plant status and water deficit are common disturbances in high traffic conditions of golf courses.

Spatial and temporal variation of soil, climate, plants and irrigation requirements are challenges for modern agriculture and complex turfgrass sites. Precision agriculture advanced to improve site-specific management based on remote sensing technologies.

Unmanned aerial vehicles (UAV) can provide valuable data to support decisions of turfgrass managers. UAV are used as a platform for collecting aerial imagery. Digital cameras collect visible light reflected from surfaces. Visible light ranges from 400-700 nm in wavelength. Most of turf stresses impact the reflectance of near-infrared (NIR) region from the electromagnetic spectrum. Near-infrared ranges from 700-1300 nm and can be recorded using a modified digital camera.

Reflectance measurements offer a quick, non-destructive technique to estimate pigment content, which reveals the plant health. For photosynthetic purposes, plants absorb and reflect sunlight, which can be measured. Three-band models are widely used to estimate accurately chlorophylls, carotenoids and other leaf pigments¹, gathering information on plant water content, disease occurrence and leaf structure^{2,3}, evaluating the physiological status of vegetation.

The visible atmospherically resistant index (VARI) introduced by Gitelson et al.⁴ estimates the green vegetation, minimizing the atmospheric effects. It provides green colour quantification similar to the human eye's perception. This index is obtained in the visible bands, reducing the cost of data acquisition process and allowing vegetative vigour evaluation with a similar accuracy to others indexes. Pedras et al.^{5,6} used this index for evaluate the Soil Water Content in a kiwifruit orchard in Guimarães, Portugal, and the irrigation water management on Royal Golf Course, Vale do Lobo, Portugal.

Normalized Difference Vegetation Index (NDVI) can reveal striking differences in spectral reflectance between the red and near-infrared (NIR) wavelengths^{2,7}. Several authors relate significant correlations between NDVI and turfgrass visual quality^{2,3}. According to them, the visual leaf quality can be estimated by red reflectance, determined by chlorophyll content, and NIR reflectance is affected by light scattering within plant cells (leaf structure). Red light influence cell production and, consequently, this affects the reflectance in the NIR. Bremer et al.³ refer that reflectance in the red and NIR are distinct biophysical phenomena that may respond differently to environmental factors such as water stress.

Imagery can provide real-time information on many aspects of turf quality important for the decision of turf managers.

The purpose of this work was to compare the use of two vegetation indices to remote sense turfgrass areas in a golf course.

¹Gitelson, A.A., Keydan, G.P., and Merzlyak, M.N.. 2006. *Three-Band Model for Noninvasive Estimation of Chlorophyll Carotenoids and Anthocyanin Contents in Higher Plant Leaves*.

² Anderson, Z. and Fermanian, T.. 2008. *Early detection of turf disease through direct sensing*. GCM: 107-110.

³ Bremer, D. J., Lee, Kemin Su, K., and Keeley, S. J.. 2011. *Relationships between Normalized Difference Vegetation Index and Visual Quality in Cool-Season Turfgrass: II. Factors Affecting NDVI and its Component Reflectances*. Crop Science, Vol. 51: 2219-2227.

⁴Gitelson, A.A., Stark, R., Grits, U., Rundquist, D., Kaufman, Y., and Derry, D.. 2002. *Vegetation and soil lines in visible spectral space: A concept and technique for remote estimation of vegetation fraction*. International Journal of Remote Sensing 23 (13) 2537-2562.

⁵Pedras, C., Valin, M.I., Fernandez H., Martins, F..2014. *Assessment of Soil Water Content and Remote Sensing Techniques—Case Study of Kiwi Orchard (Portugal)*. Journal of Agricultural Science and Technology, ISSN 1939-1250. David Publishing.

⁶Pedras, C.; Lança, R.; Martins, F.; Soares, C.; Guerrero, C.; Paixão, H.. 2015. *Remote sensing technologies applied to the irrigation water management on a golf course*. Geophysical Research Abstracts, Vol. 17, EGU2015-6800, EGU General Assembly, Vienne, Austria.

⁷Keskin, M., Y.J. Han, R.B. Dodd, and A. Khalilian. 2008. Reflectance-based sensor to predict visual quality ratings of turfgrass plots. Appl. Eng. Agric. 24:855–860.

Methods

The Royal Golf Course, Vale do Lobo, Portugal, was remote monitored using an UAV with a RGB and a NIR sensors. NDVI and VARI maps were obtained and compared to in-site observations. Spectral reflectance of the turfgrass canopy was measured by imagery collected using two cameras: a digital true color (RGB, 24bit/pixel) using a Canon Powerhot SD800IS digital camera, equipped with a 7.1 Mpixel 5.760 mm x 4.290 mm CCD, producing 24 bit/pixel, 3072 cols 2304 rows jpg images, and a second camera, of the same model, with the same characteristics, but modified by the removal of the infrared blocking filter, replaced with a blue light blocking filter. Three locations (holes 4, 13 and 18) at the golf course were overflowed and flight surveys were programmed to collect RGB and NIR images. The flight was made with 100 m altitude where, for each location were obtained about 30 photos with 5 cm of spatial resolution. The photos have 60% and 25% of longitudinal and lateral overlap, respectively, in order to make the mosaics. A photomosaic from each location was build using *Panorama Tools Graphical User Interface (PTGui) software*. These photomosaics were georeferenced at the TM06-ETRS89 coordinate system, with *ArcGIS 10.2 software*. VARI and NDVI were calculated using the following equations:

$$\text{VARI} = \frac{\rho_{\text{green}} - \rho_{\text{red}}}{\rho_{\text{green}} + \rho_{\text{red}} - \rho_{\text{blue}}} \quad (1)$$

$$\text{NDVI} = \frac{\rho_{\text{nir}} - \rho_{\text{red}}}{\rho_{\text{nir}} + \rho_{\text{red}}} \quad (2)$$

Where: ρ_{blue} , ρ_{green} , ρ_{red} , ρ_{nir} are the blue, green, red, and near infrared reflectance's, respectively. These vegetation indexes were calculated using *IDRISI Selva 17 software*.

Results and Discussion

Visible Atmospheric Resistant Index (VARI) and Normalized Difference Vegetation Index (NDVI) can be used to improve the maintenance of turfgrass. It was possible to identify and delineate site-specific management units (SSMUs), with similar patterns, especially related to irrigation. On the fairways the NDVI values ranging from 0.55 to 0.75 correspond to the VARI values from 0.00 to 0.15; on the tees the NDVI values ranging from 0.60 to 0.80 correspond to the VARI values of 0.05 to 0.20; and on the greens, the values of NDVI ranging from 0.75 to 0.85 correspond to VARI values of 0.12 to 0.22. In general, the results of VARI and NDVI indexes were the same. In the three locations the frequencies and the dispersion of VARI and NDVI values were similar for the equivalent zones (fairways, tees and greens), so there is a correspondence between the values of both indexes. Differences between the three studied locations are due the occurrence of different microclimates, where the exposition to predominant winds plays an important role.

In this study there were no differences in the determinations of the VARI and NDVI values. Further research must be carried out to confirm this results. Further work must be done to continue recording RGB and NIR images from the UAV along the year to assess the plant vigour status of the turfgrass under different weather conditions and use the acquired information to improve irrigation and optimize the water management on the golf course.

RESEEDING INTERVAL FOLLOWING METHIOZOLIN ('POACURE') APPLICATION

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Introduction

The fate and persistence of the isoxazoline herbicide, methiozolin ('PoaCure'), in turf following application has not been determined¹. Our preliminary observations indicate that PoaCure may inhibit the establishment of bentgrass for several months following application². This would be highly problematic on golf courses if turf was lost (misapplication, winterkill, disease, etc.) and the site needed to be reseeded. The effect of herbicide rate and species on reseeding interval needs to be determined to guide superintendents in how best to use this potentially new product.

Methods

A reseeding interval following PoaCure application study was conducted at the Washington State University greenhouse facility at Pullman, WA. A 90% sand:10% peat moss mix was used as the soil medium. PoaCure was sprayed onto the potted soil medium at 0, 4 L product ha⁻¹, 4 L ha⁻¹ + 4 L ha⁻¹ (2 wk interval), or 4 L ha⁻¹ + 4 L ha⁻¹ + 4 L ha⁻¹ (2 wk intervals). In a creeping bentgrass (*Agrostis stolonifera* L.) seeding study, soil was also treated with 0, 2 L product ha⁻¹, 2 L ha⁻¹ + 2 L ha⁻¹ (2 wk intervals), or 2 L ha⁻¹ + 2 L ha⁻¹ + 2 L ha⁻¹ (2 wk intervals). Seed [annual bluegrass (*Poa annua* L.), Kentucky bluegrass (*Poa pratensis* L.), perennial ryegrass (*Lolium perenne* L.), or creeping bentgrass] was planted 4, 8, 12, 16, or 20 wk after final treatment and seedling emergence counts were made 1, 2, 3, and 4 wk after each planting (only the 4 wk counts, which were converted to seedling emergence as percentage of check are presented). Plant top growth dry weights were recorded at each final emergence count. The experimental design was a randomized complete block with 4 replicates.

Results and Discussion

PoaCure inhibited emergence of annual bluegrass for 12 wk after the last treatment (WALT) and 16 wk with multiple 4 L ha⁻¹ applications (Fig. 1). Creeping bentgrass, perennial ryegrass, and Kentucky bluegrass all were showing some emergence at 16 WALT (Fig. 2-4). Although there was fair emergence at 16 WALT the seedlings were observed to be quite stunted, which was also indicated by the dry weights (data not presented) and many of these seedlings probably would not survive. Approximately 20 WALT was required for the single application of 4 L ha⁻¹ to provide consistent (> 95%) emergence (Fig. 1-4).

For creeping bentgrass the seedling emergence was also rate-specific. Creeping bentgrass emergence was greater at 2 L ha⁻¹ than at 4 L ha⁻¹ (Fig. 2 and 5). In the current trial being conducted by Moghu Research Center on golf courses in the USA, 2 L ha⁻¹ (0.63 fl oz 1000 ft⁻²) is recommended for greens and 4 L ha⁻¹ (1.26 fl oz 1000 ft⁻²) is recommended for fairways.

Conclusions

Single and multiple applications of 4 L ha⁻¹ of PoaCure, in greenhouse research, inhibited turfgrass seedling emergence for 12 WALT. At 16 WALT, a single 4 L ha⁻¹ application inhibited emergence of annual bluegrass and Kentucky bluegrass more than creeping bentgrass and perennial ryegrass. Field work is needed to verify these results. Creeping bentgrass was reduced more at 4 L ha⁻¹ than 2 L ha⁻¹ (2 L ha⁻¹ is the recommended rate for greens). Superintendents need to calibrate spray equipment and precisely apply PoaCure on bentgrass greens as reseeding may not be an option for some time following any misapplication.

¹ McCullough, P.E., and D.G. de Barreda. 2012. Cool-season turfgrass reseeding intervals for methiozolin. *Weed Technol.* 26:789-792.

² Johnston, W.J., and C.T. Golob. 2013. Reseeding timing following methiozolin ('PoaCure') application. *Northwest Turf. Assoc. Res. Rep.* 8 p.

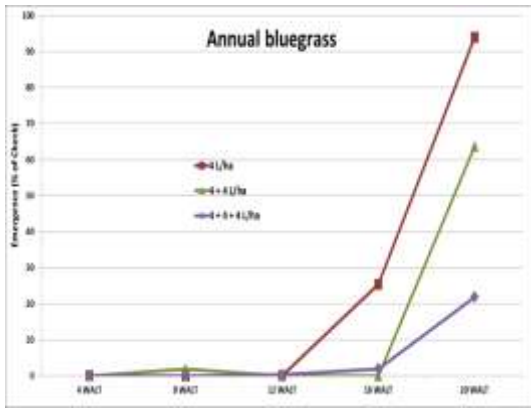


Fig. 1. Seedling emergence of annual bluegrass (weeks following last treatment) for single and multiple 4 L ha⁻¹ applications of PoaCure.

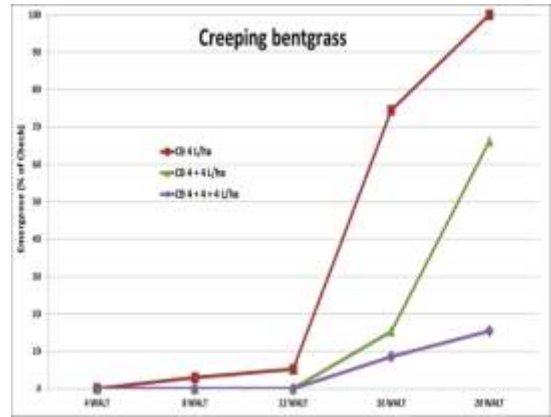


Fig. 2. Seedling emergence of creeping bentgrass (weeks following last treatment) for single and multiple 4 L ha⁻¹ applications of PoaCure.

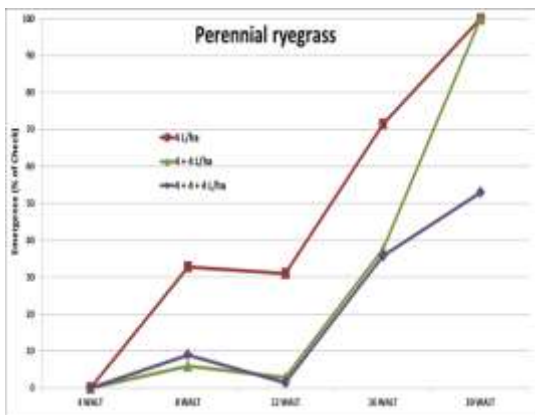


Fig. 3. Seedling emergence of perennial ryegrass (weeks following last treatment) for single and multiple 4 L ha⁻¹ applications of PoaCure.

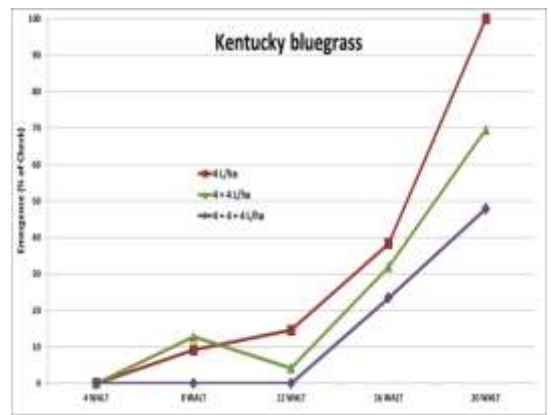


Fig. 4. Seedling emergence of Kentucky bluegrass (weeks following last treatment) for single and multiple 4 L ha⁻¹ applications of PoaCure.

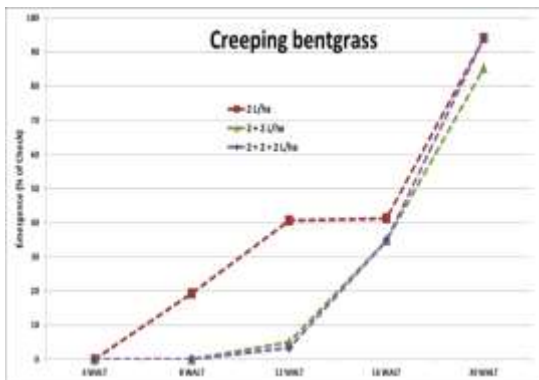


Fig. 5. Seedling emergence of creeping bentgrass (weeks following last treatment) for single and multiple 2 L ha⁻¹ applications of PoaCure.

TURFGRASS PESTS (DISEASES, INSECTS, WEEDS)

ORAL PRESENTATIONS

CONVENTIONAL FUNGICIDES VS. RESISTANCE ACTIVATORS IN TURFGRASS DISEASE MANAGEMENT

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Introduction

Fungicides are commonly used to control turfgrass diseases, but with increasingly stricter regulations regarding their use, and with governmental directives to decrease the amount of active ingredient usage, there has been increasing work on alternatives such as disease resistance-activating compounds. But how well do such compounds stack up against conventional fungicides? What types of new activator compounds are being tested, and which ones might become available on the market?

Plants possess many natural defense mechanisms against diseases that are triggered following pathogen attack. Under intensive plant maintenance systems and stressful growth conditions, these mechanisms may be insufficient to guard against disease outbreaks without significant economic loss. However, there are compounds that can activate defense mechanisms against pathogens prior to pathogen attack, resulting in faster responses upon attack¹. Unlike conventional fungicides, these compounds generally have little or no direct antifungal activity. I have previously presented some review articles on turf disease resistance activators^{2,3}. Table 1 lists several compounds which have been or are being currently tested for their systemic resistance activating properties, and references related to their activity against turfgrass diseases (if available).

Table 1. Substances known or thought to be activators of plant disease resistance

Substance	Example of Trade Name	Activity	References
Phosphites	Aliette, Apear, SwardPhite, Magallan, Alude	SAR?	4
			5
Acibenzolar	Actigard, Dac Action	SAR	6
			7
Chelated Metals	Fiesta (chelated Fe), Harmonizer (chelated Cu)	??	8
Mineral Oil	Civitas	ISR	9
Silicon	??	SAR	10
Butanediol	??	ISR	11
			9
Humic Acid	??	??	12

The two major recognized forms of pre-triggered systemic resistance are called systemic acquired resistance (SAR) and induced systemic resistance (ISR). In SAR, resistance can be activated by natural or synthetic

¹ Vallad GE Goodman RM. 2004. Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Science* 44:1920-1934.

² Hsiang T, Goodwin PH, Cortes-Barco AM. 2011. Plant defense activators and control of turfgrass diseases. *Outlooks on Pest Mgt* 22:160-164.

³ Hsiang T, Goodwin P, Cortes-Barco A and Nash B. 2013. Activating disease resistance in turfgrasses against fungal pathogens. pp. 331-342 in: Imai R, Yoshida M, Matsumoto N (eds). *Plant and Microbe Adaptations to Cold in a Changing World*. Springer, Berlin.

⁴ Landschoot PJ & Cook PJ. 2005. Sorting out the phosphonate products. *Golf Course Management* 73(11):73-77.

⁵ Dempsey JJ, Wilson ID, Spencer-Phillips TN, Arnold DL. 2012. Suppression of *Microdochium nivale* by potassium phosphite in cool-season turfgrasses. *Acta Agriculturae Scandinavica, Section B-Soil & Plant Science* 62(S1):70-78.

⁶ Lawton KA, Friedrich L, Hunt M, Weymann K, Delaney T, Kessmann H, Staub T, Ryals J. 1996. Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. *Plant Journal* 10:71-82.

⁷ Lee J, Fry J & Tisserat N. 2003. Dollar spot and brown patch incidence in creeping bentgrass as affected by acibenzolar-S methyl and biostimulants. *HortScience* 38:1223-1226.

⁸ Poschenrieder C, Tolrà R, Barceló J. 2006. Can metals defend plants against biotic stress? *Trends Plant Sci.* 11:288-95.

⁹ Cortes-Barco AM, Hsiang T, Goodwin PH. 2010. Induced systemic resistance against three foliar diseases of *Agrostis stolonifera* by (2R,3R)-butanediol or an isoparaffin mixture. *Ann Appl Biol* 157:179-189.

¹⁰ Rodrigues FA, McNally DJ, Datnoff LE, Jones JB, Labbé C, Benhamou N, Menzies JG, and Bélanger RR. 2004. Silicon enhances the accumulation of diterpenoid phytoalexins in rice: a potential mechanism for blast resistance. *Phytopathology* 94:177-183.

¹¹ Ryu C, Farag M, Hu C, Readdy M, Kloepper J, Pare P. 2004. Bacterial volatiles induce systemic resistance in *Arabidopsis*. *Plant Physiology* 134:1017-1026.

¹² Yigit F, Dikilitas M. 2008. Effect of humic acid applications on the root-rot diseases caused by *Fusarium* spp. on tomato plants. *Plant Pathol J.* 7:179-182

chemicals and avirulent or attenuated pathogens^{13,1}. These activators will locally induce resistance mechanisms that result in a signal that is translocated systemically throughout the plant. As a result, the whole plant may become more resistant to subsequent pathogen attack¹⁴. In contrast to SAR, ISR is normally induced in roots by beneficial soil-borne plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi. The substance 2,3-butanediol (listed in Table 1 above) was found to be produced by plant-growth-promoting rhizobacteria, and has been found to promote plant growth as well as induce resistance in dicotyledonous plants¹¹ and monocots⁹. Activation of ISR is associated with gene priming, in which plant defense genes are expressed more rapidly and strongly after pathogen attack¹⁵. In contrast, activation of SAR is more often associated with gene induction in which there is increased expression after treatment even without pathogen attack¹⁴.

In controlled environment tests, application of Civitas or butanediol to the soil reduced the diseased leaf area of *Agrostis stolonifera* by 20 to 40% for the fungal pathogens, *Microdochium nivale*, *Rhizoctonia solani* or *Sclerotinia homoeocarpa* compared to the water control⁹. But how well do these substances work in the field against turfgrass diseases? The following data (Table 2) are extracted from Vincelli et al.¹⁶, and show ratings on a scale of 1 to 4, with 4 showing the highest level of control. Where insufficient data are available, the rating is denoted by a "?".

Table 2: Efficacy of disease resistance activators against various turfgrass diseases (data calculated from Vincelli et al.¹⁶ on a scale of 1 (low) to 4 (high efficacy))

Turf Disease	Fungicide family			
	DMI	Strobilurin	Civitas/Harmonizer	Phosphite
Anthracnose	2.5	3.0	3.0	2.5
Brown Patch	2.6	3.8	?	?
Dollar Spot	3.8	2.0	2.0	?
Pythium Blight	0	2.3	?	2.3
Gray Leaf Spot	2.5	3.8	2.5	?
Fusarium Patch	2.3	2.8	1	?

Under some circumstances, disease resistance activators have worked as well as conventional fungicides, but because of their reliance on stimulating the plant host to produce defense reactions and chemicals against attacking pathogens, when the host is already under stress, stimulation may result in further weakening of the host. But in a world with ever increasing sentiments against synthetic pesticide use, disease resistance activators may play increasingly important roles in the management of turfgrass diseases.

¹³ Lyon G. 2007. Agents that can elicit induced resistance. In: Walters D, Newton A, Lyon G (eds). Induced resistance for plant defence: a sustainable approach to crop protection. Blackwell Publishing, Oxford, pp 9–30.

¹⁴ Sticher L, Mauch-Mani B, Métraux JP. 1997. Systemic acquired resistance. *Ann Rev Phytopathol* 35:235–270.

¹⁵ Conrath U, Pieterse CM, Mauch-Mani B. 2002. Priming in plant-pathogen interactions. *Trends Plant Sci* 7:210–216.

¹⁶ Vincelli P, Munshaw G. 2015. Chemical control of turfgrass diseases. University of Kentucky, PPA-1.

MANAGEMENT OF BENTGRASS CULTIVARS FOR ACTIVATED RESISTANCE TO MICRODOCHIUM PATCH (MICRODOCHIUM NIVALE) UNDER CLIMATE CHANGE CONDITIONS

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Introduction

The possible effects of climate change have become a growing concern. Atmospheric CO₂ levels have been on the rise and are projected to nearly double by the end of the century¹. Consequently, temperate locations such as Canada and northern Europe may experience temperature increases of 1.5-4°C within the next 50 years, allowing for less stable snow cover environments². These changes have the potential to increase plant disease severity, especially for overwintering plants such as perennial grasses.

Agrostis species (bentgrasses) are cool-season grasses commonly used as turfgrasses for fairways, tees and putting greens due to their fine texture and resilience to low mowing heights³. In temperate regions, such as Canada, creeping bentgrass (*Agrostis stolonifera*) and colonial bentgrass (*Agrostis capillaris*) are the turfgrasses most commonly used on golf course putting greens^{3,4}. Annual bluegrass (*Poa annua*) commonly invades bentgrass putting greens and can become the dominant species, especially on older greens⁵.

Bentgrasses and bluegrasses in temperate regions worldwide are susceptible to infection by the fungus *Microdochium nivale*. During the winter, particularly after prolonged snow cover, *M. nivale* will attack turfgrasses and cereals causing Pink Snow Mold⁶. The same pathogen causes Microdochium Patch (also known as Fusarium Patch) which occurs without snow cover during periods of cool, wet weather particularly in the spring and fall⁶. Significant genetic variation in resistance to *M. nivale* in turfgrass cultivars has been found under different temperature conditions⁷.

In autumn, turfgrasses undergo a cold acclimation process in response to shortened day length and temperatures around 10 °C⁸. This process may be disturbed by increased seasonal temperatures and a reduced period of optimal conditions for cold hardening^{9,10}. The unstable snow cover, particularly when temperatures dip, allows for injury, and decrease in cold hardening may make plants more prone to frost injury, ice encasement, and infection by psychrophilic pathogens. In addition, climate change may directly affect the pathogen's ability to infect, reproduce, disperse, and survive¹¹. The key to managing this disease under these changing climatic conditions is a better understanding of the host-pathogen interactions.

A recently developed control method for *M. nivale* is the use of resistance activators, which are products that are non-toxic to plants and fungi, and can cause greater activation of natural resistance responses to abiotic and biotic stresses. One such product is Civitas™ mixed with Civitas Harmonizer™ Pigment Dispersion (manufactured by Petro-Canada Lubricants, Mississauga, Ontario, Canada) which has been registered for use in the United States and Canada against a variety of turfgrass diseases. This research project examined the effect

¹ IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, 1535p.

² Feltmate, B.W. and J. Thistlethwaite. 2012. Climate Change Adaptation: A Priorities Plan for Canada. Climate Change Adaptation Project (Canada), University of Waterloo/Waterloo. Report of the Climate Change Adaptation Project, 122p.

³ Warnke, S. E. 2003. Creeping bentgrass (*Agrostis stolonifera* L.). In: Turfgrass Biology, Genetics, and Breeding (M. D. Casler and R. R. Duncan, editors), p 175-185. John Wiley & Sons, Hoboken.

⁴ Ruemmele, B. A. 2003. *Agrostis capillaris* (*Agrostis tenuis* Sibth.) colonial bentgrass. In: Turfgrass Biology, Genetics, and Breeding (M. D. Casler and R. R. Duncan, editors), p 187-200. John Wiley & Sons, Hoboken.

⁵ Harivandi, M. A., W. B. Hagan and K. N. Morris. 2008. Evaluating bentgrasses for quality, speed, thatch development and annual bluegrass invasion. *Acta Horticulture*, 783:309-315.

⁶ Lees, A. K., P. Nicholson, H. N. Rezanoo and D. W. Parry. 1995. Analysis of variation within *Microdochium nivale* from wheat: evidence for a distinct sub-group. *Mycological Research*, 99:103-109.

⁷ Tronsmo, A. M., T. Hsiang, H. Okuyama and T. Nakajima. 2001. Low temperature diseases caused by *Microdochium nivale*. In: Low temperature Plant Microbe Interactions under Snow (N. Iriki, D.A. Gaudet, A.M. Tronsmo, N. Matsumoto, M. Yoshida and A. Nishimune, editors), p 75-86. Hokkaido National Experiment Station, Sapporo.

⁸ Larsen, A. 1994. Breeding winter hardy grasses. *Euphytica*, 77:231-237.

⁹ Thorsen, S. M. and M. Höglind. 2010. Assessing winter survival of forage grasses in Norway under future climate scenarios by simulating potential frost tolerance in combination with simple agroclimatic indices. *Agricultural and Forest Meteorology*, 150 (9):1272-1282.

¹⁰ Uleberg, E., I. Hanssen-Bauer, B. van Oort and S. Dalmannsdottir. 2014. Impact of climate change on agriculture in Northern Norway and potential strategies for adaptation. *Climatic Change*, 122 (1-2):27-39.

¹¹ Garrett, K. A., S. P. Dendy, E. E. Frank, M. N. Rouse and S. E. Travers. 2006. Climate change effects on plant disease: Genomes to ecosystems. *Annual Review of Phytopathology*, 44:489-509.

of Civitas/Harmonizer on *M. nivale* inoculated onto several bentgrass turfgrass cultivars under various temperatures and two CO₂ concentrations.

Methods

Eighteen cultivars, selected from commonly used bentgrasses and annual bluegrass in Canada and Scandinavia, were grown in capped glass vials within temperature controlled chambers, inoculated with *M. nivale*, and screened for naturally occurring resistance at 10°C and 20°C (data not shown). Eight cultivars were selected from this screening and subsequently tested in 7.2 x7.2 cm pots in growth chambers with CO₂ concentrations of 400 ppm and 800 ppm at 15°C. After 10 wk of growth, the grass was treated with a foliar spray of 1ml 5% Civitas/ 0.03% Harmonizer, and inoculated 1 wk later with *M. nivale*. The grass was evaluated for percent yellowing after 7, 9, 15 and 21 days' post inoculation, and a measure of disease severity (AUDPC=area under the disease progress curve) was calculated for the entire 21 days.

Results and discussion

When treated with water under 400 ppm CO₂, the *A. capillaris* and *P. annua* cultivars exhibited 30% and 46% less disease symptoms than the group of *A. stolonifera* cultivars respectively ($p < 0.05$). Under all other treatment combinations *A. stolonifera*, *A. capillaris* and *P. annua* did not differ significantly. In addition, the *A. stolonifera* cultivar commonly used in Scandinavia did not significantly differ from the *A. stolonifera* cultivars commonly used in Canada as a group. There were significant differences between cultivars, though the application of Civitas/Harmonizer decreased the absolute range of disease values between cultivars (Table 1).

There was a significant impact of CO₂ on the disease symptoms of turfgrass cultivars ($p < 0.05$). At 800 ppm CO₂, they exhibited lower average yellowing than at 400 ppm CO₂. The increased CO₂ concentration resulted in 19-56% disease suppression in the Civitas/Harmonizer treatment, and 6-51% disease suppression in the water treatment (Table 1). And symptoms were less with activator treatment at 400 ppm CO₂ (15-52% disease suppression) and at 800 ppm CO₂ (5-61% disease suppression) compared to water (Table 1).

There is evidence to suggest that Civitas/Harmonizer resistance activation varied by cultivar, and the amount of disease suppression was higher at increased CO₂ concentrations. When incubated at 800 ppm CO₂, treated with water, the cultivar Focus showed the highest amount of disease severity, but also the highest percent disease suppression when Civitas/Harmonizer was applied under the same CO₂ conditions. The cultivar that showed the highest amount of disease under 400 ppm CO₂ and treated with water was T1, but it also showed the highest percent disease suppression caused by the increase of CO₂. Overall the *P. annua* cultivar Labelle exhibited the least amount of disease symptoms on average across all treatments, followed closely by the Scandinavian *A. capillaris* cultivar Leirin. The *A. stolonifera* cultivar with the least amount of disease symptoms on average was Alpha. This research will be useful to provide recommendations on turfgrass cultivars for northern temperate zone golf courses and on sustainable management practices to face the challenges of climate change.

Table 1. Average amount of disease (AUDPC) for turfgrass cultivars during 21 days after inoculation with *Microdochium nivale* at two CO₂ concentrations, treated with 5% Civitas/0.3% Harmonizer (C/H) or water. Average AUDPC was calculated for each treatment combination based on 4 replications. Values followed by a letter in common within a column are not significantly different ($p < 0.05$). Higher values indicate higher disease severity.

Cultivar	Species	Average yellowing (AUDPC) of turfgrasses inoculated with <i>Microdochium nivale</i> by CO ₂ concentration and Civitas/Harmonizer treatment							
		400 ppm CO ₂				800 ppm CO ₂			
		C/H		Water		C/H		Water	
Focus	<i>A. stolonifera</i>	525	A	620	AB	232	A	586	A
Tyee	<i>A. stolonifera</i>	435	AB	619	AB	216	A	518	AB
Independence*	<i>A. stolonifera</i>	285	B	594	AB	256	A	483	ABC
Penncross	<i>A. stolonifera</i>	325	AB	553	AB	240	A	354	ABCD
T1	<i>A. stolonifera</i>	378	AB	682	A	227	A	337	BCD
Alpha	<i>A. stolonifera</i>	376	AB	552	AB	258	A	273	CD
Leirin*	<i>A. capillaris</i>	289	B	423	BC	166	A	270	CD
Labelle	<i>P. annua</i>	240	B	323	C	195	A	225	D
Treatment Mean		357		546		224		381	

* Cultivars selected by Scandinavian colleagues.

A NORDIC MODEL FOR IMPLEMENTING INTEGRATED PEST MANAGEMENT (IPM) – COLLABORATION BETWEEN AUTHORITIES AND THE TURFGRASS INDUSTRY

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Introduction

Scandinavian Turfgrass and Environment Research Foundation (STERF) was established 2006 and is the Nordic golf federation's joint research body. STERF's vision is to be the leading international centre of expertise in sustainable golf course management and to make the turfgrass industry in the Nordic countries a role-model regarding responsibility for sustainable societal development. Research activities are prioritized according to the following research programs 1) integrated pest management, 2) sustainable water management, 3) turf grass winter stress management and 4) multifunctional use of golf facilities¹.

STERF's ambition is to support the golf and turfgrass industry to Take the initiative – Work proactively – Create changes. To take the initiative means, for example, to identify important challenges the sector has to face. New knowledge, delivered by STERF, helps the sector to work proactively and take a lead in improving the sustainable management of golf courses and other turfgrass areas, which means produce managed turfgrass areas and golf courses of a high standard while at the same time ensuring the sustainable use of natural resources and contributing to functioning ecosystems. By taking the lead and, entering into early constructive discussion with relevant national and international bodies, STERF has created important changes for the golf and turfgrass sector and for society as whole.

Taking a lead in making research results and new knowledge easily accessible to end-users and to provide support to implement changes in the management of golf courses and other turfgrass areas, has been an important STERF strategy for implementing EU's directive on sustainable use of pesticides².

Applied and ready-to-use research is a key to success not only in IPM but also for STERF to be recognized by the turf industry as a responsible and reliable partner. By producing or supporting objective and scientific reports on pesticide behaviour on golf greens^{3,4} turf grass' resistance to pests⁵, and biodiversity on golf courses⁶, STERF has built confidence and created a platform for dialog and cooperation with the authorities.

Implementation of EU's directive on sustainable use of pesticides

When EU's directive 2009/128 came into force, STERF and the Swedish University of Agricultural Sciences set up a workshop for Scandinavian greenkeepers, turf grass agronomists, researchers and lecturers in 2010. The aim of the workshop was to identify important challenges related to the implementation the new directive. The workshop was supported by the Swedish Board of Agriculture, which also granted money for producing and publishing the first Integrated Pest Management (IPM) library including an introduction to IPM and 17 fact sheets focusing on important turf pests and management strategies. The library and factsheets were presented to Swedish, Danish and Norwegian greenkeepers by some of the key authors at three seminars in November 2011.

EU's directive on sustainable use of pesticides has been implemented in all EU member countries and is now is a part of the Nordic national pesticide legislations. In May 2015 STERF took a new initiative to continue the dialogue with the Nordic relevant authorities. A meeting between Nordic authorities' executive officers,

¹ Scandinavian Turfgrass and Environment Research foundation. 2014. Research programme. <http://sterf.golf.se/sv/about-sterf/research-programme> 15p.

² Scandinavian Turfgrass and Environment research foundation. 2009. Research and development programme within integrated pest management. <http://sterf.golf.se/sv/about-sterf/research-programme/integrated-pest-management> 17 p.

³ Aamlid, T.S. 2014. Fungicide leaching from golf greens. A synopsis of Scandinavian studies. Popular Scientific Articles, STERF, December

⁴ Aamlid, T.S. 2012. Turfgrass species and varieties for Integrated Pest Management of Scandinavian putting greens. *Acta Agriculturae Scandinavica Section B – Soil and Plant Science*; 62: Supplement 1 10-23.

⁵ Colding, J., C. Folke. 2009. The role of Golf Courses in Biodiversity Conservation and Ecosystem Management. *Ecosystem* 12: 191-206.

⁶ Aamlid, T. 2015. More research and technology transfer on INTEGRATED PEST MANAGEMENT for turf is needed. <http://sterf.golf.se/sv/ipm/seminarier> 3p.

turfgrass researchers and representatives for the golf sector was set up in Copenhagen. Outcome of the meeting was an agreement to improve Nordic communication and education within IPM and to further develop the IPM library⁶.

Authorities in Norway and Denmark also contributed to the extension of STERF's IPM library in 2015. In Norway some of the new texts were approved as the official crop specific IPM guidelines for golf course management. In Denmark the authorities funded additional work to transform the fact sheets into workbooks for students. All fact sheets in the library have been revised by golf course managers in Sweden, Denmark and Norway before publishing. These greenkeepers are presented in the fact sheet as ambassadors for the topic and their experiences are available for colleagues.

Available IPM material STERF's IPM fact sheet library is open for everyone on www.sterf.org It is so far published only in Scandinavian languages and includes these 30 titles:

Chapter	Title /topic
General IPM	Introduction to Integrated Pest Management (13 p)
	The 8 EU principles for IPM applied on golf courses (table)
Turf grass species	Grass species and varieties for golf courses
	Grass species and varieties for amenity and parks
Turf grass agronomy	Establishing turf grass with less weed
	Fertility and IPM
	Irrigation and IPM
	Thatch control and turf disease
	Mechanical maintenance and turf pests
	Dry patches, weed and disease
Weed	The most common weed on golf courses
	Biology and control of white clover (<i>Trifolium repens</i>)
	Biology and control of thistles (<i>Cirsium sp</i>)
	Biology and control of docks (<i>Rumex sp</i>)
	Weed control in the rough
	Surveillance of weed
Insects	Reducing weed on fairways
	Registration and identification of harmful insects
	Biology and control of crane fly (<i>Tipula paludosa</i>)
	Biology and control of cockchafers (<i>Melolontha sp</i>)
Disease	Biology and control of winter diseases (<i>Microdochium nivale</i> , <i>Typhula spp.</i> , <i>Sclerotinia borealis</i> , <i>Pythium iwayamai</i>)
	Biology and control of fairy rings (<i>Basidiomycetes</i>)
	Biology and control of anthracnose (<i>Colletotrichum graminicola</i>)
	Biology and control of rings patches in turf (<i>Fusarium sp</i> , <i>Rhizoctonia solani</i> , <i>Gaeumannomyces graminis</i>)
	Biology and control of red thread and pink patch (<i>Laetisaria fuciformis</i> , <i>Limnomyces roseipelli</i>)
	Biology and control of Pythium (<i>Pythium graminicola</i> , <i>P. aphanidermatum</i>)
	Biology and control of dollar spot (<i>Sclerotinia homoeocarpa</i>)
Spraying technics	Spraying equipment – optimal use and calibration
IPM documentation	Guidelines for local turf grass experiments
	Examples on IPM documentation schemes

Work-book for students

Danish Ministry of Education has funded educational material to STERF's IPM library, and these files will be published on the official Danish web site www.undervisningsportalen.dk in the autumn 2016.

POST-APPLICATION IRRIGATION TIMING AND FORMULATION AFFECT AZOXYSTROBIN RETENTION IN HYBRID BERMUDAGRASS CLIPPINGS

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Introduction

Azoxystrobin (methyl(E)-2{2[6-(6-2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate) is a systemic strobilurin fungicide used for controlling various soil-borne diseases in turfgrass sites including athletic fields, golf courses, and residential properties¹. In 2008, azoxystrobin outranked all fungicides in gross global sales (\$900 million USD worldwide⁻¹)². Azoxystrobin plant uptake occurs via foliar and root pathways, and is acropetally translocated, making it more likely to accumulate in the upper region of treated turfgrass plants that may be removed during a mowing event, clippings³. Previous research has shown azoxystrobin residues can persist in turfgrass clippings through 32 d after treatment⁴. Additionally, previous research indicates azoxystrobin turfgrass clippings can release into water, which may cause adverse ecological effects⁴. Information from this research may improve turfgrass management with regards to azoxystrobin application and post-application practices.

Methods

Field research was initiated August 19, 2014 (Lake Wheeler Turfgrass Field Laboratory, Raleigh, NC, USA) to quantify azoxystrobin residue in clippings from hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *Cynodon transvaalensis* Burt-Davey, cv. 'Tifway 419'] maintained at 5 cm. The presented research covers two experiments. Across both experiments, azoxystrobin was applied at 0.5 kg ai ha⁻¹ (maximum single application rate) to unique 1.2 by 3 m plots at 32, 16, 8, 4, 2, 1, or 0 d before clipping collection (DBCC). Broadcast spray applications were made with a CO₂-pressurized, three-nozzle (TeeJet 8004 XR VS flat-fan) boom calibrated to deliver 814 L ha⁻¹. Granular applications were made by hand with a shaker jar. All applications were made to dry turfgrass vegetation from 12:00:00 to 15:00:00 eastern standard time. From 32 to 5 DBCC, clippings were returned to plots mown with a self-propelled rotary mower (Honda HRC 216 Rotary Mower[®], American Honda Power Equipment Division, Alpharetta, GA, USA). Plots were not mown from again until after 0 DBCC application (2 h drying period following application). Clippings were collected from each experimental unit in a plastic-lined self-propelled rotary mower bag, homogenized, and subsampled to determine pesticide concentration via high performance liquid chromatography–diode array detector methodology (limit of detection: 0.05 mg kg⁻¹). Experiment one evaluated the effect of post-application irrigation timing (PAIT) on azoxystrobin clipping residue concentration. Irrigation was simulated 0 or 4 h after treatment (HAT) with an eight-nozzle boom (TeeJet 8008 XR VS flat-fan) calibrated to deliver 0.6 cm H₂O plot⁻¹ with four passes. Experiment two evaluated the effect of formulation on azoxystrobin clipping residue concentration. Azoxystrobin was applied as a granular (Heritage G[®]) or liquid (Heritage TL[®]) and was irrigated 4 HAT identically to experiment one. Both experiments included four replications of a two-by-seven factorial treatment arranged in a randomized complete block design. Factorial levels included two PAIT (experiment one; 0 or 4 HAT) or formulations (experiment two; granular or liquid) applied at seven timings prior to turfgrass clipping collection (32, 16, 8, 4, 2, 1, or 0 DBCC). Turfgrass clipping pesticide concentrations were converted to a percent of the broadcast application rate. Data were subjected to ANOVA (P = 0.05), with PAIT, formulation, and DBCC fixed effects. Means were separated according to Fisher's protected LSD (P < 0.05) with the use of SAS general linear models.

¹ Anonymous. 2014. Heritage TL[®] herbicide label. Syngenta Crop Protection, LLC, Greensboro, NC, USA. Syngenta Publication No. SCP 1191A-L2D 1213. 19 p.

² Leadbeater, A. 2012. Resistance Risk to QoI Fungicides and Anti-Resistance Strategies. In: Thind, T.S., Fungicide Resistance in Crop Protection: Risk and Management. Centre for Agriculture and Biosciences International, Cambridge, MA, USA. p. 141.

³ EPA. 1997. United States Environmental Protection Agency, Washington DC, USA. Pesticide Fact Sheet: Azoxystrobin. 23 p.

⁴ Yelverton, F.H., M.D. Jeffries, and T.W. Gannon. 2014. Effect of turf species on pesticide clipping concentrations and subsequent release in aquatic systems. European Journal of Turfgrass Science, 45:71-72.

Results and Discussion

Analysis of variance revealed a PAIT-by-DBCC interaction on azoxystrobin residue retention in clippings. At 0, 1, 2, and 4 DBCC, 21.1, 44.5, 19.2, and 10.9% less of the applied azoxystrobin was removed via clipping collection when irrigated 0 compared to 4 HAT, respectively (Table 1). Biologically significant differences were not detected within, or between PAIT from 8 to 32 DBCC; however, it should be noted data agree with previous research showing azoxystrobin remains detectable when applied 32 DBCC⁴. This suggests clipping management practices in azoxystrobin-treated areas should consider off-target transport for at least 1 mo following application to reduce potential adverse off-target effects. Within PAIT, maximum azoxystrobin clipping residue concentration was 11.5 (0 HAT) and 51.5% (4 HAT) of the applied when collected from areas treated 0 and 1 DBCC, respectively. Following the d of application, < 7% of the applied azoxystrobin was removed via clipping collection when irrigated 0 HAT, while 14.1 to 51.5% of the applied was removed from 0 to 4 DBCC when irrigated 4 HAT. The increase in azoxystrobin removed via clipping collection from 0 to 1 DBCC when irrigated 4 HAT may be due in part to acropetal movement in treated plants, coupled with a greater portion of the treated turfgrass vegetation removed with increased growing time following treatment. Analysis of variance revealed a formulation-by-DBCC interaction on azoxystrobin residue retention in clippings. At 0, 1, 2, and 4 DBCC, 19.6, 30.9, 17.8, and 12.3% less of the applied azoxystrobin was removed via clipping collection when treated with granular compared to liquid formulation, respectively (Table 1). Biologically significant differences were not detected within, or between formulations from 8 to 32 DBCC; however, azoxystrobin residues were detected when applied 32 DBCC across formulations. Within formulation, maximum azoxystrobin clipping residue concentrations were at 1 DBCC, with 17.8 and 48.7% of the applied when collected from areas granular- or liquid-treated, respectively. As with the PAIT experiment, increases from 0 to 1 DBCC in azoxystrobin residues removed via clipping collection were observed across both formulations, further supporting the aforementioned theory. No differences were observed between 0 and 2 DBCC across granular (15.2 and 14.9% of applied, respectively) and liquid (34.8 and 32.7%) applications. The most pronounced decrease occurred from 2 to 4 DBCC with granular application (\approx 3-fold), while this was delayed from 4 to 8 DBCC with liquid application (\approx 4-fold). Azoxystrobin-clipping retention varied from 8 to 16 DBCC following granular application (4.5 and 1.6% of applied, respectively), while liquid application did not differ from 8 to 32 DBCC (0.2 to 3.8% of applied). These data suggest azoxystrobin retention in hybrid bermudagrass clippings may be significantly reduced from 0 to 4 d after treatment (DAT) by irrigating treated areas immediately after liquid application and by using a granular formulation. This is pertinent for turfgrass managers, as clipping collection 0 to 4 DAT can remove a substantial portion of the applied azoxystrobin, which is concerning from ecological and pest control perspectives. Although these management practices would not compromise soil-borne disease efficacy, azoxystrobin is also widely foliar-directed for brown patch (*Rhizoctonia solani*) control. When used for control of brown patch or other foliar diseases it would not be advisable to irrigate azoxystrobin immediately after application or apply a granular formulation due to compromising efficacy; therefore, precautions should be taken to ensure clippings from treated areas are managed to avoid displacement into off-target areas. Future research should evaluate additional best management practices to reduce potential off-target transport of azoxystrobin and other turfgrass pesticides via clipping displacement.

Table 1. Effect of post-application irrigation timing and formulation on azoxystrobin residue retention in hybrid bermudagrass clippings.^{a-c}

DBCC	PAIT		LSD0.05	Formulation		LSD0.05
	0 HAT	4 HAT		Granular	Liquid	
	% of applied			% of applied		
0	11.5	32.6	7.4	15.2	34.8	5.9
1	7.0	51.5	7.8	17.8	48.7	9.3
2	6.5	25.7	3.3	14.9	32.7	4.5
4	3.2	14.1	2.8	5.1	17.4	3.8
8	2.6	3.7	0.6	4.5	3.8	0.6
16	1.3	1.9	0.3	1.6	3.3	0.7
32	0.6	0.9	0.1	0.1	0.2	NS
LSD0.05	1.1	3.6		2.5	3.8	

^a Abbreviations: PAIT, post-application irrigation timing; DBCC, d before clipping collection; HAT, h after treatment; NS, non-significant.

^b Azoxystrobin applied (0.5 kg ai ha⁻¹) as a broadcast spray for PAIT and liquid formulation treatments, and by hand with a shaker jar for granular treatments. ^c Post-application irrigation delivered 0.6 cm H₂O over four passes. Formulation treatments irrigated 4 HAT.

INFLUENCE OF FERROUS SULFATE AND ITS ELEMENTAL COMPONENTS ON DOLLAR SPOT SUPPRESSION

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Introduction

Dollar spot is a common disease of *Agrostis stolonifera* and is especially devastating on putting greens. *Sclerotinia homoeocarpa* degrades by creating silver dollar-sized depressions of blighted and bleached turf. Sequential fungicide applications are often required throughout the growing season for adequate control. Even though many fungicides are labeled for dollar spot control, they have become limited due partly to fungicide resistance. Previous research has investigated nitrogen source for dollar spot suppression (Markland et al., 1969), but the effects of many other elements on dollar spot development are not well documented. This research was established to determine if ferrous sulfate (FeSO_4) and its elemental components are capable of reducing dollar spot epidemics.

Methods

Trials were established *in situ* and *in vitro* to address this objective. Field trials were conducted in 2012 and 2015 on creeping bentgrass putting greens built to USGA specifications (90% sand, 10% peat moss) at the Virginia Tech Turfgrass Research Center, Blacksburg, VA. Bi-weekly foliar treatments were applied from May to September in four randomized complete blocks as: control, ferrous sulfate (48.8 kg ha^{-1}), sulfur (10.3 kg ha^{-1}), and iron chelate ($11.2 \text{ kg iron ha}^{-1}$). Applications were applied as liquids with a CO_2 -pressurized sprayer with Tee Jet TTI11004 nozzles in a spray volume of 814 L ha^{-1} . Dollar spot infection centers and turf quality were recorded throughout the summer. Quality ratings were based on a 1-9 scale with 1 = dead, poor quality turf, 6 = minimally-acceptable turf, and 9 = healthy, high quality turf. Data collection from 2012 occurred on four dates when disease pressure in untreated plots reached an acceptable threshold of 30 to 60 infection centers plot⁻¹. Dollar spot infection centers and turf quality were rated 11 times in 2015 over the entire season. This alternative method was used to track disease progression over repeat applications throughout the growing season. When disease pressure became unacceptable, chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) at 12.5 kg ha^{-1} a.i. and nitrogen (46-0-0) at 7.3 kg N ha^{-1} were applied to suppress disease and enable plot recovery. The area under disease progression curve (AUDCP) was transformed for each study (ARM, v. 9.2, Gylling Data Management, Brookings, SD). and Data are presented separately because of a trial by treatment interaction. *In-vitro* trials were conducted additionally to explore the fungitoxic effect of iron sulfate against *S. homoeocarpa*. Pure cultures of *S. homoeocarpa* were plated in quarter strength PDA amended with 0, 10, 100, and 1,000 mg/kg concentrated solutions of iron sulfate with pH adjusted to 4.5, 5, 5.5, 6, and 6.5. All data were subjected to ANOVA and means separated using Fisher's protected LSD test ($\alpha = 0.05$) in JMP v. 11.0 (SAS Institute, Cary, NC).

Results and Discussion

FeSO_4 reduced dollar spot pressure each year. In 2015, dollar spot infection centers were suppressed by approximately 55% when treated bi-weekly with FeSO_4 (Figure 1). Turf quality was improved with FeSO_4 in 2012, but not 2015. Iron chelate suppressed dollar spot in 2012, but not 2015. Sulfur had no impact on dollar spot or turf quality in either year. Mycelial growth was enhanced at 10 and 100 mg kg^{-1} *in vitro*, but was completely inhibited at 1000 mg kg^{-1} (Table 1). These data suggest FeSO_4 has direct fungitoxic activity against the dollar spot pathogen when concentrations are above 100 mg kg^{-1} , but could be enhanced at lower concentrations. The effect of varying iron concentrations in the field is not well understood. Future research will address how rate impacts dollar spot progression. Additionally, research is needed to determine how iron sulfate can be integrated into a disease suppression program that can reduce fungicide application rates or extend re-application intervals required to maintain turf below acceptable turfgrass quality.

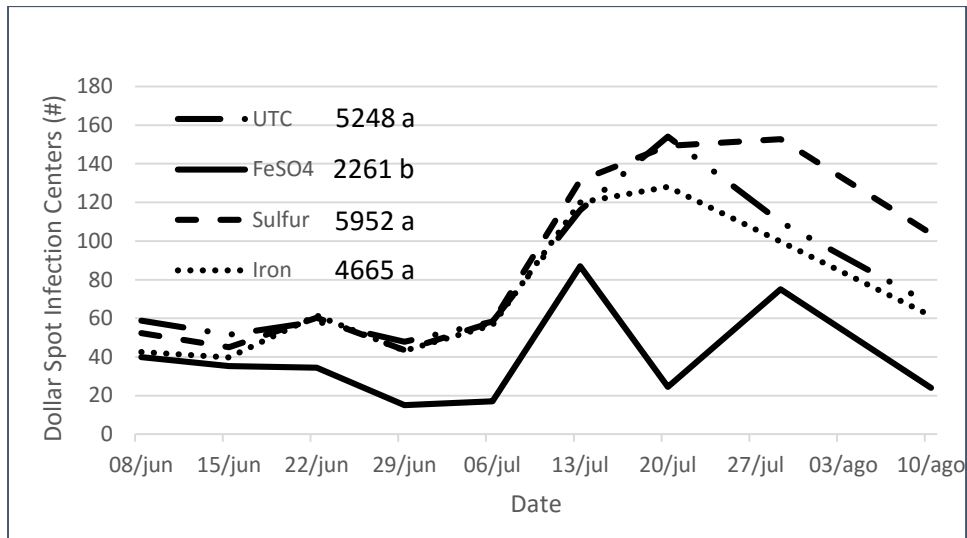


Figure 1. Dollar spot progression on creeping bentgrass putting greens, as affected by treatment in 2015. Area under disease progression curve (AUDPC) indicates seasonal accumulation of infection centers.

Table 1. ANOVA for radial mycelial growth of *Sclerotinia homoeocarpa* in nutrient agar amended with four iron concentrations at pH 6.5 using Fisher's protected LSD.

Fe (mg/ kg)	Diameter (mm)	
0	22	b†
10	44	A
100	54	A
1000	0	C

†Significant at $P < 0.0001$. Means followed by the same letter are not significantly different.

BEST MANAGEMENT PRACTICES EFFECTS ON ANTHRACNOSE DISEASE OF POA ANNUA

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Introduction

Anthracnose of annual bluegrass (ABG; *Poa annua* L.), caused by the fungus *Colletotrichum cereale* Manns sensu lato Crouch, Clarke, and Hillman, can be a devastating disease on putting greens in temperate climates across the world. Previous research indicates that several management practices including increased N fertility¹, raised mowing height², and more frequent sand topdressing^{3,4,5} can significantly reduce the severity of this disease. These earlier research studies primarily focused on the effects of individual practices on anthracnose severity of ABG putting green turf. Thus, information pertaining to the relative importance and interactive effects of multiple practices on anthracnose is limited, which has restricted our ability to fully develop recommendations for best management practices (BMP) to combat this disease.

Methods

A 3-yr field study was conducted to determine the relative impact and potential interactions of N fertility, mowing height, and sand topdressing on anthracnose severity, turf quality, and ball roll distance (BRD) of ABG putting green turf in North Brunswick, NJ USA. Treatments were arranged in a 2 x 2 x 2 factorial using a split-split-plot experimental design with four replications. Main plots were mowing height (2.3 and 3.2 mm), subplots were N fertility (100 and 200 kg ha⁻¹ yr⁻¹), and sub-subplots were sand topdressing (46.4 and 97.6 Mg ha⁻¹ yr⁻¹ in 2012 and 36.6 and 80.6 Mg ha⁻¹ yr⁻¹ during 2013 and 2014). Main plots were mowed once daily six times wk⁻¹ using a flex head walk-behind mower (model Flex 2100, Toro Co. Bloomington, MN). Nitrogen fertilizer was applied with a walk-behind spray boom (model I-1575D, The Broyhill Co., Dakota City, NE) that delivered 815 L ha⁻¹ at 379 kPa using a gasoline-powered backpack sprayer (model SHR-210, ECHO Inc., Lake Zurich, IL). Sub-angular, kiln-dried silica sand (model 310, U.S. Silica, Co., Mauricetown, NJ) with a medium-coarse particle size distribution was applied with a drop spreader (model 42H25, Gandy Co., Owatonna, MN) and incorporated into the turf canopy by pushing a medium-stiff bristle broom across plots. Rates and timings of N fertility and topdressing applications are described in Tables 1 and 2, respectively. The turf at the site was a perennial biotype of ABG grown on a sand-topdressed mat layer (50 to 60 mm deep) overlaying a Nixon sandy loam (fine-loamy, mixed, semiactive, mesic Typic Hapludults). The trial area was irrigated predominantly with hand-watering to avoid drought stress and rolled 3 times wk⁻¹ with a light-weight vibratory roller (model Vibe V, Turflite Inc., Moscow Mills, MO) to maintain surface dryness and firmness similar to a golf course putting green. Additionally, the plant growth regulator ethephon was applied at 3.81 kg a.i. ha⁻¹ three times each spring to suppress ABG inflorescence expression and trinexapac-ethyl was applied weekly at 0.05 kg a.i. ha⁻¹ from March to October each year to regulate vegetative growth. Dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) and brown patch (*Rhizoctonia solani* Kühn) diseases, and ABG weevil [*Listronotus maculicollis* (Kirby)] were controlled using pesticides having no effect on anthracnose isolates from this location. Soil pH, P and K were managed based on soil tests. Anthracnose severity was evaluated biweekly as the percentage of turf area infested with *C. cereale* using a line-intercept grid count method¹. Turfgrass quality was also evaluated biweekly. Ball roll distance measurements were taken from sub-subplots one to three times per week using a Stimpmeter (USGA, Far Hills, NJ). All data were subjected to ANOVA using the GLM procedure in the SAS software v. 9.3 (SAS Institute, 2011). Means were separated using Fisher's protected least significant difference ($p \leq 0.05$).

¹ Inguagiato, J.C., J.A. Murphy, and B.B. Clarke. 2008. Anthracnose Severity on Annual Bluegrass Influenced by Nitrogen Fertilization, Growth Regulators, and Verticutting. *Crop Sci.* 48(4):1595-1607. DOI: 10.2135/cropsci2007.06.0343.

² Inguagiato, J.C., J.A. Murphy, and B.B. Clarke. 2009. Anthracnose Disease and Annual Bluegrass Putting Green Performance Affected by Mowing Practices and Lightweight Rolling. *Crop Sci.* 49(4):1454-1462. DOI: 10.2135/cropsci2008.07.0435.

³ Inguagiato, J.C., J.A. Murphy, and B.B. Clarke. 2012. Sand Topdressing Rate and Interval Effects on Anthracnose Severity of an Annual Bluegrass Putting Green. *Crop Sci.* 52(3):1406-1415. DOI: 10.2135/cropsci2011.01.0010.

⁴ Inguagiato, J.C., J.A. Murphy, B.B. Clarke, and J.A. Roberts. 2013. Topdressing sand particle shape and incorporation effects on anthracnose severity of an annual bluegrass putting green. *Int. Turfgrass Res. Soc. J.* 12: 127-134.

⁵ Hempfling, J.W., B.B. Clarke, and J.A. Murphy. 2015. Anthracnose disease on annual bluegrass as influenced by spring and summer topdressing. *Crop Sci.* 55(1):437-443. DOI: 10.2135/cropsci2014.04.0297.

Results and Discussion

As expected, all management practices affected the severity of anthracnose. Increased N fertility was the most important practice for reducing disease severity, whereas higher mowing height was slightly more effective than greater sand topdressing rate. Turf quality was improved with greater N fertility, raised mowing height, and increased sand topdressing, and the relative importance of each factor was similar to that observed for anthracnose severity. Conversely, higher mowing height had the greatest effect on BRD (higher mowing reduced BRD), while increased N fertility and greater sand topdressing had smaller effects on BRD which would likely not have been perceptible by golfers⁶. When interactions occurred, the effect of sand topdressing depended on N fertility and/or mowing height. Increased sand topdressing was most effective at reducing disease severity under low N fertility and/or lower mowing height (2-way and 3-way interactions). These practices interacted in a similar way to affect turf quality and BRD; greater sand topdressing was most effective at improving turf quality and increasing BRD under low N fertility and/or lower mowing height. These results indicate that when adjustments in management practices are needed to increase BRD and suppress anthracnose, mowing height should be lowered rather than decreasing N fertility or reducing sand topdressing. Additionally, turf managers should recognize that sand topdressing has a greater effect on disease severity, turf quality, and BRD under low mowing height and/or low N fertility.

Table 1. Application timing, number of applications, and nitrogen (N) rates for low and high N treatments on annual bluegrass turf during 2012 to 2014.

Timing of Application	Number of Applications		Rate of Application	
	Low N	High N	Low N	High N
			----- kg N ha ⁻¹ yr ⁻¹ -----	
Mid-March to Early-April [†]	1	2	18.3	18.3
Early-April to Late-September (26 wk) [‡]	13	26	4.9 every 2-wk	4.9 every 1-wk
October [†]	1	2	18.3	18.3
Annual	15	30	100	200

[†]Fertilizer comprised of water-soluble (urea; 9.8 kg N ha⁻¹) and slow-release (methylene urea; 8.5 kg N ha⁻¹) nitrogen.

[‡]N was applied as urea.

Table 2. Application timing, number of applications, and rates of topdressing for low and high sand treatments on annual bluegrass turf during 2012 to 2014.

Timing of Application	Number of Applications		Rate of Application	
	Low Sand	High Sand	Low Sand	High Sand [†]
			----- Mg ha ⁻¹ yr ⁻¹ -----	
Late-March	1	1	19.5 (9.8) [‡]	19.5 (9.8)
April	0	2	0	9.8 (7.3)
Early-May to Early-June	1	4 (3) [‡]	4.9	4.9
Late-June/Early-July to Mid-October	1	8 (9)	0	2.4
Late-October	1	1	19.5	19.5
Annual	4	16	46.4 (36.6)	97.6 (80.6)

[†]The low sand topdressing treatment emulated the rates and application frequency of a minimal program in the northeastern U.S.A. The high sand topdressing treatment applied sand at rates and frequency that matched growth of the turf (thatch accumulation).

[‡]Values in parentheses were the number and rates of topdressing applications during 2013 and 2014. The number and rate of applications were adjusted to match the growth of the turf during 2013 and 2014.

[‡]Values in parentheses were the number and rates of topdressing applications during 2013 and 2014. The number and rate of applications were adjusted to match the growth of the turf during 2013 and 2014.

⁶ Karcher, D., T. Nikolaj, and R. Calhoun. 2001. Golfer's perceptions of greens speeds vary: Over typical Stimpmeter distances, golfers are only guessing when ball-roll differences are less than 6 inches. *Golf Course Manage.* 69(3):57-60.

OVERSEEDING OF FAIRWAY

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Introduction

Overseeding fairways is not common in the Nordic countries and optimal strategies are needed in order to justify the costs. Overseeding to create a strong turf might be helpful in order to minimize weed competition in situations with no or very limited use of herbicides. Thus, the aim of our project was to evaluate the effects of overseeding time (spring or autumn), additional fertilization at overseeding, and turfgrass species (perennial ryegrass (*Lolium perenne*), red fescue (*Festuca rubra*) or Kentucky bluegrass (*Poa pratensis*)) in relation to cultural grass introduction, weed occurrence and general appearance on golf course fairways. The cost of overseeding was also evaluated.

Methods

Repeated overseeding of the same plots was performed on an experimental fairway at Landvik (NIBIO, Norway) and on three golf courses in Denmark (Korsør, Skovbo and Hornbæk) in 2011, 2012 and 2013 and with registrations in 2011, 2012, 2013 and 2014.

Seeding procedure in Norway; Mowing height was reduced the week before seeding to 10 cm. A walk behind JD Aerocore was used to make hollow tine cores and seeding was performed with a Scot's drop spreader. The trial was irrigated and top-dressed after seeding.

Seeding procedure in Denmark; Fairways were mowed before seeding. Seeding of subplots was conducted using a BLEC Disc Seeder 1800 (Working width 1,8m, 5 cm between each slit). No topdressing or irrigation was conducted after the overseeding.

In both locations the sowing rate of all species was 30 kg ha⁻¹. The species and variety used were; Red fescue; var. Nikky and var. Valdora, Perennial ryegrass; var. Dickins and var. Columbine, Kentucky bluegrass; var. Julius and var. Yvette (Table 1).

Table 1. Experimental design

No	Grass for overseeding	Time for overseeding	Fertilizer when overseeding
1	None	No overseeding	No fertilizer
2	None	No overseeding	50 Kg N/ha, May
3	None	No overseeding	50 Kg N/ha, September
4	Red fescue	May	No fertilizer
5	Red fescue	May	50 Kg N/ha, May
6	Kentucky bluegrass	May	No fertilizer
7	Kentucky bluegrass	May	50 Kg N/ha, May
8	Perennial ryegrass	May	No fertilizer
9	Perennial ryegrass	May	50 Kg N/ha, May
10	Red fescue	September	No fertilizer
11	Red fescue	September	50 Kg N/ha, September
12	Kentucky bluegrass	September	No fertilizer
13	Kentucky bluegrass	September	50 Kg N/ha, September
14	Perennial ryegrass	September	No fertilizer
15	Perennial ryegrass	September	50 Kg N/ha, September

Results and Discussion

The main finding was that overseeding of perennial ryegrass was successful, both on the three Danish golf courses and on the Norwegian experimental fairway. On the Norwegian experimental fairway there was a tendency for overseeding to be more successful in autumn, especially when combined with extra fertilizer, but all in all, perennial ryegrass could be introduced both in spring and autumn and irrespective of extra fertilizer.

On the Norwegian experimental fairway red fescue was also successfully introduced, but this could not be demonstrated on the three golf course fairways in Denmark. For Kentucky bluegrass there was no indication of any establishment after three years of overseeding in either Denmark or Norway.

The amount of weeds was not reduced by overseeding in any of the experiments during the three-year period. However, this might not indicate that there isn't an effect on weeds in the long run. A greenkeeper experience from another overseeding trial in Denmark years ago suggests that the effect on weed will be more pronounced after 7 - 10 years.

Registrations of general appearance and density etc. in the Norwegian trial did not demonstrate persistent effects of any of the treatments, but the best color was recorded in plots overseeded with perennial ryegrass. The visual appearance of the fairway could be improved temporarily by introducing perennial ryegrass, but the effect was not clear throughout the growing season.

The costs for overseeding will be significant for most golf courses especially if topdressing is included in order to increase the success. But the discussion should be if it is necessary to overseed the whole golf course? A solution if money is limited, could be to overseed selected areas where the turf is poor and open. Sherratt (2012)¹ claimed that overseeding of dense turf outside high-traffic areas is a waste of time and money because the mature, existing grass prevents seedlings to establish. Overseeding should be restricted to high-traffic areas. Lifting the quality of the poorest fairways might have a large effect on overall player satisfaction.

TURFGRASS PESTS (DISEASES, INSECTS, WEEDS)

POSTER PRESENTATIONS

AN INVESTIGATION OF TURFGRASS AS A POSSIBLE ROUTE FOR POLLINATOR EXPOSURE TO LAWN-APPLIED IMIDACLOPRID

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Introduction

There are numerous routes for pollinator exposure to insecticides, including treatments to lawns and flowering plants within gardens, subsequent contact with treated grass or flowering weeds, and by ingestion of tainted pollen, nectar, or guttation water^{1,2,3}. Guttation, the exudation of xylem-transported sap, has been identified as a route of insecticide exposure in several monocots including winter rape (*Brassica napus* L. var. *napobrassica* (L.) Rchb.)⁴ as well as triticale (\times *Triticosecale*) and maize⁵. Imidacloprid is a common neonicotinoid insecticide used for the control of turf and ornamental insect pests that can be translocated into guttation fluid, potentially harming non-target insects. Previous research found imidacloprid residue within creeping bentgrass (*Agrostis stolonifera* L.) guttation was high enough to cause intoxication of a minute pirate bug [*Orius insidiosus* (Say); average 88 ng mL⁻¹, 1 week after treatment]; however, levels were transitory and not likely hazardous by three weeks after treatment³. The majority of studies on the ecotoxicological interactions between pollinators and turfgrasses have been conducted in cool-season grasses with far less emphasis on those species that predominate warm-season climates. Two warm-season grasses, St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze) and bermudagrass (*Cynodon* spp.), are commonly used in residential and commercial lawns in the southeastern US⁶. Research objectives were to investigate the fate of soil applied imidacloprid within 'Tifway' hybrid bermudagrass (*C. dactylon* \times *C. transvaalensis*) and 'Palmetto' St. Augustinegrass.

Methods

A field experiment was conducted as a completely randomized design (six replicates) of bermudagrass and St. Augustinegrass placed within 0.13 m² plastic flats. Flats were treated 20 October, 2014. Imidacloprid was applied at standard use rates (443 g ai ha⁻¹) to soil-only via sub-irrigation with 1.0 L of water containing 5.76 mg imidacloprid. Overhead irrigation was withheld for the duration of the study. Dew fluid (approximately 300 μ L per 0.13 m² flat) was collected 48 hours after treatment (HAT). Imidacloprid content was identified and quantified by Liquid Chromatography-Mass Spectrometry. Results were analyzed as a completely randomized design. Means were separated based upon adjusted 95% confidence intervals (CI).

Results and Discussion

In 2014, dew collected 48 HAT from soil-only treated bermudagrass and St. Augustinegrass contained 15.8 and 9.2 ng imidacloprid mL⁻¹, respectively. These levels are less than concentrations reported to be lethal to the European honeybee (LD₅₀ 43 ng mL⁻¹)⁷. However, it has been suggested that similarly low concentrations could be associated with sub-lethal effects in honey-bees (reportedly >10 ng mL⁻¹) depending upon the amount of contaminated water transported into hives from contaminated dew or guttation⁵. Sub-irrigated treatments may not be field realistic but were intended to avoid the confounding effects of foliar residues with translocated imidacloprid. Furthermore, sub-irrigated treatment likely mimics a best case scenario where

¹ Gels, J.A., D.W. Held, and D.A. Potter. 2002. Hazards of insecticides to the bumble bees *Bombus impatiens* (Hymenoptera: Apidae) foraging on flowering white clover in turf. *J Econ Entomol*, 95:722-728.

² Larson, J.L., C.T. Redmond, D.A. Potter. 2013. Assessing insecticide hazard to bumble bees foraging on flowering weeds in treated lawns. *PLoS ONE*, 8:e66375.

³ Larson, J.L., C.T. Redmond, and D.A. Potter. 2015. Mowing mitigates bioactivity of neonicotinoid insecticides in nectar of flowering lawn weeds and turfgrass guttation. *Environmental Toxicology and Chemistry*, 34:127-132.

⁴ Shawki, M.A., D. Titěra, J. Kazda, J. Kohoutková, and V. Táborský. 2006. Toxicity to honeybees of water guttation and dew collected from winter rape treated with Nurelle D[®]. *Plant Prot Sci*, 42:9-14.

⁵ Retz, J.E., S. Zühlke, M. Spittler, and K. Wallner. 2011. Neonicotinoid insecticides translocated in guttated droplets of seed-treated maize and wheat: a threat to honeybees?. *Apidologie*, 42:596-606.

⁶ Duple, R.L. 2001. Turfgrasses: Their management and use in the southern zone. Vol. 20. Texas A&M University Press. College Station, TX, USA.

⁷ Environmental Protection Agency (EPA). 2016. Preliminary pollinator assessment to support the registration review of imidacloprid. p 103.

imidacloprid has been dislodged from leaf tissue with post-application irrigation. Results from a 2015 follow-up study will also be presented.

DEVELOPMENT OF A MULTIPLEX END-POINT PCR REACTION FOR THE DETECTION OF *OPHIOSPHAERELLA* SPP.

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Introduction

Bermudagrass is an important turf type grass that grows in warm regions throughout the world. The two most common types are *Cynodon dactylon* and interspecific hybrids of *Cynodon dactylon* x *Cynodon transvaalensis*. The fine textured, dark green interspecific hybrids are widely used on golf courses, lawns, and sport fields due to their high shoot density, aggressive growth, pest and disease tolerance¹.

Spring dead spot (SDS) is one of the few damaging diseases of Bermudagrass. It is caused by three fungal species in the ascomycete genus *Ophiosphaerella*: *O. herpotricha*, *O. korrae* and *O. narmari*. *Ophiosphaerella* is a member of Pleosporales family which includes plant pathogens and facultative saprophytes. During periods of cool temperatures, the fungi colonize the roots, stolons, and rhizomes of the grass, which can lead to necrosis and lesions prior to cold temperature induced dormancy of the plants². As the grass resumes growth in the spring, symptoms appear as dead plants in circular patches that can extend from a few centimeters to more than a meter in diameter³. Field tests have shown that *O. herpotricha* is the most virulent of the three species, followed by *O. korrae*, and *O. narmari*⁴.

Traditional morphological based identification is often unattainable for *Ophiosphaerella* that cause SDS, since they rarely produce ascospores or other diagnostic structures. To achieve species-specific diagnosis, three pairs of primers were designed to identify the fungi that cause SDS and to detect the pathogen in infected roots using a multiplex end-point PCR.

Methods

Different loci served as templates for the design of each specific primer pair, for *O. korrae* the ITS region, for *O. herpotricha* the translation elongation factor 1- α , and for *O. narmari* the RNA polymerase II second largest subunit were selected. Multiple sequence alignments, including sequences from SDS causing species and closely related fungi, were performed for each individual locus to identify polymorphisms that were exclusive for each species. Primers were designed that included such polymorphisms at the 3' end. To achieve uniform melting temperatures, three to five random nucleotide extensions (flaps) were added in the 5' end of some of the designed specific primers. Primers were tested *in silico* for the formation of secondary structures. Specificity of the primers was initially assessed by blasting them against the GenBank database.

Sensitivity and specificity of the primers were assayed *in vitro*. Specificity was tested using isolates of each SDS-causing species of *Ophiosphaerella*, closely related fungal species, and other common fungal pathogens of Bermudagrass. Pure cultures of each species, that were previously identified using PCR, were obtained from the culture collection of the turfgrass pathology laboratory at Oklahoma State University. Fungi were grown on liquid media at room temperature for 2 weeks. Mycelia were lyophilized and used for DNA extraction using a CTAB conventional method. Extracted DNA served as template for multiplex PCR reactions, and the amplicons were resolved in 2% agarose gels using SB electrophoresis buffer. Cycling conditions and PCR components were standardized to optimize the concentration and quality of the amplicons. Serial dilutions of DNA, decreasing in one order of magnitude from 10 ng/ μ L to 10⁻⁶ ng/ μ L, were prepared, and served as template for testing the sensitivity of the reaction on each species.

The standardized multiplex PCR was validated on field collected plant samples. Plants were collected in the field from specific locations where *O. herpotricha* was previously inoculated, from locations where the disease was not present previously, and from a fairway with a history of severe SDS caused by both *O. korrae* and *O.*

¹ Tredway L.P., Tomaso-Peterson M., Perry H., Walker N.R. 2009. Spring dead spot of bermudagrass: a challenge for researchers and turfgrass managers. Plant Health Progress at <http://www.plantmanagementnetwork.org>. (doi:10.1094/PHP-2009-0710-01-RV).

² Walker, N. R., Mitchell, T. K., Morton, A. N., and Marek, S. M. 2006. Influence of temperature and time of year on colonization of bermudagrass roots by *Ophiosphaerella herpotricha*. Plant Dis. 90:1326-1330.

³ Wadsworth D.F., Young H.C. 1960. Spring dead spot of bermudagrass. Plant Disease Reporter. 44:516-518.

⁴ Iriarte, F. B., Wetzell, H. C., Fry, J. D., Martin, D. L., Vincelli, P., Dixon, E. W., and Tisserat, N. A. 2005. Aggressiveness of spring dead spot pathogens to bermudagrass. Intl. Turfgrass Soc. Res. J. 10:258-264.

herpotricha. Roots were surface sterilized, the DNA was obtained as described above and used as template for the multiplex PCR assay.

Results and discussion

Three species-specific primer pairs were designed: OHERFW (5'CGTAATCTCCAAAGATGGCCAA 3') and OHERRV (5' CACGCAGTTGGTAGAAACGT 3') for *O. herpotricha*, OKORFW (5' GGACACCCATTGAACCTATTT 3') and OKORRV (5'GTTATCAGACGCAGTGGAGTG 3') for *O. korrae*, and ONARFW (5' GGACTCTTCGATAGGGATATCAG 3') and ONARRV (5' GCCACTCATCAAGATCTTCCG 3') for *O. narmari*. *In silico*, all primers hybridized only with sequences corresponding to the target species. Primers for *O. herpotricha* and *O. narmari* hybridized with all sequences corresponding to their respective species, while primers for *O. korrae* hybridized with 22 out of 30 entries available for this species in GenBank.

Final concentrations for the multiplex PCR on a 60 μ L reaction were, 1x of Green GoTaq (Promega, WI, USA), 0.08 μ M of each primer, and 0.58 ng/ μ L of template DNA. Standardized cycling conditions were 97 $^{\circ}$ C for 5 minutes followed by 30 cycles of 95 $^{\circ}$ C for 45 s, 56 $^{\circ}$ C for 45 s, 72 $^{\circ}$ C for 45 s, and a final extension of 72 $^{\circ}$ C for 5 minutes. The reaction resulted in specific amplicons for each species. Products were 272, 247, and 205 bp in size for *O. korrae*, *O. herpotricha*, and *O. narmari*, respectively (Fig. 1). It was found that those primers with the lowest probability of dimer formation had the best sensitivity, however, all primers could detect as low as 0.1 ng/ μ L of template DNA. Reactions using DNA from closely related fungi as template resulted in no amplification. During validation, multiplex PCR of total genomic DNA from Bermudagrass roots was positive for inoculated samples and several of the fairway collected plants, but negative for plants from locations without a previous history of SDS.

Results show that the multiplex end-point PCR herein developed is capable of producing a unique amplicon for each one of the three SDS-associated *Ophiosphaerella* species. There is no cross-reaction with closely related fungi or fungi commonly found associated with Bermudagrass and the test is sensitive enough to detect the pathogens directly from field samples, which makes this method a very useful tool for timely diagnosis.

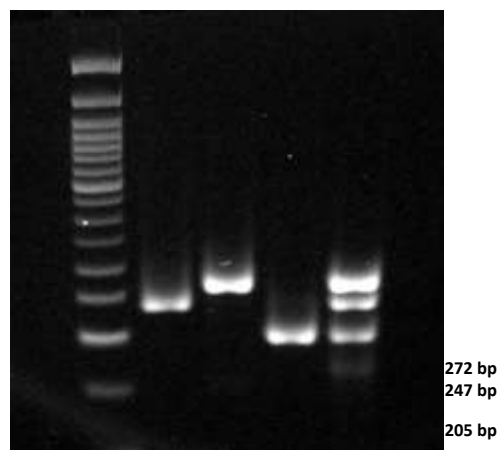


Figure 1. PCR products of the multiplex reaction for the identification of SDS-associated *Ophiosphaerella* species. DNAs used as template came from (1) *O. herpotricha*, (2) *O. korrae*, (3) *O. narmari*, and (4) *O. herpotricha*+*O. korrae*+*O. narmari*. L: 50 bp ladder. NTC: No template control.

OZONE APPLICATION FOR DOLLAR SPOT INCIDENCE REDUCTION IN A BENTGRASS GOLF GREEN

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Introduction

Dollar spot, incited by *Sclerotinia homeocarpa* F.T. Bennett, is one of the main disease problems on close-cut creeping bentgrass (*Agrostis stolonifera* L.)¹. Dollar spot can be successfully controlled by DMI fungicides with greater advantage over other classes of fungicides², but chemical control options might be reduced or banned by authorities in several regions of Europe, therefore other control alternatives should be evaluated. Cultural control of *S. homeocarpa* in bentgrass greens implies several practices like dew removal to reduce leaf wetness duration, trees and shrubs prune or removal to increase air circulation and solar radiation, thatch layer removal, avoidance of bentgrass moisture stress by maintaining soil moisture around 75% of field capacity and a correct nitrogen fertilization programme to avoid nitrogen lack³. Extensive research has been conducted into the biological control of dollar spot, Goddman and Burpee⁴ tested 24 potential antagonist (fungi and bacteria) with four promising ones inhibiting *S. homeocarpa* growth. Other researchers found promising products against dollar spot but only in areas with moderate infestations. Ozone (O₃) is the strongest commercially available disinfectant and it is also very effective at destroying bacteria, fungi and viruses⁵. Turfgrass species can be injured when exposed to an atmosphere containing ozone. Youngner and Nudge⁶, and Richards et al⁷, reported that C3 turfgrass species were more sensitive to fumigated ozone than C4 species. Brennan and Halisky⁸ found creeping bentgrass (cv Pencross) and annual bluegrass (*Poa annua* L.) more sensitive to an ozone atmosphere than other turfgrass species. However Hsieh⁹ found ozone useful in order to inhibit the conidial germination of several fungi affecting turfgrass seedling development like *Bipolaris australiensis*, *Curvularia pallescens* and *Exerohilum rostratum*. The main concern when using total disinfectants is that beneficial microorganisms are controlled as well. However, due to the short half-life of ozone (several minutes at 15-25°C), soil could be re-inoculated with beneficial microorganisms competing with *S. homeocarpa*. The objective of the present research is to test an ozone treatment followed or not by an application of a microbial concentrate that contains a mixture of four different bacteria species (*Azotobacter vinelandii* 20x10⁶ UFC.ml⁻¹, *Bacillus licheniformis* 20x10⁶ UFC.ml⁻¹, *Bacillus megaterium* 20x10⁶ UFC.ml⁻¹ and *Pseudomonas fluorescens* 20x10⁶ UFC.ml⁻¹) in a creeping bentgrass golf green.

Methods

An experiment was conducted in July-August 2015 on a bentgrass (cv L-93) golf course green in Valencia (Spain). The experimental design was a randomized complete block with one factor and four replicates with the elemental subplot area of 1 m². The tested factor (dollar spot treatment) had four levels: control (no treatment); ozone treatment at 10 mg.L⁻¹; ozone treatment at 10 mg.L⁻¹ + inoculation with a microbial concentrate (MC) at 2 mL.m⁻² in 1 L of water, at 30 minutes and 7 and 14 days after the initial ozone treatment; iprodione fungicide at 1.2 mL.m⁻² in 100 mL of water and repeated 7 days after. Ozone gas was generated on-site using an electrically powered corona discharge ozone generator and directly incorporated into the soil through 40 L.m⁻² of water with a hose equipped with a thumb 7-pattern nozzle. Applications of MC and the fungicide were made with a CO₂-pressured sprayer calibrated to deliver 325 L.ha⁻¹ with a single flat-fan nozzle (9504 EVS flat-fan; TeeJet Spraying Systems) at 206 kPa. Turfgrass quality was visually evaluated weekly after initial treatment (WAT) on a 1 to 9 scale where 1 equalled dead turf and 9 equalled dense, uniform turf.

¹ Bonos, S. A. 2006. Heritability of dollar spot resistance in creeping bentgrass. *Phytopathology* 96:808-812.

² Miller, G. L., Stevenson, K. L., and Burpee, L. L. 2002. Sensitivity of *Sclerotinia homeocarpa* isolates to propiconazole and impact on control of dollar spot. *Plant Dis.* 86:1240-1246.

³ Walsh, B., Ikeda, S.S. and Boland, G.J. 1999. Biology and Management of Dollar Spot (*Sclerotinia homeocarpa*); an Important Disease of Turfgrass. *HortScience* 34: 13-21.

⁴ Goodman, D.M. and Burpee, L.L. 1991. Biological Control of Dollar Spot Disease of Creeping Bentgrass. *Phytopathology* 81:1438-1446.

⁵ Ebihara, K., Strykewska, H.D., Mitsugi, F., Ikegami, T., Sakai, T. Joanna Pawlat, J. and Teii, S. 2012. Recent development of ozone treatment for agricultural soil sterilization and biomedical prevention. *Przegląd Elektrotechniczny*, 88: 92-94.

⁶ Youngner, V.B. and Nudge, F.J. 1980. Air Pollution Oxidant Effects on Cool-Season and Warm-Season Turfgrasses. *Agronomy Journal*. 72: 169-170.

⁷ Richards, G.A, Mulchi, C.L. and Hall, J.R. 1980. *Journal of Environmental Quality*, 9: 49-53.

⁸ Brennan, E. and Halisky, P.M. 1970. Response of Turfgrass Cultivars to Ozone and Sulfur Dioxide in the Atmosphere. *Rutgers University Journal Series Paper No.* 3953.

⁹ Hsieh, S., Ning, S. and Tzeng, D. 1998. Control of Turf Grass Seedborne Pathogenic Fungi by Ozone. *Plant Pathology Bulletin* 7:105-112.

Disease evolution (number of dollar spot infection spots and percentage of disease affected area) was also evaluated weekly. All statistical analysis were made with Statgraphics Plus 5.1.

Results and Discussion

Symptoms of ozone phytotoxicity were soon detected. 2 days after treatment, turfgrass in all ozone treated subplots bleached (Figure 1). The same symptoms were reported by Brennan and Halisky, 1970 in several bentgrass cultivars with injuries very dependant on the ozone atmosphere rate, 0,23 vs 0,30 mg.L⁻¹. Surprisingly, the above mentioned ozone effect was transitory and 1 WAT ozone treated subplots recovered, at least to the control subplots level. Ozone treated plots were better than control plots only from late July to mid August and inoculated ones after ozone treatment never performed better than control plots. Fungicide treated plots were better than the rest of plots almost in every evaluation day. Non-treated turfgrass general aesthetics constantly decayed along the experiment whilst fungicide treated plots had a constant good quality, which was statistically significant better than control and ozone + MC subplots from 22nd July to 18th August. There was not a positive effect on the MC inoculated subplots, in fact ozone treated plots without MC inoculation had better aspect than inoculated ones at least for 28th July and 4th August, which suggest bacteria colonization was not successful.

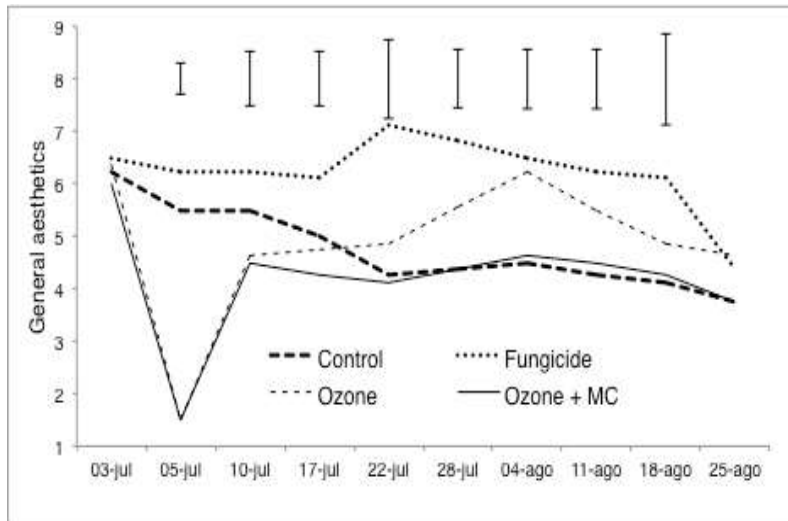


Figure 1: Creeping bentgrass general aesthetics evolution after treatments. MC: Microbial Concentrate. Vertical bars are LSD values indicating significant differences between treatments at 5% probability level.

Evolution of the number of Dollar Spot infection points is showed in Figure 2, where fungicide had an excellent effect over the disease during the first month after the initial treatment. Ozone treated subplots (inoculated or not) had the same trend, in between control and fungicide evolution. There were 3 dates (22nd and 28th July and 4th August) in which ozone treated subplots had a lower number of Dollar Spot infection points than the control subplots. There was a general increase in Dollar Spot infection points from 4th August onwards suggesting that another treatment on that date would have inhibit the disease development.

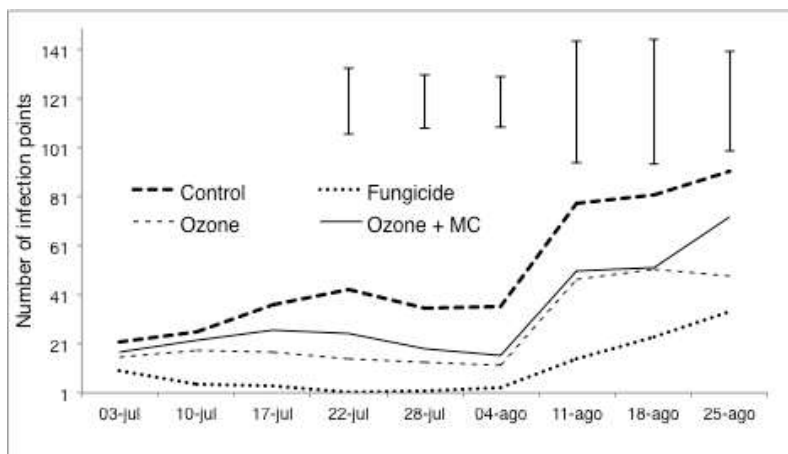


Figure 2: Evolution of Dollar Spot number of infection points in a creeping bentgrass golf green after treatments.

MC: Microbial Concentrate. Vertical bars are LSD values indicating significant differences between treatments at 5% probability level.

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Introduction

In recent years Golf popularity has been increasing all over the world. In many regions, golf and its related industry are a major component in the local economy¹. Golf contributes to the life quality of many residents and to the Algarve region visitors. Golf also generates billions of euros for the portuguese economy. Some criteria that are used to designate a good course, are speed and evenness of the playing surface and green healthy grass. Each of these quality parameters can be negatively affected by plant-parasitic nematodes. From all the pests that commonly affect golf courses turfgrass in Algarve, nematodes are probably the least understood and the most difficult to manage. Due to Algarve climate, nematode problems are common and can be very severe. The soils also provide a perfect habitat for the most destructive nematode species. Plant-parasitic nematodes are microscopic roundworms that feed exclusively on plant tissues. Damage produced by nematodes activity depends on several factors, such as nematodes species, populations levels, plant susceptibility, environmental conditions and the presence of other microorganisms mainly fungi². Most species are plant roots parasites. As plant-parasitic nematodes, they damage the root system and reduce the ability of the plant to obtain water and nutrients from the soil. Nematodes also cause a reduction in the fine feeder-roots that are important in water and nutrient uptake by the plant. They survive in soil and plant tissues and several different species may coexist in turf. They have a wide host range and their environmental requirements are diverse as well the symptoms they cause. Some plant-parasitic nematodes are related to the health of turfgrass. Fleming et al.³ referred increasing levels of nematode damage at United Kingdom and Irish sports turf industries. So populations of nematodes must be identified in the diagnosis of turfgrass diseases. The main goal of this study was to identify the plant-parasitic nematodes that occurs in the golf courses located in the Algarve region and evaluate their populations levels.

Methods

Samples were collected from the golf greens healthy-appearing but suspected to have a possible pathogenic nematode invasion. The sampling method was based in the cores collection in a zig-zag pattern across the area. Turf samples were obtained by cutting cores with a soil sampler (2 cm diameter and 10 cm depth). The samples were constituted by multiple cores and were placed into a plastic bag, sealed and labeled. Samples were transported under protection of direct sunlight and heat due to the high nematode sensitivity to high temperatures and UV radiation. The method used for the nematode extraction was the sieving and sugar centrifugal⁴. Plant-parasitic nematodes were identified to genus based on their morphological characteristics. Damage threshold (number/100 cm³ soil) used to determine if nematode numbers reach the levels that could be harmful to the turfgrass depends on site, time of the year, biotic and abiotic stresses. In the present work damage thresholds used were the ones referred by Crow² and Fleming et al.³. If needed and according to the obtained results, golf course managers get advises for plant parasitic control measures.

Results and discussion

The presence of plant-parasitic nematodes was analysed in 132 samples in which 18 genera were recorded. The plant-parasitic nematodes genus identified from the golf greens in Algarve were: *Meloidogyne*; *Tylenchorhynchus*; *Helicothylenchus*; *Heterodera*; *Paratrichodorus*; *Criconemoides*; *Pratylenchus*; *Tylenchulus*; *Aphelenchoides*; *Hemicyclophora*; *Hemicriconemoides*; *Trichodorus*; *Aphelenchus*; *Subanguina*; *Xiphinema*; *Globodera*; *Belonolaimus* and *Longidorus*. The obtained results were shown in table 1 where the identified genus are described. Their occurrence and their occurrence rate were indicated in the same table.

¹ Crow, W.T. 2005. Plant-parasitic nematodes on golf course turf. *Outlooks on Pest Management*. 16: 10-15.

² Crow W.T. 2005. How bad are nematode problems on Florida's golf courses. *Florida Turf Digest*. University of Florida, USA. 22: 10-12.

³ Fleming, C. C.; Craig, D. and MacDowell, M. H. 2008. Plant-parasitic nematodes: a new turf war. *Biologist*. 55 (2): 76 – 82.

⁴ Jenkins, W.R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Reporter*, 48: 692

Zeng et al. (2012)⁵ reported in North and South Carolina (USA) golf courses turfgrass, populations of *Helicotylenchus dihystra*, *Mesocriconema xenoplax*, *Hoplolaimus galeatus*, *Tylenchorhynchus claytoni*, *Belonolaimus longicaudatus*, *Meloidogyne graminis* and *Paratrichodorus minor* as the most prevalent and abundant species. Vau (2011)⁶ reported that *Helicotylenchus*, *Meloidogyne* and *Tylenchorhynchus* were the most common genus in studies conducted in golf courses from de Algarve region.

Four identified genus (*Meloidogyne*, *Helicotylenchus*, *Paratrichodorus* and *Tylenchorhynchus*), in the present study, were common to the isolates described by Zeng et al.⁵ and Vau⁶. *Meloidogyne* and *Helicotylenchus* were the most isolated genus in the studied samples. The obtained results suggest that further studies should be done to contribute for the identification of the plant-parasitic nematodes.

Table 1. Occurrence rate of plant-parasitic nematodes in 132 turf samples from greens of golf courses in Algarve region.

Genus	Commun name	Type	Occurrence (number of samples)	Occurrence Rate (%)
Meloidogyne	Root knot nematode	Endoparasite	124	94
Helicotylenchus	Spiral nematode	Ecto-endoparasita	121	92
Paratrichodorus	Stubby root nematode	Ectoparasite	82	62
Pratylenchus	Lesion nematode	Endoparasite	69	52,3
Tylenchulus	Citrus nematode	Semi-entoparasite	65	49
Criconemoides (Criconemella)	Ring nematode	Ectoparasite	46	35
Tylenchorhynchus	Stunt nematode	Ectoparasite	8	6
Hemicycliophora	Sheath nematode	Ectoparasite	7	5,3
Hemicriconemoides	Sheathoid nematode	Ectoparasite	6	4,5
Aphelenchus	Corn root worm	Endoparasite	5	3,4
Trichodorus	Stubby root nematode	Ectoparasite	4	3
Subanguina	Root gall nematode	Endoparasite	4	3
Aphelenchoides	Wood borer nematode	Endoparasite	3	2,3
Belonolaimus	Sting nematode	Ectoparasite	3	2,3
Heterodera	Cyst nematode	Endoparasite	3	2,3
Globodera	Cyst nematode	Endoparasite	2	1,5
Xiphinema	Dagger nematode	Ectoparasite	2	1,5
Longidorus	Needle nematode	Ectoparsite	1	0,8

⁵ Zeng Y.; Ye W.; Martin, S. B.; Martin, M. and Tredway, L. 2012. Diversity and Occurrence of Plant-parasitic Nematodes Associated with Golf Course Turfgrasses in North and South Carolina, USA. J. Nematol. 44 (4): 337-347.

⁶ Vau, S. J. S. O. 2011. Estudo comparativo de géneros de nematodos fitoparasitas nos "greens" dos campos de golfe da região algarvia. Dissertação de Mestrado em Gestão e Manutenção de campos de golfe. 151 pp.

TRICHODERMA GAMSII AS A BIOLOGICAL CONTROL AGENT OF TURFGRASS DISEASES

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Introduction

Golf is a growing and economically important activity in Portugal with direct and indirect incomes respectively over 350 and 1.450 million of euros, corresponding nearly 1.25% of the Gross National Product. The correct maintenance of lawns in a sustainable manner is required. Sport lawns are quite susceptible to soil-borne diseases¹, which cause enormous economic losses, making environmental friendly disease control methods a practice to develop. The increasing interest regarding environment and public health protection have been increased the search for alternatives, leading researchers to seek strategies to reduce the use of synthetic products, such as fertilizers and pesticides². Also, the European legislation is directing the reduction of pesticides³, facing itself towards more sustainable policies.

The use of synthetic fungicides to control plant pathogens in the soil has been generating problems, such as fungi resistance, ecosystem imbalance by toxic effects of residues and human and animal health hazards⁴.

Biocontrol, through the use of microorganisms, offers an alternative and attractive approach for disease control, without the negative impact of chemicals^{1,3}, and it has proved to be economically sustainable and environmentally compatible. The biocontrol agents are easy to deliver, may activate plant resistance mechanisms, like systemic or induced resistance, and thereby indirectly improve plant growth. *Trichoderma* spp. are widely used and several strains inhibit a wide range of plant pathogens⁵. *Trichoderma* spp. has different mechanisms of action against plant pathogens, including competition for nutrients, mycoparasitism and antibiosis by hydrolytic enzymes and metabolites and substances that promote plant growth.

Trichoderma spp. is common in the soil⁶; isn't pathogenic to plants and may be beneficial for agriculture, protecting the plants from certain diseases³.

Dollar spot (*Sclerotinia homeocarpa*) is one of the most important diseases that affect turfgrass, which can cause considerable damage, particularly to highly maintained golf course putting greens, closely mown fairways and bowling greens. *Sclerotinia homeocarpa* cause diseases in lawns at temperatures between 15 and 27 °C⁷, with an optimum development temperature of 26 °C⁷. In general *Sclerotinia homeocarpa* is active from autumn until spring, when days are hot and humid and the nights with dew⁸.

The typical dollar spot symptoms on a closely mowed turf are small, circular, sunken, straw-colored patches of 25 to 50 mm in diameter. With severe attacks, the individual spots may coalesce to form larger, irregular-shaped patches. Lesions may be seen on infected leaves. They initially appear as small chlorotic spots, but typically the lesion has a reddish-brown to tan margin and will enlarge across the full **width of the leaf blade**⁹.

In the literature there are few studies in which the application of biological control agents are used in curative treatments, with the disease already installed on lawns.

In this work it was proposed to test the effect of *Trichoderma gamsii* as a curative biological control agent against *Sclerotinia homeocarpa* in turfgrass with different degrees of infection.

¹ Barker, A.V. 2001. Compost utilization in sod production and turf management. In: Stoffella, P.J. and B.A. Kahn. (eds.): Compost utilization in horticultural cropping systems. Lewis Publications, Boca Raton, 201-225.

² Bonanomi, G., V. Antignani, M. Capodilupo and F. Scala. 2010. Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. *Soil Biology & Biochemistry* 42, 136-144.

³ Trillas, M.I., E. Casanova, L. Cotxarrera, J. Ordovás, C. Borrero, and M. Avilés 2006. Composts from agricultural waste and the *Trichoderma asperellum* strain T-34 suppress *Rhizoctonia solani* in cucumber seedlings. *Biological Control* 39, 32-38.

⁴ Johnson, D.A. and Z.K. Atallah 2006. Timing fungicide applications for managing *Sclerotinia* stem rot of potato. *Plant Disease* 90, 755-758.

⁵ Mendez-Vilas, A. 2010. A review on contributions presented at the BioMicroWorld2009 conference. *American Journal of Agricultural and Biological Sciences* 5, 486-487.

⁶ Harman G.E., C.R. Howell, A. Viterbo, I. Chet and M. Lorito 2004. *Trichoderma* species-opportunistic, a virulent plant symbionts. *Nature Reviews Microbiology* 2, 43-56.

⁷ Endo, R.M. 1963. Influence of temperature on rate of growth of five fungal pathogens of turfgrass and on rate of disease spread. *Phytopathology* 53, 857-861.

⁸ Smiley, R. W., P.H. Dernoeden, and B.B. Clarke 1992. *Compendium of turfgrass diseases*. 2nd edition. Amer. Phytopath. Soc., St. Paul, Minn.

⁹ Beard, J.B., 2002. *Turf management for golf courses*. Ann Arbor Press. Chelsea, Michigan, second edition.

Methods

The experiment was carried out in the *Campus* of Gambelas at the University of Algarve, Portugal. Pots, with an area of 56.7 cm², were filled with 766 cm³ of fine sterile sand typically used on golf courses. *Agrostis stolonifera* cv. *palustris* T1 was sown at a density of 5 g m⁻². The pots with turfgrass were left outdoors, under similar climatic conditions to those of a golf course. So, ideal conditions for the development of the typical diseases of turfgrass for the season were reproduced. Visual control was carried out to observe disease symptoms appearance. After 67 days, the pots were divided into five treatments designated as follows: M1 (fungicide treatment, very low plant infection), M2 (control, very low infection), M3 (sprayed with a known concentration of *T. gamsii*, very low infection), M4 (sprayed with a known concentration of *T. gamsii*, low infection) and M5 (sprayed with a known concentration of *T. gamsii*, heavy infected). *T. gamsii* to be sprayed was obtained from a suspension of water and spores with 2.5 x 10⁸ spores mL⁻¹. The fungicide applied was Rovral (BASF, Portugal) being the active substance the iprodione and a concentration of 1.5 g L⁻¹. On a daily basis, the number of spots caused by the disease was counted, measured the area of these spots and estimated the caused damage. A classification from 0 to 5 was given: 0 corresponding to healthy plants; 1 for an affected area of 1 to 24 %; 2 for an affected area of 25 to 49 %; 3 for an affected area of 50 to 74 %; 4 for an affected area of 75 to 99 % and 5 for an affected area of 100 %.

Microbiological analyzes were performed at the beginning and at the end of the test to confirm the presence of *Sclerotinia homeocarpa* and *T. gamsii*.

The fungi growth was carried out on PDA medium with incubation for 7 days at 25 ± 2 °C. The identification was based on cultural and microscopic characteristics of the fungi structures. Air temperature, relative humidity and rainfall were recorded every day.

The number of disease spots, the affected area and the damage level values were submitted to a variance analysis (ANOVA); differences were considered significant when p < 0.05. Normality of sample distribution and homogeneity of variances were verified before ANOVA (Zar, 1999). The comparative analysis of the treatment averages was realized through the New Multiple-Range Test. For the statistical analysis it was used the SPSS ver. 19.0 (SPSS Incorporation, 1989-2010, U.S.A.) and Microsoft Excel (Office 2013).

Results and Discussion

The experiment started when the presence of *S. homeocarpa* was detected in all treatments. Initially, the highest number of spots was observed in M1 and M4 treatments. M1 had a significant higher number of spots at day 4 (p < 0.05). In all treatments the number of spots decreased after the inoculation of *T. gamsii*, including treatments with higher degree of disease (M4 and M5). The infected average area was statistically different between treatments at day 4 (p < 0.05), day 5 (p < 0.05), day 7 (p < 0.01) and day 12 (p < 0.01); M5 treatment had, in all sampling days, the significant highest infected area. At the end of the experiment *S. homeocarpa* was observed in all treatments, but with less severity. *T. gamsii* was recovered in M3, M4 and M5 treatments, where it had been previously inoculated.

The affected area developed according to the degree of infection caused by *S. homeocarpa*. It was observed that only the affected area of M3 treatment started to decrease from day 1 to day 4, showing a positive effect of *T. gamsii* treatment. In M4 there was a slight increase on day 4, then starting to decline. In M5, the affected area increased significantly during the first days, decreasing only on day 12.

During the experiment it was observed that *T. gamsii* prevented the spread of the disease on M3 treatment. In M4 disease increased but didn't reach 50% of the pot area. In M1, the fungicide did not prevent the development of disease, exceeding 75% of damage. In this case only on day 12 the damaged area decreased. A similar situation was found in M5. The positive effect of the antagonist *T. gamsii* was verified against *S. homeocarpa* decreasing the disease symptoms.

The results indicate that the application of *T. gamsii* was effective as a biological control agent of *S. homeocarpa* when the level of infection is not the most severe one. Although this observation it can be an advantageous alternative to the use of chemical fungicides.

MICROBIOLOGICAL PRODUCTS FOR CONTROL OF MICRODOCHIUM NIVALE ON GOLF GREENS

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Introduction

The most important turfgrass pathogen in Scandinavia is *Microdochium nivale*. Many golf courses spray their greens routinely with fungicides before winter, but EU directive 2009/128 calls for a reduction in pesticide use through the introduction of Integrated Pest Management (IPM) and replacement of pesticides with low risk alternatives. Biological control of microdochium patch and other diseases may be possible by the application of microbial inoculants¹, but registration of such products in EU requires the same efficacy tests as ordinary fungicides. Thus, the objective of this research, coordinated by STERF and funded by STERF's industrial partner Interagro BIOS AB through a grant from the Danish Environmental Protection Agency, was to provide documentation according to 'Good Experimental Practice' standard for potential registration of Turf G+/WPG, a fungal product containing *Gliocladium catenulatum*, and Turf S+/WPS, a bacterial product containing *Streptomyces* spp., for use on Scandinavian golf courses.

The efficacy of Turf G+ (new formulation Turf WPG released in 2012) and Turf S+ (new formulation Turf WPS released in 2014) was compared with fungicides both *in vitro* and in field trials. In the *in vitro* trials, Turf G+/WPG, Turf S+/WPS and their mixture gave almost the same inhibition of *M. nivale* as the fungicides fludioxonil (Medallion) and prothioconazole + trifloxystrobin (Delaro) when all products were applied at the full (recommended) dose in PDA agar in petri dishes incubated at 16 °C. In contrast, Turf S+ was less efficient than the fungicides when applied at 1/10 or 1/100 of the recommended dose, and Turf G+/WPG was counterproductive and increased the radial growth of *M. nivale* when the Petri dishes were incubated at 6 °C.²

Methods

Field trials were established in October 2011 at Rungsted GC, Eastern Denmark (56 °N, 12°E), Kävlinge GC, Southern Sweden (56 °N, 12°E) and NIBIO Landvik Turfgrass Research Center, Southern Norway (58 °N, 9°E). The trials were laid out on putting greens in ordinary play at Rungsted GC and Kävlinge GC and on an experimental green exposed to artificial wear from a pedestrian-type wear machine at NIBIO Landvik. In order to establish and maintain the desirable flora of antagonistic microorganisms, the treatments and registrations at Kävlinge and Landvik were repeated over three experimental years until 1 Oct. 2014, but the trial at Rungsted GC had to be discontinued and replaced with a new trial on a putting green at Sydsjælland GC (55 °N, 12°E) after the first year.

The trials were established according to a randomized complete block design with three or four replicates and five treatments: (1) Unsprayed control, (2) Fungicide control (two applications in October-November; products varied between countries depending on registration status), (3) Turf G+/WPG (two applications in October-November and two applications when the air temperature exceeded 5 and 10 °C in spring), (4) Turf S+/WPS (monthly applications from May to September), and (5) Turf G+/WPG at low temperatures in the late fall and early spring combined with Turf S+/WPS from May to September (i.e. treatment 3+4). Weather conditions varied among sites and years from virtually no snow cover at the Danish and Swedish sites in 2011-12 and 2013-14 to four months of snow and three months of ice encasement resulting in complete winter kill and thus the need for reseeded at Landvik in 2012-13. Per cent of plot area affected by *M. nivale* was assessed from October to April in all trials, but the total number of observations varied depending on snow cover and other factors.

Results and Discussion

Results showed a strong variation in disease between trials and years that could mostly be explained by the predominant grass species³ (Table 1). Two fungicide applications before winter gave excellent control of *M. nivale* except at Kävlinge GC in 2011-2012. Both microbial products and their combination significantly reduced

¹ Nelson, E.B. 1997. Microbial inoculants for the control of turfgrass diseases. International Turfgrass Society Research Journal 8: 971-811.

² Espevig, T., A. Tronsmo & T.S. Aamlid 2014. Evaluation of microbial agents for control of *Microdochium nivale* *in vitro*. European Journal of Turfgrass Science 45(2): 49-50.

³ Aamlid, T.S., G. Thorvaldsson, F. Enger & T. Pettersen. 2012. Turfgrass species and varieties for Integrated Pest Management of Scandinavian putting greens. Acta Agriculturae Scandinavica Section B Soil & Plant Science 62 (Supplement 1): 10-23.

M. nivale on the *Festuca rubra* dominated green at Sydsjælland GK in 2012-13, and Turf G+/WPG significantly reduced *M. nivale* in *Agrostis capillaris* at Landvik in 2013-14, but in both cases the control levels were significantly less than for the fungicides. No effect of the microbial products could be observed on the *Poa annua* dominated greens at Rungsted GC and Kävlinge GC.

In summary, our results suggest that the efficacy and consistency of Turf G+/WPG and Turf S+/WPS is too low to replace fungicide applications on Scandinavian golf greens. According to the *in vitro* trials², one reason for this may be that *G. catenulatum* requires higher temperature for control of *M.nivale* than what is usually encountered in Scandinavia during the late fall and winter. Despite the fact that new batches of inoculum were delivered for each experimental year, determination of the number of cell forming units (CFU) in two of the batches also suggested that inoculum was less concentrated than specified by the manufacturer. Microbial control of pathogens is always a numbers game⁴, and laboratory experiments have shown declining levels of control if the CFU of the antagonistic microorganisms drops below 10⁶ per gram soil.¹

Table 1. Effect of fungicide and microbial inoculants on percent of plot area showing symptoms of *M. nivale*. Mean of observations from October to April in four trials. Grass species have been indicated.

	Rungsted GC, Denmark	Sydsjælland GC, Denmark		Kävlinge GC, Sweden			NIBIO Landvik, Norway		
	10 % <i>F. rubra</i> 45 % <i>A. capillaris</i> 45 % <i>P. annua</i>	60 % <i>F. rubra</i> 29 % <i>A. capillaris</i> 11 % <i>P. annua</i>		28 % <i>A. stolonifera</i> 72 % <i>Poa annua</i>			2011-2013: 100 % <i>A. stolonifera</i> 2013-2014 † 100% <i>A. capillaris</i>		
	2011- 2012	2012- 2013	2013- 2014	2011- 2012	2012- 2013	2013- 2014	2011- 2012	2012- 2013	2013- 2014
1. Untreated control	27.8 a ¹	1.5 a	1.4 a	22.6 a	31.6 a	21.0 a	0.8 a	0.1 a	8.2 a
2. Fungicide control	1.5 b	0.0 c	0.0 c	11.2 a	1.1 b	1.1 b	0.0 a	0.0 a	0.5 c
3. Turf G+ / WPG	26.8 a	0.8 b	0.8 b	20.8 a	35.4 a	16.3 a	1.0 a	0.1 a	5.2 b
4. Turf S+ / WPS	28.1 a	0.5bc	0.8 b	16.3 a	38.1 a	18.8 a	0.4 a	0.1 a	9.5 a
5. Turf G+/WPG + Turf S+/WPS	28.7 a	0.8 b	0.8 b	18.0 a	33.0 a	18.3 a	0.3 a	0.2 a	9.0 a
<i>P</i> -value	<0.001	<0.001	<0.001	0.08	<0.001	<0.001	>0.10	>0.10	<0.001

¹Within each column, means followed by the same letter are not significantly different according to LSD_{0.05}.

⁴ Horvath, B. & Vargas, J. jr. 2000. Biological control: It's a numbers game. Golf Course Management 68(6): 55-58.

IRRIGATION AND SOIL SURFACTANTS AFFECT ABAMECTIN DISTRIBUTION IN SOIL

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Introduction

Nematodes are microscopic, soil-dwelling organisms that are among the most abundant global metazoan and adversely affect many turfgrass systems, including golf course putting greens¹. Nonchemical control practices on golf course putting greens are limited due to inconsistent efficacy. Abamectin ($\geq 80\%$ avermectin B_{1a} and $\leq 20\%$ avermectin B_{1b}), a fermentation byproduct from the bacterium *Streptomyces avermitilis*, offers nematode control in varying agricultural commodities including golf course putting greens; however, its physicochemical properties suggest it does not readily distribute in soil and may be sorbed in the thatch layer of established turfgrass systems. Specifically, it has low water solubility ($K_s = 7.8 \mu\text{g L}^{-1}$), high soil-organic carbon partition coefficient ($K_{oc} = 4,000 \text{ mL g}^{-1}$), and short to long soil persistence ($T_{1/2} = 10$ to 134 d)^{2,3}. Currently, recommendations to promote downward abamectin movement on golf course putting greens include sprayer setup (nozzle selection and carrier volume) and post-application irrigation inputs (0.25 cm H₂O 1 h following treatment). From anecdotal field observations, soil surfactant application in tandem with abamectin treatment is recommended; however, research to date has not confirmed this influences abamectin soil distribution. The objectives of this research were to evaluate the effect of irrigation and soil surfactant inclusion on abamectin distribution in soil.

Methods

Field research was initiated 15 Jul. 2013 and 19 Aug. 2014 on golf course putting greens in Wilmington, NC and Pinehurst, NC, respectively, to evaluate the effect of irrigation and soil surfactant inputs on abamectin soil distribution. Experiments were conducted in both runs on 'A1/A4' creeping bentgrass (*Agrostis stolonifera* L.) and 'Champion' ultradwarf bermudagrass (*Cynodon dactylon* L. x *C. transvaalensis* Burt-Davy) putting greens. Evaluated treatment regimens all included four broadcast abamectin spray applications ($37 \text{ g a.i. ha}^{-1}$) every 14 d and evaluated abamectin alone, abamectin alone followed by (fb) irrigation, irrigation fb abamectin alone fb irrigation, and abamectin tank-mixed with soil surfactant fb irrigation. Abamectin was applied as a broadcast spray with a CO₂-propelled, three-nozzle boom calibrated to deliver 814 L ha^{-1} at 179 kPa. Revolution[®], a soil surfactant, was applied at 2 L ha^{-1} . Soil cores were collected and divided into above-ground foliage, 0 to 2.5 cm, 2.5 to 5 cm, and 5 to 10 cm depth increments. Samples were collected 28 and 56 d after last treatment (DALT) in run one; however, due to limited abamectin detection, collection dates were adjusted to 7 and 28 DALT in run two. Abamectin residues were quantified by high performance liquid chromatography-mass spectrometry methodology, with 0.01 and 0.001 mg kg⁻¹ limit of detection for avermectin B_{1a} and B_{1b}, respectively.

Laboratory research was initiated 21 Aug. 2015 in Raleigh, NC to further evaluate the effect of irrigation and soil surfactant inclusion on ¹⁴C-abamectin soil distribution. ¹⁴C-abamectin was applied ($70 \text{ g a.i. ha}^{-1}$; $1.17 \mu\text{Ci column}^{-1}$) to the soil surface of polyethylene terephthalate columns (17.8 cm length by 5.7 cm i.d.) comprised of United States Golf Association specification putting green rootzone mix (90:10 sand:peat by vol). Evaluated treatments were comprised of various combinations of simulated irrigation amounts (0.3 + 0.3 or 0.6 cm H₂O), irrigation timing with respect to abamectin application (24 h prior to, 0 h after, or 24 h after), soil surfactant (Dispatch[®], Qualibra[®], or none), and soil surfactant application timing with respect to abamectin application (applied alone 24 h before or as tank-mixture). Dispatch and Qualibra were applied at 1.6 and 20 L ha⁻¹, respectively, per label recommendations. Destructive sampling occurred 3 and 7 DAT, at which time 0 to 2.5 cm, 2.5 to 5 cm, 5 to 7.5 cm, 7.5 to 10 cm, 10 to 12.5 cm, and 12.5 to 15 cm depths were collected. Following collection, soil was homogenized and frozen until analysis. ¹⁴C-abamectin soil analysis was conducted by combusting 2 g soil depth⁻¹ for 4 min at 900 °C. ¹⁴C was quenched and collected in scintillation cocktail (20 mL) and activity was determined by liquid scintillation counting.

Field research experimental design was a split-plot randomized complete block design with four replications. Whole plot factor was turfgrass species and subplots were broadcast spray-irrigation treatment combinations.

¹ Kiontke, K., and D.H.A. Fitch. 2013. Nematodes. *Current Biology*. 23:862-864.

² USEPA. 1993. Environmental Fate and Ground Water Branch Review of Abamectin. USEPA Rep. D187271. US Gov. Print Office, Washington, DC. 8 p.

³ Wislocki, P.G., L.S. Gross, and R.A. Dybas. 1989. Environmental aspects of abamectin use in crop protection. In: W.C. Campbell, editor, Ivermectin and abamectin. Springer-Verlag, New York, NY. p. 182-200.

Laboratory research experimental design was a completely randomized design, with three replicates. Data were subjected to ANOVA ($P = 0.05$) using general linear models (Statistical Analysis Software, Version 9.2, SAS Institute, Cary, NC) and means were separated according to Fisher's Protected LSD ($P < 0.05$)

Results and Discussion

Analysis of variance did not detect significant treatment or turfgrass species main effects or interactions from field research in 2013 or 2014; therefore, data were pooled over turfgrass species and treatments. At 28 DALT in 2013, B_{1a} was not detected in the foliage and $> 99\%$ of the recovered abamectin was in the 0 to 2.5 cm depth. Furthermore, 0.1% of the applied was recovered in the 2.5 to 5.0 cm depth. At 28 DALT, avermectin B_{1b} was not detected while neither B_{1a} or B_{1b} were detected at 56 DALT. At 7 DALT in 2014, distribution of avermectin B_{1a} and B_{1b} followed similar trends. With the exception of abamectin alone, greater avermectin B_{1a} and B_{1b} was recovered in 0 to 2.5 cm depth than foliage and deeper depths. With abamectin fb irrigation, abamectin tank-mixed with Revolution fb irrigation, irrigation fb abamectin fb irrigation, 2.6, 1.9, and 2.2% of the applied were recovered in foliage, respectively, while 29.3, 35.1, and 32.4% were recovered in the 0 to 2.5 cm depth, respectively. With abamectin alone, similar amounts were recovered in foliage and the 0 to 2.5 cm depth (7.8 and 4.5%, respectively). Regardless of application regimen, $\leq 2\%$ of the applied abamectin was recovered deeper than 2.5 cm. Within treatments, greater abamectin was recovered in foliage when applied alone (7.8%) compared to abamectin fb irrigation, irrigation fb abamectin fb irrigation, and abamectin tank-mix with Revolution fb irrigation (2.6, 2.2 and 1.9%, respectively).

Analysis of variance revealed a significant soil surfactant and irrigation treatment by depth interaction and data are presented accordingly as percent of recovered at 3 and 7 DAT. Regardless of soil surfactant or irrigation treatment regimen, highest abamectin was present in the 0 to 2.5 cm depth (77 to 100%) with less present in the 2.5 to 5.0 cm depth (1 to 18% of recovered) and $< 4\%$ of the recovered was deeper than 5 cm at 3 DAT. Radioactivity was not detected deeper than 10 cm. Qualibra applied and irrigated 24 h before abamectin application (8.2%) enhanced distribution into 2.5 to 5.0 cm depth compared to Dispatch (0.5%) and regimens that did not include a soil surfactant (0.9 to 1.7%). Within irrigation treatment regimens, Qualibra enhanced abamectin distribution into the 2.5 to 5.0 cm and 5.0 to 7.5 cm depths greater than Dispatch, which was similar to abamectin alone. Similarly, at 7 DAT, Qualibra increased abamectin distribution in the soil profile compared to Dispatch and regimens that did not include a soil surfactant. Regardless of soil surfactant or irrigation treatment regimen, greater abamectin was present in the 0 to 2.5 cm depth (64 to 96%) compared to the 2.5 to 5.0 cm depth (4 to 29%), which was greater than 5.0 to 7.5 cm and deeper depths at 7 DAT (Table 5). Qualibra applied and irrigated 24 h before abamectin application enhanced distribution into 2.5 to 5.0 cm depth 6 to 9% compared to Dispatch and regimens that did not include a soil surfactant. The most profound increase in abamectin distribution was observed with Qualibra irrigated immediately or 24 h after abamectin application. Irrigated (0.6 cm) immediately after application, Qualibra increased abamectin distribution in the 2.5 to 5.0 cm (19 to 24%) and 5.0 to 7.5 cm (7%) depths compared to Dispatch and abamectin alone. Similarly, irrigated 24 h after application (0.6 cm), Qualibra increased abamectin distribution in the 2.5 to 5.0 cm (16 to 21%) and 5.0 to 7.5 cm (2 to 3%) depths compared to Dispatch and abamectin alone, respectively. The authors note delaying irrigation is not advised and not supported by the product label.

Abamectin is an effective nematicide although distribution into the profile is an inherent challenge in established turfgrass systems that may limit efficacy. This research highlights practices that may be utilized to enhance distribution into the profile, thereby increasing bioavailability and efficacy. Future research should evaluate additional soil surfactants, application timings and programs, as well as other cultural practices that may aid distribution in established turfgrass systems.

ENTOMOPATHOGENIC FUNGI FOR BIOLOGICAL CONTROL OF TURFGRASS DISEASES

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Introduction

The use of pesticides is the most common way to control harmful organisms of turfgrass. The growing concern with the negative effects of pesticides on the environment and on public health, leads to the development and the implementation of environmentally friendly alternatives. Biological control of turfgrass diseases with fungal antagonists is an issue of growing interest. For turfgrass ecosystems the most important are phytopathogenic fungi that causes diseases to plants and entomopathogenic fungi that causes diseases to insect pests of turfgrasses. Although entomopathogenic fungi are known for their effects on insects, in ecosystems they can interact with other organisms and exhibit also harmful effects against them. These additional effects of entomopathogenic fungi, when directed against phytopathogenic fungi, can be an important issue leading to control simultaneously insect's pests and plant diseases. *Beauveria bassiana* (Balsamo) Vuillemin is an entomopathogenic fungus with a long action spectrum against insect pests and commercialized as a biopesticide. *B. bassiana* is one of the most widely studied fungus, for its effect on insects and for its endophytic capacity in several plants species. More recently, few studies have investigated the potential of *B. bassiana* as plant disease antagonist^{1,2}. Fungi of the genus *Rhizoctonia* are foliar pathogens of turfgrass. They cause "brown patch", in the case of *R. solani* (Kuhn); "yellow patch" in the case of *R. cerealis* (Hoeven). Both species can also cause damping off, root rot and seed rot. *Sclerotinia homeocarpa* (Bennett) is also a phytopathogenic fungus which infects foliar tissues and causes dollar spot³. The present work aimed to evaluate the antagonistic effect of this entomopathogenic fungus on turfgrass fungal pathogens.

Methods

Fungi were grown on Potato Dextrose Agar (PDA; Oxoid) and on Soil Extract Agar (SEA). SEA culture media was prepared as described by Fragoeiro and Magan⁴, using a soil from Faro (Algarve). Soil has a sandy texture and was collected from an area free of pesticides treatments. Antagonistic activity was determined against *R. solani*, *R. cerealis* and *S. homeocarpa* – all were isolated from golf courses in the Algarve region (south of Portugal). The experimental protocol used in this study was direct opposition, where *B. bassiana* plugs (6.5 mm in diameter) were taken from actively growing colonies on PDA and SEA media and placed on Petri dishes (9 cm diameter) with respectively PDA and SEA. One day after, plugs of actively growing colony of the plant pathogen were placed at a distance of 3 cm from the antagonist *B. bassiana*. Pathogenic fungi and *B. bassiana* were also grown alone. Each treatment was done in triplicate and incubated at 25° ± 1°C. For each phytopathogenic fungi, the fungus growth radius in the presence of *B. bassiana* (R2) were compared with the radius of the fungus grown alone along the timeline of the experiment (R1). Percentage of inhibition was calculated using the following formula:

$$PI = \frac{R1 - R2}{R1} \times 100$$

PI = percentage of inhibition; R1 = radius of pathogenic fungus grown alone;
R2 = radius of pathogenic fungus grown in the presence of *B. bassiana*

Assays to evaluate *B. bassiana* endophytic ability were conducted in *Agrostis stolonifera* var. *palustris* in pots under field conditions.

Data were analysed through a factorial analysis of variance (two-way ANOVA). To homogenise variances, data were transformed using $\sqrt{x + 0.5}$.

¹ Ownley B.H., M.R. Griffin, W.E. Klingeman, K.D. Gwinn, J.K. Moulton and R.M. Pereira 2008. *Beauveria bassiana*: endophytic colonization and plant disease control. *Journal of Invertebrate Pathology*, 3:267-270.

² Jaber L.R. 2015. Grapevine leaf tissue colonization by the fungal entomopathogen *Beauveria bassiana* s.l. and its effect against downy mildew. *BioControl* 60:103-112.

³ Beard, J.B. 2002. Turf management for golf courses. 2nd edition. Ann Arbor Press. Chelsea, Michigan.

⁴ Fragoeiro, S. and N. Magan 2005. Enzymatic activity, osmotic stress and degradation of pesticide mixtures in soil extract liquid broth inoculated with *Phanerochaete chrysosporium* and *Trametes versicolor*. *Environmental Microbiology*, 7 (3): 348-355.

Results and discussion

Results in Figure 1 indicate that *B. bassiana* had antagonistic activity against the tested pathogens. In PDA, the percentage of growth inhibition (PI) of *S. homeocarpa* and *R. solani* by *B. bassiana* was 58.5% and 35.2%, respectively. *R. cerealis* was the least affected. Several authors described the production several metabolites by *B. bassiana*⁵, with antagonistic activity⁶. Our results showed the possible presence of inhibitory metabolites that could diffuse in agar and had as a consequence the growth inhibition of *S. homeocarpa* in contact with *B. bassiana*. *R. solani*, *R. cerealis* and *S. homeocarpa* as soil pathogenic fungi, were more competitive in SEA than *B. bassiana*. In SEA probably *B. bassiana* couldn't produce enough metabolites to inhibit the growth of the tested fungi. The existence of an antagonistic effect opens a possibility to use *B. bassiana* as biological control agent of turfgrass pathogens. It was demonstrated that *B. bassiana* could behave as an endophytic organism⁷, colonizing plant tissues and preventing the growth of pathogens. Our results from the field assays suggested that *B. bassiana* could colonize turfgrass foliar tissues. As an endophytic, this entomopathogenic fungus may be useful to limit the severity of sportive turfgrass diseases. Plant microbiota may affect plant resistance and microorganisms with antagonistic activity could play a role in protecting plants against pests^{5,8}.

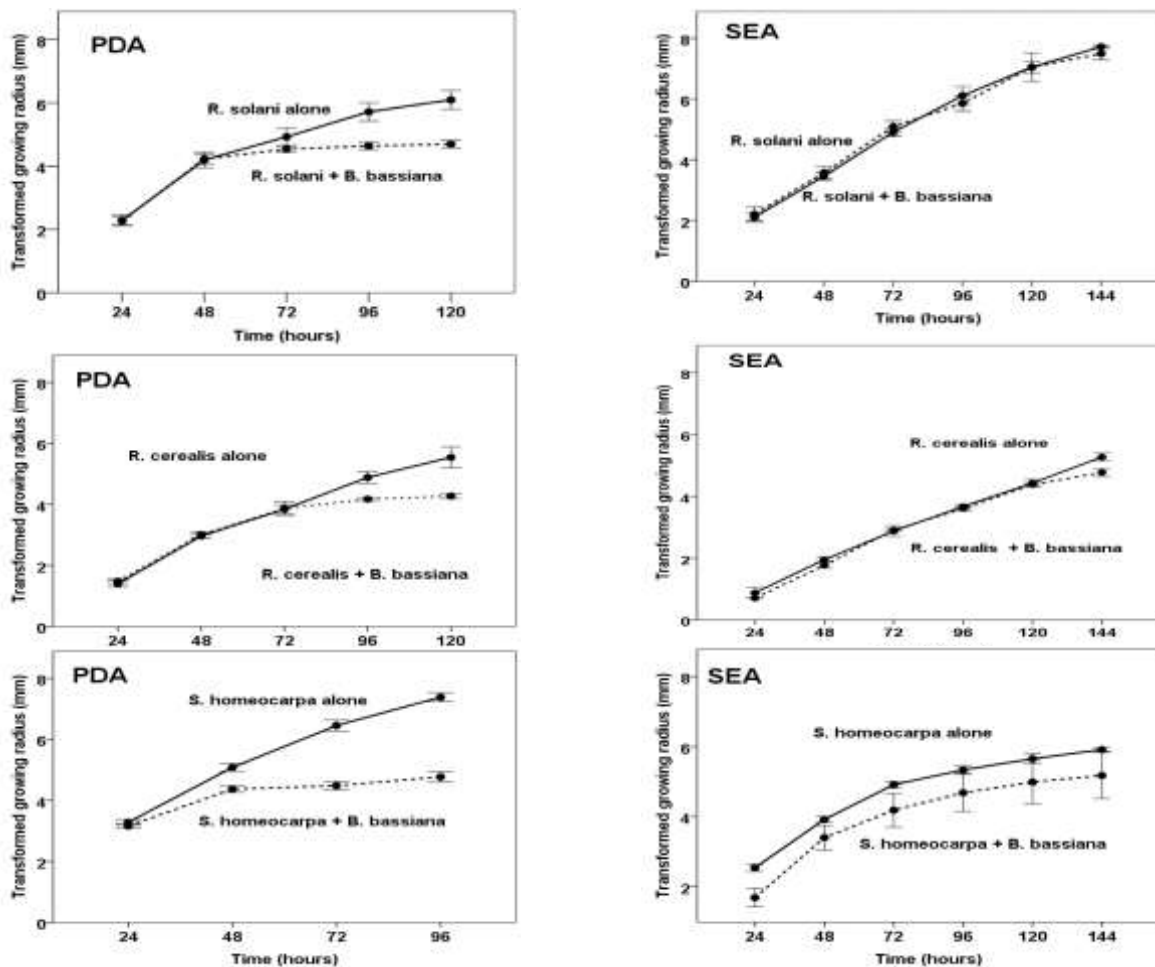


Figure 1: Growth of plant pathogenic fungi alone (radius R1) and in the presence of the antagonist *B. bassiana* (radius R2) in PDA and SEA culture media. Values are means \pm standard deviation after a data transformation by $\sqrt{(x + 0.5)}$. PI means Percentage of Inhibition.

⁵ Ownley, B.H., K.D. Gwinn and F.E. Vega 2009. Endophytic fungal entomopathogens with activity against plant pathogens: ecology and evolution. *BioControl*, 55 (1): 113-128.

⁶ Sahab, A. F. 2012. Antimicrobial Efficacy of Secondary Metabolites of *Beauveria bassiana* against selected bacteria and phytopathogenic fungi. *Journal of Applied Sciences Research*, 8 (3): 1441-1444.

⁷ Wagner, B. L. and L.C. Lewis 2000. Colonization of Corn, *Zea mays*, by the entomopathogenic fungus *Beauveria bassiana*. *Applied Environmental Microbiology* 66 (8): 3468.

⁸ Porrás-Alfaro, A. and P. Bayman 2011. Hidden fungi, emergent properties: endophytes and microbiomes. *Annual review of phytopathology*, 49: 291-315.

AN INTEGRATED NUTRITIONAL AND CHEMICAL APPROACH TO POA ANNUA SUPPRESSION IN CREEPING BENTGRASS GREENS

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Introduction

Creeping bentgrass (*Agrostis stolonifera* L.) provides an ideal putting surface for golf courses located in temperate climates because it provides a uniform playing surface, moderately tolerates heat and disease pressure, and is aesthetically pleasing. Monocultures of creeping bentgrass greens are invaded by annual bluegrass (*Poa annua* L.) and silvery thread moss (*Bryum argenteum* Hedw.), which can be difficult to consistently eliminate. Introduced in the 1980's, the plant growth retardant paclobutrazol has been shown to suppress annual bluegrass growth more than creeping bentgrass, favoring creeping bentgrass dominance. The standard recommendation for using paclobutrazol (22.9% ai, Trimmit 2SC) to control annual bluegrass is to apply 0.04 to 0.27 kg ai ha⁻¹, every fourteen days, spring through the fall¹. Xu and Mancino² studied iron rates of 0, 2, 4, 6, and 8 mg L⁻¹, reporting that creeping bentgrass shoot and root growth was favored over those of annual bluegrass. Sequential growing season foliar applications of alkaline extracts of *Ascophyllum no²osum* L., known as seaweed extracts (SWE) have been shown to result in a reduction in summer bentgrass decline³. Our objectives were i) to determine the effects of repeated high rates of ferrous sulfate on transitioning an annual bluegrass-infested creeping bentgrass putting green to a monoculture; ii) to determine if SWE with ferrous sulfate safens the transition to a creeping bentgrass monoculture; and iii) to determine if sequential high paclobutrazol rates, with or without ferrous sulfate, is a safe and effective combination for annual bluegrass suppression.

Methods

The trial was arranged as a randomized complete block split-plot design with four main plots consisting of four ferrous sulfate rates (0.0, 12.2, 24.4, and 48.8 kg ha⁻¹) and two subplots consisting of SWE (12.8 L ha⁻¹) or paclobutrazol (0.37 kg ai ha⁻¹ (March 1 to June 1 and September 1 to October 31) and 0.18 kg ai ha⁻¹ (June 1 to August 31)). Ammonium sulfate was sprayed uniformly over the entire study area every two weeks from March through November for a total of 18 treatments at 4.8 kg N ha⁻¹ each. No other fertility inputs were applied over the course of the study. Ferrous sulfate, SWE and paclobutrazol were applied bi-weekly starting on March 2, 2011 and March 4, 2012; final annual applications were October 24, 2011 and October 26, 2012. The study was on a 25-year-old 'Penneagle' creeping bentgrass putting green at the Virginia Tech Turfgrass Research Center in Blacksburg, VA built with a 20 cm sandy loam root zone over 5 cm of pea gravel. Irrigation (pH 7.6) was applied as needed to prevent visual signs of wilt. The green was mowed five times per week at 3.2 mm. Disease were controlled on a preventative basis. The trial site was core cultivated in the spring (Mar. 21, 2011 and Mar. 14, 2012) and fall (Sept. 9, 2011 and Oct. 10, 2012), totaling 15% surface area removal per year, followed by pure sand topdressing that was brushed in. Annual bluegrass infestation percentage was estimated each May when seedheads were very apparent. Percent infestation was estimated using a grid measuring 0.9 m x 1.8 m with 512 squares each square being 25 cm². A count of silvery thread moss colonies (measuring between 5 to 30 mm in diameter) was taken at the end of each growing season, in December. Data were subjected to a combined analysis of variance using the general linear models procedure in SAS version 9.1 with sums of squares partitioned to reflect the split-plot treatment design and the two years as a random factor. Mean squares were tested as appropriate for the split-plot design and random years. Interactions and main effects were separated with Fisher's protected LSD test ($\alpha = 0.05$) or described with linear or polynomial regressions where appropriate.

¹ McCarty, L.B. 2011. Best Golf Course Management Practices. Third edition. Pearson Education, Inc. Upper Saddle River, NJ. 776 pages.

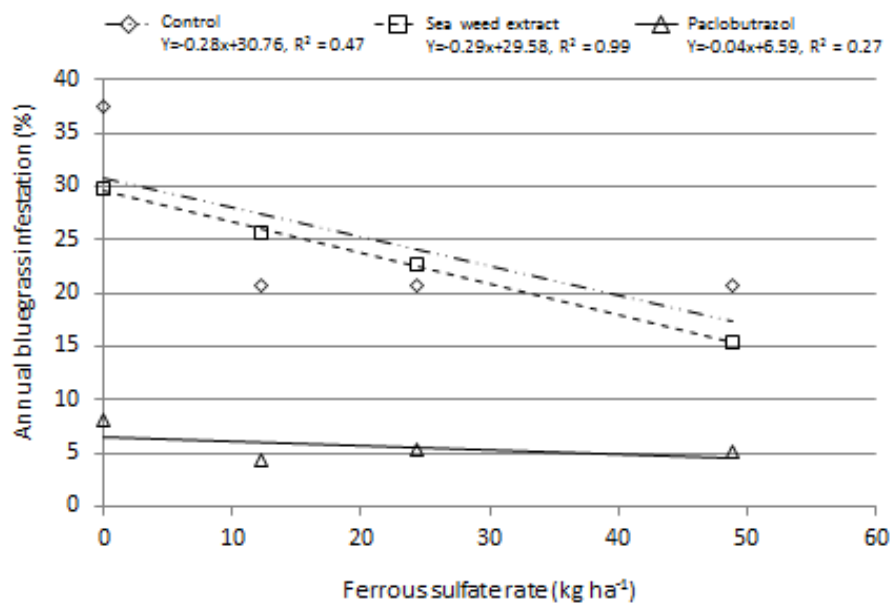
² Xu, X. and C.F. Mancino. 2001. Annual bluegrass and creeping bentgrass response to varying levels of iron. HortSci. 36:371-73.

³ Zhang, X. and E.H. Ervin. 2004. Cytokinin-containing seaweed and humic acid extracts associated with creeping bentgrass leaf cytokinins and drought resistance. Crop Sci. 44:1737-45.

Results and Discussion

The ANOVA test for annual bluegrass infestation percentage was affected by ferrous sulfate rates, SWE or paclobutrazol treatments or their combination over time on a creeping bentgrass putting green indicated a significant interaction between main and split plots but no effect of year; therefore, the data are pooled over year and presented as a regression. Annual bluegrass percent infestation was estimated to be approximately 45% at trial initiation. With each 10 kg ha⁻¹ increase in ferrous sulfate, annual bluegrass infestation decreased 2.8 and 2.9% when no PGR and SWE were applied, respectively (Figure 1). The aggressive paclobutrazol program effectively controlled annual bluegrass regardless of ferrous sulfate rate (Figure 1). It should be noted that a 15% decline in annual bluegrass occurred in plots that were untreated, possibly due to creeping bentgrass competition being favored by biweekly application of ammonium sulfate and exclusion of phosphorus and potassium fertilizers.

Figure 1. Interaction of ferrous sulfate rate and plant growth regulator on annual bluegrass infestation percentage on a creeping bentgrass putting green.



INFLUENCE OF GRASS COVERAGE ON TRIAZINE HERBICIDE TRANSPORT DURING SURFACE RUNOFF

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Introduction

Triazine herbicides, such as atrazine and simazine, are often applied to turfgrasses grown for home lawns in the southern United States. Triazine herbicides provide effective control of many broadleaf and some annual grassy weeds. However, home lawns can vary in turfgrass coverage from differences in cultural and environmental conditions that often necessitate herbicide application to prevent weed encroachment. As a result, pesticides, such as triazines, applied to adjacent water bodies could potential pose an increased risk from surface runoff.

The triazine herbicide atrazine (6-chloro-*N*-2-ethyl-*N*-4-isopropyl-1,3,5-triazine-2,4-diamine) is among one of the most commonly identified pesticide pollutants in water bodies¹. As a result of triazine prevalence in water supplies, the USEPA has developed maximum contamination levels of 3 and 4 $\mu\text{g L}^{-1}$ for atrazine and simazine (6-chloro-*N*-2,*N*-4-diethyl-1,3,5-triazine-2,4-diamine) within the United States, respectively.

According to Caron et al.² moderate to high water soluble pesticides such as atrazine are transported via surface runoff compared to sediment bound transport for weakly soluble pesticides like simazine. This suggests vegetative groundcover should affect atrazine and simazine losses from surface runoff as a result of decreasing total solid movement with denser canopies. Therefore, the objective of this research was to determine the effect turfgrass coverage has on triazine runoff to develop better best-management procedures.

Methods

Surface runoff research commenced in 2010 and 2011 with experimental units consisting of six turfgrass coverages (0, 25, 50, 75, and 100%) and untreated bare soil. Atrazine or Simazine were applied at recommended label rates, 2.24 kg ha⁻¹, 24 hours prior to rainfall simulation, a period of time that met or exceeded manufacturer's herbicide labeling for rain-fastness. Rainfall was simulated at a one-hour precipitation event of 7.32 cm h⁻¹, using a Tlaloc 3000 rainfall simulator (Joern's Inc., West Lafayette, IN) unit, based on designs of Miller³. For a thirty-minute runoff period. Runoff volume was collected and weight recorded in addition to 1-L composite samples collected for total solids and pesticide analysis. Pesticides were analyzed using gas chromatography and quantitated through mass spectrometry. Differences in total atrazine and simazine losses were compared as well as the portion of pesticide losses in water and sorbed to total solids.

Results/Discussion

Dense vegetation such as turfgrasses absorb raindrop kinetic energy preventing splash erosion and sediment dislodging, and also acts as a filter to trap suspended solids. Data from this research concur with these conclusions as total runoff volume and TS losses decreased with increasing turfgrass coverage. Previous research has indicated this reduction in water and sediment losses can in turn decrease herbicide runoff losses from turfgrass area suggesting why grasses have been adopted as vegetation portion of BMPs.

Total herbicide loss and loss pattern observed during this study indicate the influence chemical characteristics have on surface transport. Total atrazine POA runoff losses were greater than total POA runoff losses for simazine at 50, 75, and 100% turfgrass coverage. However, the greatest losses observed for both herbicides occurred at 50% turfgrass coverage 23.2 and 14.6 POA lost for atrazine and simazine, respectively. These higher losses observed for 50% turfgrass coverage were attributed to water channeling on the research experimental units. Similar observations from 70% vegetative coverage compared to bare soil have resulted in higher runoff volume as a result of bare areas connecting to one another. Interestingly, no difference in total

¹ Giroux, I. 2002. Contamination de l'eau par les pesticides dans les régions de culture de maïs et de soya au Québec, Campagnes d'échantillonnage de 1999, 2000 et 2001 et évolution temporelle de 1992 à 2001. Direction du suivi de l'état de l'environnement, envirodoc no EN/2002/0365, report no QE/137. Ministère de l'Environnement, Québec City, Québec, Canada.

² Caron E., P. Lafrance, J.C. Auclair. 2010. Impact of grass and grass with poplar buffer strips on atrazine and metolachlor losses in surface runoff and subsurface infiltration from agricultural plots. 39:617-629.

³ Miller, W.P. 1987. A solenoid-operated, variable intensity rainfall simulator. Soil Science Society of America Journal. 51: 832-834.

POA atrazine losses occurred between the different turfgrass coverages. At 100% turfgrass cover 18.2% of total atrazine applied was lost and did not differ from the 12.5% lost for 0% coverage. Higher water soluble pesticides such as atrazine can move into and through the soil profile with less restriction compared to lower water soluble pesticides. Therefore, without the presence of turfgrass to impede soil contact, lower herbicide total losses for 0% coverage were attributed to adsorption and infiltration.

Physical partitioning processes such as soil-organic-carbon partition coefficient (K_{oc}) and *n*-octanol-water partition coefficient (K_{ow}) along with pesticide water solubility have been utilized in prediction models for pesticide mass transport. According to Lickfeldt and Branham⁴ pesticide solubility and K_{oc} influence pesticide distribution between leaves, soil, and thatch however, factors such as post treatment irrigation, spray volume and pesticide formulation can alter pesticide fate. However, the majority of research evaluating sediment, nutrient and pesticide runoff from turfgrass systems has primarily focused on bare soil and highly managed well-established turfgrass sites. Singular comparisons such as these may not accurately characterize turfgrass effects on pesticide transport during surface runoff given the effect of turfgrass on runoff occurrence and severity.

Total simazine and atrazine losses were partitioned into water and sediment adsorbed losses in order to understand more specifically surface coverage influence on runoff losses. Atrazine water losses were greater than simazine water losses at all coverages. Atrazine water losses constituted >98% of total atrazine losses at all coverages compared to highest soluble simazine water losses constituting < 54% of total simazine losses at 100% coverage. Interestingly, simazine water losses for 0, 25, 50 and 75% turfgrass coverage range constituted 31 to 42% of total simazine losses. Simazine soil losses constituted a majority of simazine total losses and ultimately decreased as surface coverage increased. Sediment simazine losses for bare soil, 25, 50 and 75% turfgrass coverage ranged from 5.9 to 6.7% applied with a decrease to 2.6% observed at 100% turfgrass coverage. This pattern of herbicide loss suggests the filtering capacity of turfgrasses is an important component in reducing TS transport but may not be as effective in reducing dissolved pollutant transport.

Understanding turfgrass coverage influence on runoff occurrence and pesticide loss forms should help devise more site specific strategies to reduce potential losses into adjacent surface water bodies. This research evaluated losses occurring 24 h post herbicide application however also indicated the importance of soil and sediment sorption. Timing atrazine and simazine applications around rainfall and increasing residual time between rainfall events could decrease losses. The data presented also indicates the role herbicide selection can have on mitigating runoff losses and should be considered when creating BMP strategies.

⁴ Lickfeldt, D.W., and B.E. Branham. 1995 Sorption of nonionic organic compounds by kentucky bluegrass leaves and thatch. J. Environ. Qual. 24:980-985.

IDENTIFICATION OF ZOYSIAGRASS GENOTYPES WITH RESISTANCE TO LARGE PATCH UNDER CONTROLLED ENVIRONMENTAL CONDITIONS

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Introduction

Zoysiagrasses (*Zoysia* spp.) are perennial, warm-season turfgrass species well-adapted for home lawns, commercial landscapes, and golf courses in warm or transitional climates. They are versatile species with great potential as low-input turf because of their moderate drought and shade tolerances. While zoysia species have been found to have fewer major diseases than other turfgrasses, they are very susceptible to large patch, caused by the fungal pathogen *Rhizoctonia solani* Kuhn. During periods of active disease development, large areas of turf can be severely damaged and recovery can take weeks or even months. Chemical control is available but must be applied on a preventative basis. Therefore, genetic resistance to the fungus represents a very effective approach for management of large patch. The objective of this study was to screen 17 cultivars, five plant introductions, and 79 collections of zoysiagrass for resistance to *Rhizoctonia solani* in order to identify sources of resistance for cultivar development.

Methods

Plant sources: Vegetative material from each entry was collected from the NCSU germplasm collection and planted in Styrofoam cups filled with calcined clay (PROFILE Products, LLC). Plants were maintained at the Southeastern Environment Laboratory (Raleigh, NC) under controlled environmental conditions at 12hr light, 28°C/24°C. Plants were mowed twice a week to a height between 0.75 and two inches until inoculation.

Inoculations: Two isolates were tested in separate trials, isolate 1 belongs to North Carolina State University and isolate 2 is a University of Florida isolate, both with known pathogenicity to zoysiagrass. Each isolate was grown on potato dextrose agar (PDA). Plugs were then transferred to flasks containing sterile rye grain seeds and incubated in darkness for seven days at a constant temperature of 25°C. Plants were then inoculated with 15 rye grain seeds previously colonized by the fungus. Inoculated plants were placed in plastic containers enclosed with plastic bags to increase relative humidity¹ (Figure 1). Growth chamber conditions were 12hr light, 23°C/20°C, 100% HR.



Figure1. Experimental setup in walk-in growth chamber.

The experimental design corresponded to a randomized complete block design (RCBD) with three replications. Disease severity was estimated 14 days after inoculation (DAI) (Figure 2) using the Horsfall-Barratt scale². Genotypes were evaluated against isolates 1 and 2 separately in two runs each. After inoculation and data collection, plants were sprayed with Headway 1.4ME at 3fl oz. /1000 sq. ft. and allowed to recover for eight weeks. A second run of inoculation was conducted after this period of time for each isolate. Data was subject to an ANOVA using the PROC GLM procedure in SAS v9.2³. Least square (LS) means were generated with a Tukey-Kramer adjustment⁴.

Results and Discussion

Average disease severity for isolate 1 ranged from 7.8 to 72.5% with a mean of 31.8% and standard deviation of 20.3%. Likewise, isolate 2 ranged from 10 to 73.3% with a mean value of 42.6% and standard deviation of

¹ Green, D.E., J.D. Fry, J. C. Pair, and N.A. Tisserat. 1993. Pathogenicity of *Rhizoctonia solani* AG-2-2 and *Ophiosphaerella herpotricha* on zoysiagrass. *Plant Disease* 77(10): 1040-1044.

² Horsfall, J.G. and R.W. Barratt. 1945. An improved grading system for measuring plant diseases. *Phytopathology* 35: 655.

³ SAS Inst. Inc. 2008. SAS/STAT 9.2 User's Guide. SAS Inst. Inc., Cary, NC.

⁴ Kramer, C.Y. 1956. Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics* 12: 307-310.

24.3%. Some variability was observed in terms of severity by isolate (Figure 3). The peak of the distribution was between 20 and 40% for isolate 1 whereas isolate 2 accumulated a higher frequency of genotypes for values between 30 and 60%. Isolate 2 was evidently more virulent than isolate 1; visually this was reflected in the percentage of disease symptoms observed (the amount of mycelia covering the inoculated foliage).



Figure 2. Mycelia covering zoysiagrass plant at 15 DAI

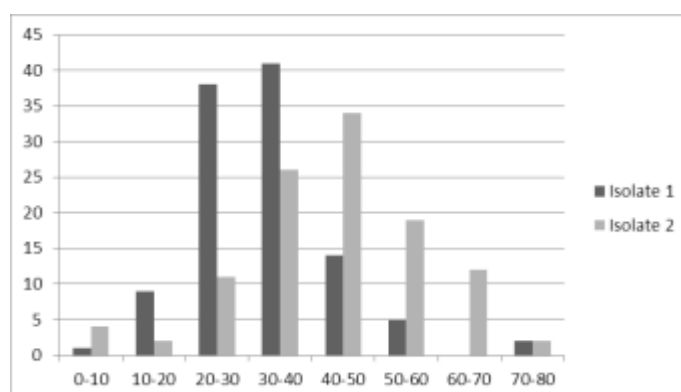


Figure 3. Frequency distribution of disease severity observed for two isolates among 110 zoysiagrass genotypes.

ANOVA results (Table 1) showed significant differences among entries ($P < 0.001$), between isolates ($P < 0.001$), as well as for the interaction of isolate and entry ($P < 0.001$). These differences are explained by the variable disease symptom response depending on the entry and isolate tested.

Table 1. Results of the analysis of variance (ANOVA) for zoysiagrass genotypes inoculated with two different *Rhizoctonia solani* isolates under controlled environmental conditions.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Isolate	1	3.825	3.825	113.920	<.0001
Entry	109	10.953	0.100	2.990	<.0001
Isolate*Entry	109	10.828	0.099	2.960	<.0001

Table 2. LS Means and mean separation for the top performing genotypes, cultivars and PIs inoculated with two different *Rhizoctonia solani* isolates.

Entry	Isolate 1	Isolate 2
Cashmere	20.17 abcd	23.50 abcdef
Cavalier	14.33 abc	27.67 bcdefg
Diamond	25.00 abcde	52.50 hijkl
L1F	25.00 abcde	30.00 bcdefgh
Matrella	38.33 cde	39.17 efghij
PI 231146	7.83 a	9.50 ab
PI 553019	41.67 de	13.33 abcd
PI 553020	33.33 bcde	11.83 abc
PristineFlora	29.33 abcde	23.33 abcdef
Rollmaster	40.00 de	62.50 jkl
Royal	19.50 abcd	35.83 cdefghi
Shadowturf	21.83 abcd	8.50 ab
Ultimate Flora	19.17 abcd	45.00 fghijkl
VJ	28.33 abcde	47.50 fghijkl
Z09005	12.67 ab	42.50 efghijk
Z09040	19.17 abcd	37.50 defghi
Z09063	41.67 de	1.00 a
Z09064	20.00 abcd	34.17 cdefghi
Zeon	35.00 bcde	68.33 l
Zorro	12.67 ab	44.167 efghijkl

Pathogenicity results for both isolates identified PI 231146 as the most resistant genotype (Table 2). Among cultivars, Cashmere, Shadowturf and Pristine Flora had the highest levels of resistance with either isolate. Accessions Z09005, Z09040 and Z09064 had levels of resistance comparable to those of the most resistant genotypes when inoculated with isolate one but showed an increase in susceptibility when inoculated with isolate two. Cultivars Zeon and Rollmaster were not significantly different from the worst performers with either isolate (Table 2). These results are an indication of the specific plant-pathogen interactions present when using inoculum sources collected at different geographic locations. However, PI 231146 constitutes a valuable germplasm source for large patch resistance breeding efforts in zoysiagrass given its stable resistance response across the pathogen isolates used in this study. Future breeding efforts will include the use of PI 231146 to elucidate the genetic control of large patch resistance in this genotype and to map genes associated with this and other traits of interest by means of genotyping by sequencing (GBS).

OBSERVING THE IMPACT OF FUNGICIDE APPLICATIONS ON THE PHYLLOSHERE MICROBIAL COMMUNITY

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Introduction

Turfgrasses frequently encounter disease-causing fungi that can greatly impact turfgrass health and survival. Uniform and disease-free turfgrass sites are not only valued for their aesthetics, but also for their utilization as a sports surface. This is particularly important in golf course putting greens, which are often considered the focal point of golf course design. Cultural control for disease may provide some relief, but in many cases, unfavorable environmental conditions cause turfgrass managers to rely on pesticide applications to maintain turfgrass health and function. Many chemistries offer broad-spectrum control of many microbes through various biochemical modes of action; although, there are concerns of these chemistries altering the beneficial microflora of turfgrass ecosystems^{1,2}. Smiley and Craven observed that fungicide combinations applied to suppress fungi often stimulated bacteria and actinomycetes in the soil³. In a similar study by Harman et al., fungicide applications did not impact the overall number of microbes on turfgrass foliage, but did impact the composition of microbial groups; chlorothalonil applications favored yeasts and *Penicillia* while triadimefon applications favored *Trichoderma* growth⁴. The recent rise in reports of bacterial disease in golf course putting greens has raised questions regarding the impact of fungicide applications on microbial communities^{5,6}. With the wealth of fungicide chemistries available, it is possible that certain products may be impacting specific microbial groups that reside on turfgrass leaves. The objective of this research was to determine the impact of repeated fungicide applications across a growing season on culturable microbial groups residing in the turfgrass phyllosphere.

Methods

In 2013, a 2-yr field trial was initiated in order to understand the impact of fungicide applications on phyllosphere microbiota. Experimental plots were established on a 7-yr-old creeping bentgrass (*Agrostis stolonifera* L. cv. 'A-1') putting green maintained on a sand-based rootzone constructed according to USGA specifications. Turf was mown 5-6 times per week using a Toro Greensmaster 3150 with a bench setting of 3.2-4.1 mm. Fertilization was applied using soluble sources (i.e., 46-0-0, 12-0-0, 25-5-18, 18-3-4, and 25-6-12) with the site receiving elemental N, P, and K at 87, 6, and 37 kg ha⁻¹ in 2013 and 137, 44 and 75 kg ha⁻¹ in 2014, respectively.

The site received wetting agents' applications every 21-28 d throughout each growing season to maintain water infiltration and irrigation was supplied to support turfgrass growth. With the exception of chlorantraniliprole (Acelepryn, Syngenta Crop Protection) applications to reduce insect pests, all maintenance pesticide applications were omitted from the experimental area.

Experimental plots (0.9 by 1.3 m) were arranged side-by-side in both years of study; although separate field plots were used each year. Fungicide treatments [fluzinam (Secure, Syngenta Crop Protection) applied at 637 g a.i. ha⁻¹, fluxapyroxad (Xzemplar, BASF Corp., Raleigh, NC, USA) applied a 244 g a.i. ha⁻¹, chlorothalonil (Daconil Ultrex, Syngenta Crop Protection) applied a 8056 g a.i. ha⁻¹, fosetyl-aluminum (Chipco Signature, Bayer Environmental Science, Research Triangle Park, NC, USA) applied a 9783 g a.i. ha⁻¹, pyraclostrobin (Insignia) applied a 488 g a.i. ha⁻¹ (2014 Only), and no fungicide] were applied every 14 d using a CO₂ pressurized sprayer to deliver the appropriate rate 816 L H₂O ha⁻¹. A 0.3 m buffer separated experimental plots in order to prevent contamination from treatment applications. Phyllosphere samples were extracted 5 days post-application of

¹ Alexander, M. 1969. Microbial degradation and biological effects of pesticides in soil. In: Soil Biology. pp. 209-240. UNESCO, Rome.

² McCallan, S.E., and L.P. Miller. 1958. Innate toxicity of fungicides. In: Advances in Pest Control Research (R.L. Metcalf, Editor), Vol. II, pp. 107-134. Interscience, New York.

³ Smiley, R.N., and M.M Craven. 1979. Microflora of turfgrass treated with fungicides. Soil Biol. Biochem. 11:349-353.

⁴ Harman, G.E., E.B. Nelson, and K.L. Ondik. 2006. Non-target effects of fungicide applications on microbial populations of putting greens. USGA Green Sec. Rec. 44(4):9-12.

⁵ Giordano P.R., A.M. Chaves, N.A. Mitkowski, and J.M. Vargas. 2012. Identification, characterization, and distribution of *Acidovorax avenae* subsp. *avenae* associated with creeping bentgrass etiolation and decline. Plant Disease, 96:1736-1742.

⁶ Roberts J., L. Tredway, and D.F. Ritchie. 2014. First report of *Xanthomonas translucens* causing etiolation on creeping bentgrass turf in IL, KY, and NC. Plant Disease, 98:839.

fungicides. Four randomized-replicate samples were taken from 171 (0.025 m²) sampling locations in each fungicide treatment. Sampling sites were re-randomized for each sampling date. Phyllosphere samples included five individual turf plants removed from the treated area, with roots and senescent material removed. All turf samples were placed in a pre-weighed 1.5 ml microcentrifuge tubes, and transported on ice from the field to laboratory to prevent decomposition of microbial populations present. Turf samples were pulverized using a plastic pestle in microcentrifuge tubes containing in 1 ml of sterile deionized water (DI water) and 0.3 cm³ glass beads (450-600 μm). The resulting suspension was vortexed for 10 sec and diluted in a 10-fold series. Once diluted, 10 μl of each dilution were plated and lawned across four different isolation media including Actinomycete isolation agar [VWR, Radnor, PA, USA), acidified potato dextrose agar (Becton, Dickinson, and Company, Franklin Lakes, NJ, USA), nutrient agar (Becton, Dickinson, and Company) + 1% sucrose, and Kings Medium B (Becton, Dickinson, and Company). All four media were included to isolate different microorganisms including actinomycetes, general bacteria, fungi, and fluorescent *Pseudomonads*. All plates were maintained in a dark box at room temperature (27° C). Microbial growth was quantified by visual counts of colony forming units (CFUs) at 48 and 168 hours after plating. Plate quantifications were normalized to the original plant tissue weight for determination of CFUs g⁻¹ of turf tissue. Data was subjected to analysis of variance using SAS 9.4 for Windows and treatment means were separated using Tukey's Honest Significant Difference.

Results and Discussion

Phyllosphere microflora were impacted by repeated applications in both years, although effects observed were not always negative (Figure 1). General fungi were not impacted by any fungicide in 2013, but chlorothalonil and pyraclostrobin significantly decreased fungi in 2014. Surprisingly, actinomycetes were increased by fluazinam applications in 2013 whereas these applications decreased populations compared to the non-treated control in 2014. Chlorothalonil and fosepyl-AI also lowered actinomycetes in 2014 whereas all other treatments had no effect. General bacteria were not impacted by any fungicide in 2013, but fluazinam applications did cause a reduction compared to the non-treated in 2014. Fluorescent *Pseudomonads* were negatively impacted by fluxapyroxad applications in 2013 while no differences were observed amongst treatments in 2014.

Overall, it seemed that populations of all microorganisms were impacted less than originally expected. This was not surprising as Harman was not able to detect differences in total fungi or total bacteria impacted through repeated fungicide applications⁴. Similarly, Smiley and Craven also observed that collective microbial groups were often impacted less than individual species from individual and combinations fungicide products³. In our research, weather fluctuations across the season (i.e., temperature and rainfall) seemed to impact microbial populations and additional research is needed to explain the impact of environment on microbes in the turfgrass phyllosphere (data not shown). Future research to incorporate next-generation sequencing technologies will benefit our ability to detect differences in non-culturable organisms.

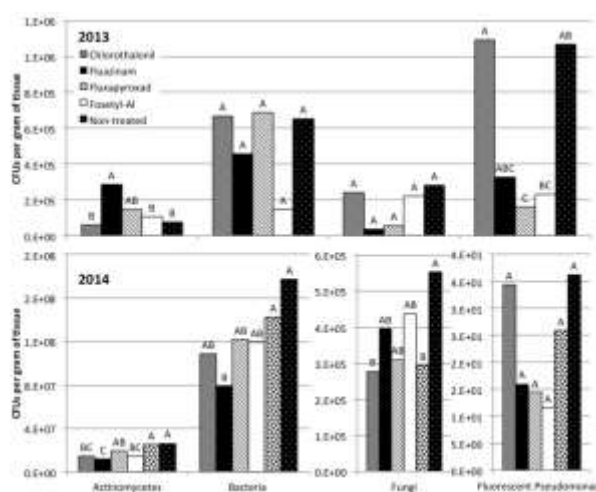


Figure 1. Impact of repeated fungicide applications on individual microbial groups sampled in a creeping bentgrass putting green across 2013(upper) and 2014(lower). Values represent cumulative means with letter designations indicating significant differences at the 0.05 significance level using Tukey's HSD.

TURFGRASS NUTRITION AND PHYSIOLOGY

ORAL PRESENTATIONS

BERMUDAGRASS DORMANCY AVOIDANCE USING A COMMERCIAL MICROBIAL CONCENTRATE

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Introduction

Bermudagrass (*Cynodon dactylon* (L.) Pers) is probably the most common warm season turfgrass used in urban parks and private gardens in tropical, subtropical and even in the transition zone to temperate climates. When cultivated in the transition zone, it gets dormant when temperatures approach 10 °C and leaves turn brown. A winter dormancy period that prevents year-round green colour is currently the main impediment to a more widespread use of warm-season grasses in the transition zones^{1,2}. Late-season N fertilization is an alternative way for reduction of the dormancy period³. Another possible way to reduce winter dormancy could be a biostimulant application in late season, particularly if it contains nitrifying bacteria releasing N to the rhizosphere. The objective of this research was to evaluate the effect of a biostimulant (commercial microbial concentrate) that contains a mixture of four different bacteria species on bermudagrass in order to avoid, or at least delay, its autumn-winter dormancy and, as a consequence, improve turfgrass general aesthetics during cold periods.

Methods

Three experiments were conducted in bermudagrass during 2014-2016 in Valencia (Spain). Two of them were equal but carried out in different years, in pots and under open-air conditions, and the third one on a golf course in field conditions. The pot experiments were sown with 'Princess 77' and 'Sovereign' bermudagrass varieties in July (2014 and 2015) in 16 cm of diameter and 41 cm height pots, filled with a 90/10 weight mixture of 0.6 mm sand and peat. Turf management (irrigation and mowing) was normal during the first 3 months after sowing. On the second week of October a turfgrass fertilizer (20-5-8-2Mg) was applied to half of the pots at 5 different doses (0, 50, 100, 200 and 400 g.m⁻²) with 350 mL of water added just after fertilizer application. The other half of the pots received on the same day a microbial concentrate product containing a mixture of four different bacteria species (*Azotobacter vinelandii* 20x10⁶ UFC.ml⁻¹, *Bacillus licheniformis* 20x10⁶ UFC.ml⁻¹, *Bacillus megaterium* 20x10⁶ UFC.ml⁻¹ and *Pseudomonas fluorescens* 20x10⁶ UFC.ml⁻¹) at 5 different doses (0, 50, 100, 200 and 450 mL.m⁻²). The biostimulant was diluted in 300 mL of water and another 50 mL of water were added to wash the biostimulant from treated leaves. Biostimulant treatment was repeated 2 more times in a 14 day interval. Turfgrass in the pots was not mown anymore until the end of the experiment 5 months later and irrigation was performed equally in all pots. Evaluation of both experiments consisted in i) weekly turf quality of each pot with a subjective visual 1 to 9 scale where 1 equalled dead turf and 9 equalled an ideal turf; ii) weekly turf colour with the same 1 to 9 scale where 1 equalled straw brown colour turf and 9 dark green colour turf; iii) turfgrass growth from treatment to the end of the experiment by measuring turf height 5 times per pot with a rule (1 mm accuracy); iv) weight of the aerial part of turfgrass, oven dried at 60 °C 5 months after initial treatment. Both experiments consisted in four (2 varieties x 2 products applied) complete randomized designs with one factor (product dose) and four replicates. The golf course field experiment consisted of 'Princess 77' bermudagrass sowings on a 50 cm depth sand bed at 3 different sowing times, on the 22nd of July, 4th of August and 18th of August 2015. In each sowing time, eight 1 m² subplots were seeded at 8 g.m⁻². Normal turfgrass management (irrigation and mowing) was performed from sowing time to early November when the same biostimulant used in the pot experiments was applied to half of the subplots at 50 mL.m⁻² diluted in 200 mL of water. Biostimulant treatment was made with a CO₂-pressured sprayer calibrated to deliver 325 L.ha⁻¹ with a single flat-fan nozzle (9504 EVS flat-fan; TeeJet Spraying Systems) at 206 kPa and repeated 2 more times in a 14-day interval. Evaluation of the field experiment consisted in i) weekly turf quality and colour and growth along the experiment, rated as in the pot experiments; ii) turfgrass subplot cover with a visual percentage estimation; iii) dark green colour index⁴ obtained from digital images taken on the subplots

¹ Geren, H., Avcioglu, R. and Curaoglu, M. 2009. Performances of some warm-season turfgrasses under Mediterranean conditions. *Afr. J. Biotechnol.* 8: 4469-4474.

² Macolino, S., Serena, M., Leinauer, B. and Ziliotto, U. 2010. Preliminary Findings on the Correlation between Water-soluble Carbohydrate Content in Stolons and First Year Green-up of Seeded Bermudagrass Cultivars. *Hortecchnology* 20: 758-763.

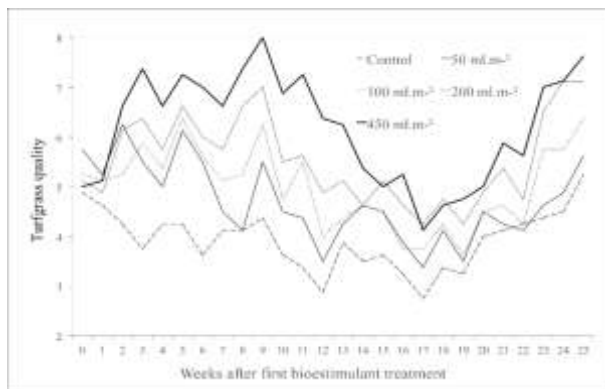
³ Pompeiano, A., Volpi, I., Volterrani, M. and Guglielminetti, L. 2013. N source affects freeze tolerance in bermudagrass and zoysiagrass. *Acta Agriculturae Scandinavica Section B.* 63: 341-351.

⁴ Karcher, D.E and Richardson, M.D. 2003. Quantifying Turfgrass Color Using Digital Image Analysis. *Crop Sci.* 43:943-951.

on January and February with a Canon PC1089 camera and a black box to provide uniform lighting conditions iv) weight of the aerial part of turfgrass mown at 1.4 cm and oven dried at 60 °C at the end of the experiment. Each sowing time experiment on the golf course consisted in a randomized complete block experiment with one factor (treatment) and 4 replicates. All statistical analysis was made with Statgraphics Plus 5.1.

Results and Discussion

Winter in Valencia during 2014-2015 and 2015-2016 was very mild and inconsistent with temperatures dropping below 10 °C only late in the winter and unusual warm mean temperatures in autumn and winter. Maybe this was the reason as the pot experiments were so different each other. In the first pot experiment, when the biostimulant was applied to 'Princess 77' bermudagrass the turf quality significantly improved (Figure 1), above all with the higher biostimulant doses. In the same figure it can be observed how the control grass evolution was always found less than 5 points in the quality scale whereas the highest biostimulant dose only dropped below 5 during weeks 17 to 19 (February). In the whole experimental period there were 14 weeks out of 25, in which treated turfgrass was statistically better than control turfgrass. The same pattern was obtained



when evaluating 'Princess 77' colour and the 'Sovereign' variety: a big difference between the highest biostimulant dose and the control cultivars and some dose-response statistical differences among biostimulant doses. However, little effect was observed when both varieties were fertilized, although dormancy avoidance was detected as well. In the following year the experiment was replicated and big differences were observed in respect to the first year, firstly when fertilized pots showed better quality than biostimulated ones and secondly because 'Sovereign' performed better than 'Princess 77' in terms of growth and yield.

Figure 1: 'Princess 77' general aesthetics evolution after biostimulant treatment in the first pot experiment.

In the golf course field experiment the biostimulant was applied 3 times: the first one on the 4th of November and the rest in 14-day interval, over 3 different bermudagrass sowings. The biostimulant did not help covering the subplot surface, neither a turfgrass quality increase was detected whatever it was the bermudagrass sowing time. The only statistically significant positive effect induced by the biostimulant was colour retention visually evaluated. In that case, in the period between 4th November and 4th February, treated subplots of the first, second and third sowings had statistically significant darker green colour than control ones in 5 out of 12, 5 out of 12 and 8 out of 12 dates respectively, mainly at the beginning. However when analysing dark green colour index with digital images, although treated subplots always had darker green colour whatever it was the sowing time and image date, differences were not statistically different (Figure 2).

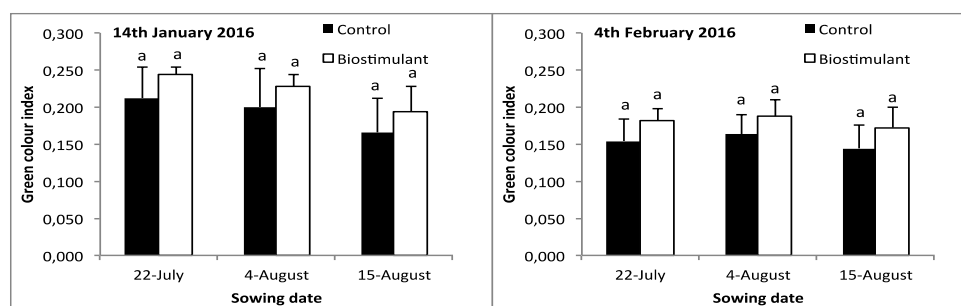


Figure 2: Dark green colour index obtained from digital image analysis over bermudagrass in the field experiment.

Acknowledgments

Authors would like to acknowledge José Manuel Iserte, greenkeeper of Real Club de Golf Manises (Spain), Santiago Gámiz from the Drone Services Company Hidronico CB, and the companies Semillas Dalmau S.L. and Biotecnología del Mediterráneo S.L. for providing seeds and the biostimulant respectively.

PLANT COLORANTS INTERFERE WITH REFLECTANCE BASED VEGETATION INDICES

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Introduction

Reflectance-based vegetation indices are commonly used to quantify turfgrass color, estimate chlorophyll content, and make inferences about plant health. The chlorophyll index is calculated by comparing reflectance of the canopy at 700 and 840 nm. Similar vegetation indices include the normalized difference vegetation index (NDVI) and the normalized difference red edge (NDRE). These indices typically correlate well with visual color ratings, but the application of colorants or iron may impact the reliability of these measurements. The reflectance characteristics of these products have not been characterized in the turfgrass literature, but they may overlap with the reflectance spectrum of chlorophyll. The objective of this study was to evaluate the reliability of non-destructive color assessment techniques for plots treated with iron and plant colorants at experimental research stations in Verona, WI and Ithaca, NY.

Methods

Creeping bentgrass, *Agrostis stolonifera*, in Verona, WI was treated with ammonium sulfate (2.4 or 9.8 kg N ha⁻¹), Turf Screen (untreated or 4.0 L ha⁻¹), or ferrous sulfate (untreated or 61.0 kg Fe ha⁻¹) with four replications. Products were applied every 14 days for a total of five applications, and applications were made with a CO₂-pressurized backpack sprayer calibrated to deliver 815 L water carrier ha⁻¹. Three trained individuals rated visual turfgrass color on a 1-9 scale, where 6 was minimally acceptable, 9 was the highest possible, and 1 represented dead turf. Chlorophyll index was measured with a FieldScout CM1000 (Spectrum Technologies, Inc., Aurora, IL), and the normalized difference vegetation index (NDVI) and normalized difference red edge (NDRE) were measured with a Crop Circle ACS-470 (Holland Scientific, Lincoln, NE) at a height of 30 cm above the leaf canopy. The CI, NDVI, and NDRE were calculated as:

$$CI = R_{840}/R_{700} \quad (\text{Eq. 1})^1$$

$$NDVI = (R_{800} - R_{670})/(R_{800} + R_{670}) \quad (\text{Eq. 2})^2$$

$$NDRE = (R_{790} - R_{720})/(R_{790} + R_{720}) \quad (\text{Eq. 3})^3$$

In Ithaca, NY, plots of *A. stolonifera*, were treated on a 14-day interval with maximum-labeled rates of the colorants Harmonizer, Foursome, Turf Screen, or Green Lawngr and compared to a non-treated control with four replications. Subjective visual color ratings (1-9 scale), DGCI, chlorophyll index, and chlorophyll fluorescence were measured weekly. Chlorophyll and carotenoids were measured monthly by digesting tissue in dimethylformamide. For each response variable at both sites, treatment means were separated using Analysis of Variance (ANOVA), treatment means were separated using the Student's *t* test.

Reflectance characteristics were measured for the plant colorants Harmonizer, Mirage, Turf Screen, Foursome, Pigment Green, and Green Lawngr. Each product was diluted 1:1000 in water, and absorbance was measured in triplicate from 400 to 999 nm in increments of 1 nm with a microplate reader (Synergy HT, Biotek, Winooski, VT). Each raw absorbance value was divided by the maximum absorbance for a given scan to normalize the absorbance intensities, which varied due to the different formulations of the products. Absorbance was converted to reflectance with the following equation:

$$\text{Reflectance} = 1/10^{\text{Absorbance}} \quad (\text{Eq. 4})$$

¹ Read, J.J., E.L. Whaley, L. Tarpley, and K.R., Reddy. 2003. Evaluation of a hand-held radiometer for field determination of nitrogen status in cotton. Dig. Imaging Spec. Techniques: Appl. Prec. Agric. Crop Phys. digitalimaginga, 177-195.

² Haboudane, D., J.R. Miller, E. Pattey, P.J. Zarco-Tejada, and I.B. Strachan. 2004. Hyperspectral vegetation indices and novel algorithms for prediction the green LAI of crop canopies: Modeling and validation in the context of precision agriculture. Rem. Sens. Environ. 90:337-352.

³ Rodriguez, D., G.J. Fitzgerald, R. Belford, and L.K. Christensen. 2006. Detection of nitrogen deficiency in wheat from spectral reflectance indices and basic crop eco-physiological concepts. Crop Pasture Sci. 57:781-789.

Results and Discussion

In the Verona, WI study, plots that were not treated with Fe had correlations between visual color (1-9) and NDRE ($R=0.78$), NDVI ($R=0.49$), and CI ($R=0.72$). Plots that were treated with Fe had lower correlation coefficients between visual color and NDRE ($R=0.42$), NDVI ($R=0.20$), and CI ($R=0.66$). Plots that were not treated with Turf Screen had correlations between visual color and NDRE ($R=0.45$), NDVI ($R=0.36$), and CI ($R=0.62$). Plots that were treated with Turf Screen had lower correlation coefficients between visual color and NDRE ($R=0.17$), NDVI ($R=0.24$), and CI ($R=0.35$). The application of Fe resulted in significantly lower CI, NDRE, and NDVI values, but greater visual color ratings. Plots treated with Turf Screen had greater CI, NDRE, NDVI, and visual color. In the Ithaca, NY study, all colorants improved visual color ratings, DGCI, and CI, but did not affect plant chlorophyll content, carotenoids, or chlorophyll fluorescence.

Each of the colorants reflected light in the green (495 to 570 nm), red (650 to 750), and the near infrared (750 to 1000) portions of the spectrum (Fig. 1). Non-destructive color measurements including CI, NDVI, and NDRE utilize reflectance data from wavelengths ranging from 670 to 840. The six colorants described in Fig. 1 all exhibit reflectance in this range, potential leading to overestimation of vegetation indices provide that the ratio of R_{840}/R_{700} increased with the application of colorants. The amount of interference would depend on the application rate of the colorant. We were not able to measure reflectance of ferrous sulfate because its dark color is associated with its redox status as it dries on the plant leaf. Under the conditions of this study, interpretation of vegetation indices by themselves could have led to incorrect inferences about plant health. These results suggest that plant colorants interfere with reflectance-based vegetation indices.

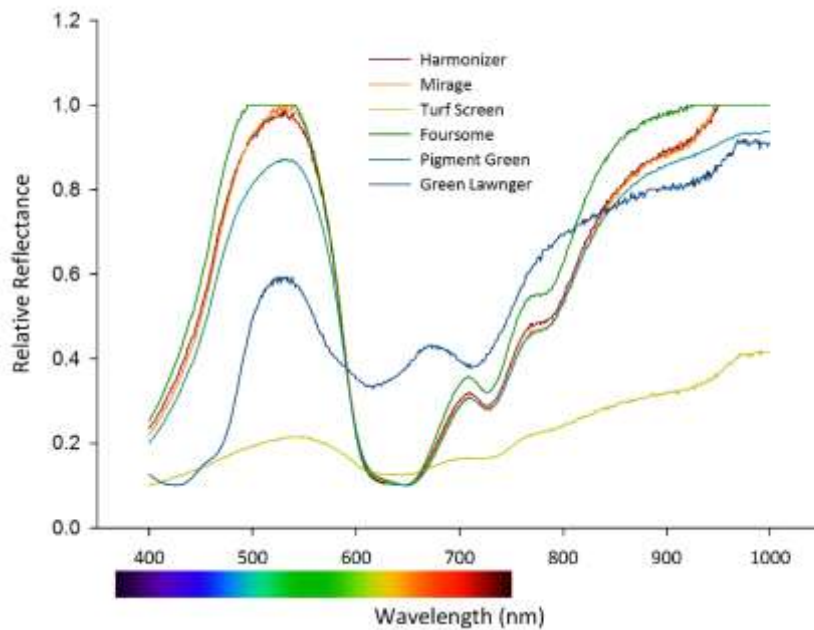


Figure 1. Reflectance characteristics for six plant colorants.

THE EFFECT OF ICE ENCASEMENT AND TWO PROTECTIVE COVERS ON THE WINTER SURVIVAL OF SIX TURFGRASSES ON PUTTING GREENS

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Introduction

Winter damage on putting greens is a significant economic burden for golf courses at northern latitudes, and management strategies which reduce damages are required. During the winter, plants are subjected to a multitude of stresses, which differ from region to region and from year to year. Grass plants may have to tolerate one or a combination of the following stress factors: lack of photosynthetic light, prolonged exposure to freezing temperature, flooding or ice encasement, fungal diseases that develop under snow, soil heaving, solar radiation and wind that can cause desiccation. Ice encasement (IE) is the most economically important winter stress in Scandinavia, however little is known about IE tolerance of the different putting green species/subspecies. Protective covers have been utilized in Canada to protect golf greens against low temperatures and desiccation, with promising results^{1,2,3}. A number of Scandinavian golf courses have started using protective covers, with the intention to avoid IE. It was therefore of interest to investigate further the response of protective covers under Scandinavian conditions. The objective of this study was to assess the impact of IE and two protective covers on the winter survival of the most common turfgrass species used on greens in Scandinavia.

Methods

The experiment was conducted on a USGA-green seeded with *Agrostis capillaris*, *A. canina*, *A. stolonifera*, *Festuca rubra* ssp. *commutata*, *F. rubra* ssp. *litoralis* and *Poa annua* during two winters. The two protective covers (non-permeable plastic and non-permeable plastic covering a 10 mm woven mat to create an air space between the green surface and plastic) were installed in November once the ground was frozen. IE was established on 22 Nov. 2011 and 4 Dec. 2012 by adding small amounts of water over a period of three days, to plots surrounded by an aluminium frame. IE conditions persisted for 98 and 119 d in 2011/12 and 2012/13, respectively. The control treatment consisted of natural winter conditions with lasting snow cover.

Core samples (8 cm diameter, 10 cm deep) were taken from the plots at the time of cover installation, and then on 16 Jan., 13 Feb. and 12 Mar. in 2012, and 28 Jan. and 22 Feb. in 2013. During the winter of 2013 additional samples were taken from the IE treatment on 11 Jan., 8 Feb., 8 Mar. and 20 Mar. Core samples were thawed for two days at 4°C in the dark, and potted. The samples were placed in a growth chamber at 18°C, 18 h photoperiod, and after 21 days, the percent coverage of the sample with healthy grass was registered. Turf coverage, overall impression and disease levels of field plots were also registered in the spring.

Results and Discussion

The first winter 2011-12 was unusually short, with warmer temperatures and an earlier snowmelt than normal. Mild temperatures beginning in January caused a layer of ice to accumulate under the snow. Despite efforts to avoid water seepage under the protective covers, a thin layer of ice (approx. 5 mm) also developed in these treatments. Under these conditions, the plastic cover with and without air space improved the coverage of *P. annua* in the spring of 2012 by 47 and 39 per cent units, respectively, compared to the control. The improvement was due to the avoidance of complete IE under the plastic covers as *P. annua* is very sensitive to IE^{4,5}. The tendency to better survival with than without the mat under the plastic could be explained by the observation that the crystals were formed in the woven mat that provided the air space, thus not restricting gas exchange.

¹ Rochette, P., et al., *Atmospheric composition under impermeable winter golf green protections*. Crop Science, 2006. 46: p. 1644-1655.

² Asher, D., T. Paquette, and J. Ross, *Survivability of Annual Bluegrass under Impermeable Winter Covers - The Glendale Study*. 2009, Alberta Turfgrass Research Foundation.

³ Tompkins, D.K., P. Rochette, and J. Ross, *Mitigation of anoxia under ice and impermeable covers on annual bluegrass putting greens*. 2009, Canadian Turfgrass Research Foundation.

⁴ Aamlid, T.S., W.M. Waalen, and T. Espevig, *Fungicide strategies for the control of turfgrass winter diseases*. Acta Agriculturae Scandinavica, Section B — Soil & Plant Science, 2014. 65: p. 161-169.

⁵ Tompkins, D.K., J. Ross, and D.L. Moroz, *Effects of ice cover on annual bluegrass and creeping bentgrass putting greens*. Crop Sci, 2004. 44: p. 2175-2179.

The second winter 2012-13 was a more normal winter, with 141 days of snow cover and little ice development under the snow. In this year, the two plastic cover treatments had no impact on coverage measurements taken throughout the winter, but the assessment in spring showed a negative response in *A. capillaris* because of more *Microdocium nivale* under the covers than in the control treatment.

Sampling from the IE treatment during the winter of 2012-13, as shown in Fig. 1, revealed large differences in the average turfgrass coverage for the different species. *P. annua* was completely dead after 40 days of IE. The tolerance of *A. capillaris* was also poor, with coverage dropping to 50% after approximately 50 days of IE. *A. canina* on the other hand showed superior tolerance to IE, and even after 119 days of IE the coverage had not dropped to below 75%. The differences between the turfgrass coverage following IE in 2012-13 was determined to be: *A. canina* (a) > *A. stolonifera* (b), *F. rubra* ssp. *commutata* (b), *F. rubra* ssp. *litoralis* (bc) ≥ *A. capillaris* (c) > *P. annua* (d) (P ≤ 0.0001).

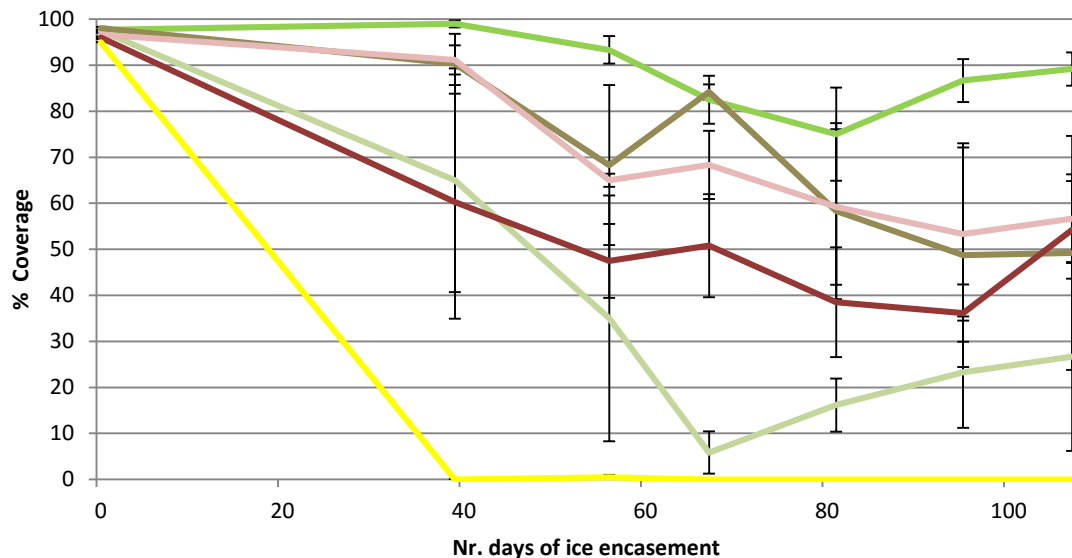


Figure. 1. The effect of duration of IE on percent coverage of *A. canina* (green), *A. capillaris* (light green), *A. stolonifera* (brown), *Festuca rubra* ssp. *commutata* (pink), *F. rubra* ssp. *litoralis* (red) and *Poa annua* (yellow) registered after the removal of IE and 21 days of regrowth during the winter of 2012/13. Bars indicate standard errors of the means (SE).

In conclusion, winter conditions during the experimental period did not justify the use of covers on the six turfgrass species tested, despite small improvements for *P. annua* in the first year. However, in regions with less snow cover, lower temperature or longer IE periods, the use of covers may be beneficial for species with low tolerance to IE, such as *P. annua* and *A. capillaris*, or species that are slow to establish, such as the *Festuca* species. To avoid negative effects due to water seepage under the covers and snow mould growth, proper installation and appropriate fungicide strategies are necessary. This study presents evidence that *A. canina* and *A. stolonifera* have superior IE tolerance, and are species especially suited to areas prone to IE conditions.

PIGMENT-CONTAINING PRODUCT EFFECTS ON CREEPING BENTGRASS AND BERMUDAGRASS

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Introduction

With the ever-changing market of the golf course industry, turfgrass managers are constantly exploring options of promoting healthier turf while maintaining a favorable budget¹. One constant problem is the management and relief of summer stress on creeping bentgrass [*Agrostis stolonifera* L. var *palustris* (Huds.)] putting greens². Application of pigmented products is an increasingly popular management practice attempting to relieve plant stress associated with high temperatures and light intensity. Several of these products are also marketed for use on warm-season grasses such as bermudagrass (*Cynodon dactylon* (L.) Pers.) as a means of improving turf quality during winter dormancy or hastening spring green-up. Research on using these products on creeping bentgrass has increased in recent years but is still limited, while research on warm-season grasses is almost nonexistent^{3,4}. Therefore, the objective of this experiment was to investigate the impacts of pigment-containing products on turfgrass physiology in both warm- and cool-season grasses during periods of respective stress.

Methods

Three pigment-containing products and three pigment-free products were selected for testing alone and in combinations for two field studies in 2013 and 2014 on creeping bentgrass and hybrid bermudagrass: Turf Screen (zinc oxide + titanium dioxide); PAR (copper-based pigment); Chipco Signature (fosetyl-aluminum + a copper-based pigment); Title Phyte (potassium phosphite); Turf Screen + Title Phyte; PAR + Title Phyte; and, Fosetyl-Al (fosetyl-aluminum). Products were applied bi-weekly for twelve weeks. Civitas (mineral oil) + Harmonizer (copper based pigment) and Harmonizer alone were added for 2014 field study on hybrid bermudagrass^{5,6}. All products were also used in bermudagrass spring green-up study.

Results and Discussion

In field studies, application of products caused a general increase in canopy temperatures (~0.5 to 3°C) compared to untreated controls of both grass species (Table 1). Bentgrass treated with products exhibited greater (~6 to 20 $\mu\text{mol CO}_2 \text{ cm}^{-2} \text{ s}^{-1}$) carbon dioxide exchange rates (CER) than the untreated control indicating a reduction in photosynthesis. Applications of Signature to hybrid bermudagrass in year two resulted in a more negative CER (-28.295 $\mu\text{mol CO}_2 \text{ cm}^{-2} \text{ s}^{-1}$) indicating greater photosynthetic activity. Decreased (24 to 50 relative) chlorophyll content in creeping bentgrass was observed in both study years by all treatments compared to the untreated while no effect was observed in bermudagrass. Normalized difference vegetation index (NDVI) reflectance ratio (greening)⁷ was reduced by all treatments except for Fosetyl-Al and Title Phyte in Study 1.

Root mass was unaffected following product use in either grass species. Creeping bentgrass tissue and soil zinc concentrations increased by ~820 ppm and ~4.75 kg ha⁻¹, respectively, following Turf Screen and Turf Screen + Title Phyte applications (Table 2).

¹ McCarty, L.B. 2011. Best Golf Course Management Practices. 3rd ed. Prentice-Hall Inc. Upper Saddle River, New Jersey. 776pp.

² Huang, B., X. Liu, and J.D. Fry. 1998. Effects of high temperature and poor soil aeration on root growth and viability of creeping bentgrass. *Crop Sci.* 38:1618-1622.

³ McCarty, L.B., J.R. Gann, C.E. Wells, and P.D. Gerard. 2014. Creeping bentgrass field response to pigment-containing products. *Agron. J.* 106:1533-1539.

⁴ Reynolds, W.C., G.L. Miller, and T.W. Ruffy. 2013. Athletic field paint color differentially alters light spectral quality and bermudagrass photosynthesis. *Crop Sci.* 53:2209-2217.

⁵ Lucas, L.T., and L.C. Mudge. 1997. Fungicidal compositions for the enhancement of turf quality. U.S. Patent 5 643 852. Date issued: 3 April

⁶ Kreuser, W.C. and F.S. Rossi. 2014. The Horticultural Spray Oil, Civitas™, Causes Chronic Phytotoxicity on Cool-season Golf Turf. *Hort. Sci.* 49:1217-1224.

⁷ Bremer, D.J., H. Lee, K. Su, and S.J. Keeley. 2011. Relationships between normalized difference vegetation index and visual quality in cool-season turfgrass. *Crop Sci.* 51:2212-2218.

Table 1. Canopy temperature, chlorophyll content, and normalized difference vegetation index (NDVI) of 'L-93' creeping bentgrass following various treatments and rates applied bi-weekly in two field studies conducted in 2013 and 2014 at Clemson University, Clemson, SC.

Treatment	Rate	Canopy Temperature	Chlorophyll Content		NDVI	
	L ha ⁻¹	C	Study 1	Study 2	Study 1	Study 2
—	—	Study 2	Study 1	Study 2	Study 1	Study 2
untreated	—	37.6 b	325 a	315 a	0.755 a	0.730 a
Turf Screen (TS)	7.97	39.0 a	295 bc	275 c-d	0.725 cd	0.695 b-d
PAR	1.17	39.1 a	280 c	265 de	0.710 d	0.685 cd
Title Phyte (TP)	12.57	38.3 ab	292 bc	290 b	0.745 ab	0.715 b
TS + TP	7.97 + 12.57	38.7 ab	285 de	268 de	0.725 cd	0.681 d
PAR + TP	1.17 + 12.57	39.3 a	286 bc	262 e	0.720 cd	0.682 d
Signature	19.13	38.6 ab	295 b	282 bc	0.727 b-d	0.705 bc
Fosetyl-Al	12.57	38.5 ab	287 bc	277 b-d	0.735 a-c	0.715 ab

Turf Screen = zinc oxide + titanium dioxide + pigment; PAR = copper phthalocyanine pigment; Title Phyte = potassium phosphite; Signature = Fosetyl-Al 80WG + Stress Gard. A 0-0-25 was added to all non- Title Phyte treatments at 12.6 L ha⁻¹. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.05$).

Table 2. Carbon dioxide exchange rate plus zinc tissue and soil levels of 'L-93' creeping bentgrass following various treatments and rates applied bi-weekly in two field studies conducted in 2013 and 2014 at Clemson University, Clemson, SC.

Treatment	Rate	CO ₂ Exchange	Tissue Zn		Soil Zn	
	L ha ⁻¹	$\mu\text{mol cm}^{-2} \text{s}^{-1}$	mg kg ⁻¹		kg ha ⁻¹	
—	—	Study 2	Study 1	Study 2	Study 1	Study 2
untreated	—	10.5 c	0 b	5 b	1.5 b	0.95 b
Turf Screen (TS)	7.97	22 ab	850 a	975 a	6.1 a	6.5 a
PAR	1.17	27 ab	10 b	10 b	0.95 b	1.15 b
Title Phyte (TP)	12.57	18.5 bc	5 b	10 b	0.5 b	1.6 b
TS + TP	7.97 + 12.57	29.5 ab	875 a	950 a	5.25 a	6.0 a
PAR + TP	1.17 + 12.57	30.5 a	12 b	5 b	1.0 b	0.85 b
Signature	19.13	20.5 a-c	12 b	5 b	0.5 b	1.1 b
Fosetyl-Al	12.57	27.5 ab	7.5 b	2 b	0.6 b	1.05 b

Turf Screen = zinc oxide + titanium dioxide + pigment; PAR = copper phthalocyanine pigment; Title Phyte = potassium phosphite; Signature = Fosetyl-Al 80WG + Stress Gard. A 0-0-25 was added to all non- Title Phyte treatments at 12.6 L ha⁻¹. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.05$).

Applications of PAR, PAR + Title Phyte, and Turf Screen + Title Phyte had an increase of ~27 ppm of copper in bentgrass shoot tissue. Applications to bermudagrass had similar results with zinc in soil and tissue analysis (data not shown). Spring green-up study revealed no differences in the effect of treatments on earlier breaking of dormancy of hybrid bermudagrass. The increased CER of treated bentgrass indicates a reduction in net photosynthesis while increased canopy temperatures promote a more stressful environment. Results suggest that several products investigated may promote greater heat stress on creeping bentgrass during times of hot, humid weather. Applications to bermudagrass during same time period did not show negative effects, however, the concentration of heavy metals could create future toxicity problems⁸.

⁸ Faust, M.B., and N.E. Christians. 2000. Copper reduces shoot growth and root development of creeping bentgrass. *Crop Sci.* 40:498-502.

INFLUENCE A SINGLE HIGH RATE NITROGEN APPLICATION OF POLYMER COATED UREA ON THE FATE OF NITROGEN

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Introduction

There has been interest in reducing the number of fertilizer application made by individual property owners or commercial management firms to save time and labor costs, while not sacrificing turfgrass quality or increasing the risk of groundwater contamination. The concept involves applying one season long fertilizer application, most often for nitrogen) at the beginning growing season with a controlled release source. However, whenever N is applied beyond plant use and soil storage, it has the potential to be lost to the environment by volatilization, runoff, leaching and nitrification. High single application rates of water soluble N sources have been shown to cause excessive N leaching^{1,2}, while controlled release sources had less N leaching³. Thus, the objective of this project is to determine if a high single N application rate (120 or 196 kg N/ha) application made once will a polymer coated urea (PCU) results in N leaching greater than 4 applications of a high urea containing fertilizer (total applied 196 kg N/ha) as well as influencing turfgrass quality and shoot growth.

Methods

The site is at the leaching field facilities at the Cornell University Turfgrass Research Lab-Bluegrass Lane, Ithaca, NY-USA. The study was composed of 20 plastic lined plots (5 fertilizer treatments and 4 replicates) that were 2.1 m. in dia, 0.5 m deep with a single drain used to collect all drainage water. Most of the plots were established in the mid-1990. The existing turf was removed in April 2012 and sodded with Kentucky bluegrass sod (cultivars: 30% Award, 30% Blueberry, 30% Bedazzled, and 10% Washington) on March 2012. Following a short establishment period, fertilizer treatments were started on April 2012 applied again in April 2013 and 2014. The plots were mowed weekly with a rotary mower set at 5 cm, clippings were removed. Treatments included: non-fertilized control, standard program of 4 applications/y at 49 kg N/ha (April, June, August, October) with 60-75% urea/25-40% PCU, single application made in April at 196 kg N/ha with PCU (Duration SIFI 35-0-10), single application in April at 120 kg N/ha with PCU (Duration SIFI 35-0-10) and PCU (Duration SIFI 35-0-10) applied in September at 120 kg N/ha (and small amounts of N as urea during the rest of the year). The amount of drainage water from each plot was recorded by using tipping buckets with data logger. Leachate samples were frozen until analysis for NO₃-N by a discrete analyzer. Clippings were collected monthly from May to October, dried, weigh for yield and analyzed for N content. Monthly color (0-100) was determined by digital photo analysis.

Results

The extent of nitrate leaching was related to the amount of total N applied, the amount of water soluble N applied and only during periods of heavy rain events during the 3 years of the study (Table1). In general, there was very low nitrate leaching, less than 0.25% of the amount of N applied. There were higher amounts of nitrate leaching from the standard program with the high amount of soluble N and the higher rate of PCU applied in the spring. These two fertilizer program also had the highest amount of N recovered in the monthly clipping sampling (Fig. 1). Turfgrass color response (Fig 2.) reflected N rate and timing. All spring applied N treatments had higher color for the first 3 months after application with the standard program having a higher color in the fall in 2012. The fall applied PCU had no effect on color in 2012 but did increase color in part of 2013. In summary, applying a high single rate of slow release N (PCU) once at the beginning of the growing season at a moderate rate (120 kg N/ha) did not increase nitrate leaching, had similar turfgrass quality (color) and nitrogen recovery in clippings as high N rate standard program.

¹ Huang, Z.T. and A.M. Petrovic. 1994. Clinoptilolite zeolite influence on nitrate leaching and nitrogen use efficiency in simulated sand based golf greens. *J. Environ. Qual.* 23:1190-1194.

² Kopp, K.L. and K. Guillard. 2005. Clipping contributions to nitrate leaching from creeping bentgrass under varying irrigation and rates. *International Turfgrass Society Research Journal.* 10:80-85.

³ Brown, K.W., R.L. Doble and J.C. Thomas. 1977. Influence of management and season on the fate of N applied to golf greens. *Agron.J.* 69:667-671.

Table 1. Nitrate⁻ leaching (kg ha⁻¹) as a function of N source and rate over the 3-year study period

Treatment	N Rate yr ⁻¹	2012	2013	2014	Total
----- Kg ha ⁻¹ -----					
Unfertilized control	-	0.04a	0.06b	0.05b	0.15b (-) ¹
Polymer coated urea	120	0.09a	0.12b	0.05b	0.27b (0.08)
Polymer coated urea	196	0.13a	0.16b	0.36a	0.65a (0.11)
Polymer coated urea (fall)	120	0.09a	0.13b	0.12b	0.34b (0.09)
Standard program	196	0.21a	0.40a	0.10b	0.71a (0.12)

¹ Percent of applied N that leached over the 3-year study

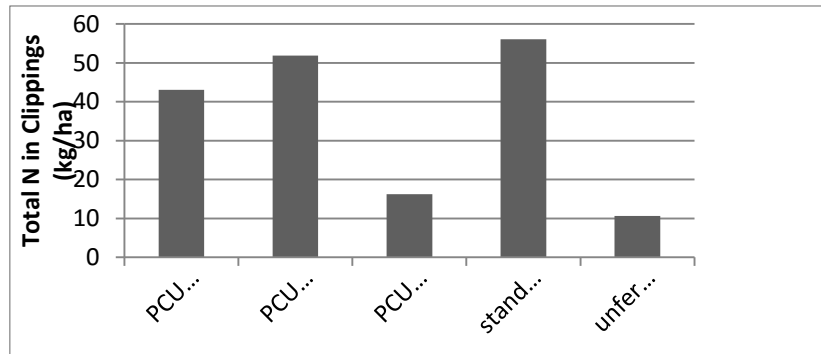


Figure 1. The total amount of nitrogen recovered in clippings for 2012 and 2013 from monthly sampling

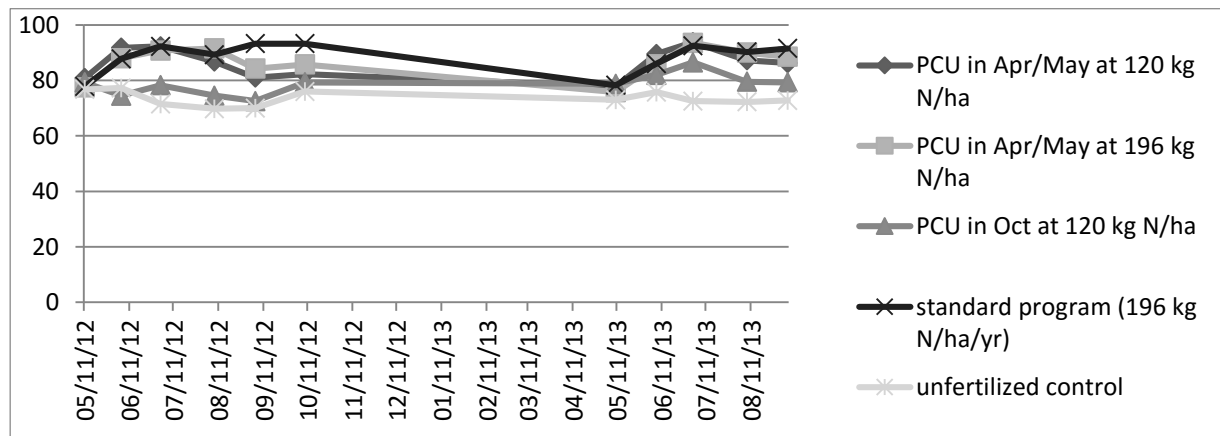


Figure 2. Turfgrass color values (0-100) for 2012 and 2013 from monthly sampling

CARBON SEQUESTRATION IN CENTRAL CHILE: THE EFFECT OF NEWLY ESTABLISHED TURFGRASS COVER AND ITS RELATIONSHIP WITH PHOTOSYNTHESIS

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Introduction

Climate change has given importance to research on terrestrial ecosystems as carbon (C) sinks. Photosynthesis is the main process where plants sequester CO₂¹. An important characteristic of urban land-use conversion with respect to soil organic carbon (SOC) storage is the introduction of turfgrass cover and management^{2,3,4}. Since 1950, the Chilean urban population increased by 30%. Currently, 89% of the total Chilean population is urban, and this percentage is expected to increase to 93% by 2050⁵. Under this context, turfgrasses, as a main surface of Chilean urban green areas, play an important function in providing suitable spaces for human and nature interaction and, at the same time, sequestering C. The objectives of this study were to assess and compare the magnitude of carbon sequestration on seven turfgrass species and bare soil using seasonal organic carbon stocks measurements above ground (Aerial Organic Carbon (AOC) and below ground (Soil Organic Carbon (SOC)) and to associate these data with turfgrass seasonal photosynthetic behavior.

Methods

Festuca arundinacea Schreb (cultivars Bingo and Cochise), *Festuca rubra* L. ssp *rubra* (cv Cindy Lou), *Cynodon dactylon* L. (common cv), *Cynodon dactylon* L. x *Cynodon transvaalensis* Burt Davy (cv Tifway II), *Poa pratensis* L (cv Kenblue), *Poa trivialis* L (Sabre II) and *Lolium perenne* L (cultivars Derby Xtreme and Premier II), and areas with bare soil were established during 2010. Soil samples from 10 cm, 20 cm and 30 cm were collected seasonally (Spring 2010, Autumn 2011, Spring 2011, Autumn 2012). Cuttings from each turfgrass species were also collected seasonally after reaching the 4-cm cutting height. Soil Organic Carbon and Aerial Organic Carbon (data not shown) were assessed using the Modified Mebius method. The CO₂ assimilation rate, stomatal conductance and photosynthetic water use efficiency were measured seasonally over two years using an Infrared Gas Analyzer. In order to compare the SOC storage of different turfgrass species, the organic carbon percentage was transformed to SOC ton ha⁻¹ using the soil bulk density per layer and layer thickness as described by Wairu and Lal⁵. After checking for normality and homogeneity of variances, the data were analyzed using Proc GLM by SAS®. One overall analysis of variance (ANOVA) considering season, turfgrass species and soil depth as factors and was conducted to show the changes in SOC over time. A repeated measures ANOVA was used to analyze the SOC by season using depth as the repeated factor. Mean SOC treatments were compared by depth and season using Fisher's LSD (p<0.05).

Results and Discussion

The comparison of the total SOC as the mean sum of three soil depths (Table 1) by the seasons of measurement showed that the SOC increased from spring 2010 to autumn 2012 for all turfgrass cultivars, but not bare soil. In addition, high assimilation rates, between 40 and 50 μmol m⁻² s⁻¹, were detected from *Cynodon dactylon* during summer 2011 and 2012. Comparatively, the cool season turfgrass species showed lower assimilation rates, between 5 and 20 μmol m⁻² s⁻¹ (Figure 1). In general, lower CO₂ assimilation values occurred during 2012 when compared to 2011. This study shows, turfgrass species with high photosynthetic activity during the summer, such as *Cynodon dactylon* and *Festuca arundinacea* 'Bingo' (Figure 1), resulted in a high total SOC of 2.4 ton SOC ha⁻¹ and 2.7 ton SOC ha⁻¹, respectively, when compared with bare soil (0.9 ton SOC ha⁻¹) at the end of the study (Table 1).

¹ Lal, R. 2004. Carbon emission from farm operations. *Environ Int* 30: 981-990.

² Kaye, J., R. McCulley, and I. Burke. 2005. Carbon fluxes, nitrogen cycling, and soil microbial communities in adjacent urban, native and agricultural ecosystems. *Global Change Biology* 11: 575-587.

³ Milesi C, S. Running, C. Elvidge, J. Dietz, B. Tuttle, and R Nemani. 2005. Mapping and modeling the biogeochemical cycling of turf grasses in the United States. *Environ Manage* 36: 426-438.

⁴ Wairiu, M., and R. Lal. 2003. Soil organic carbon in relation to cultivation and topsoil removal on sloping lands of Kolombangara, Solomon Islands. *Soil Till Res* 70(1): 19–27.

⁵ United Nations Division of the Department of Economic and Social Affairs. 2014. World Urbanization Prospects The 2014 revision Highlights: Accessed January 20, 2015: <http://esa.un.org/unpd/wup/Highlights/WUP2014-Highlights.pdf>

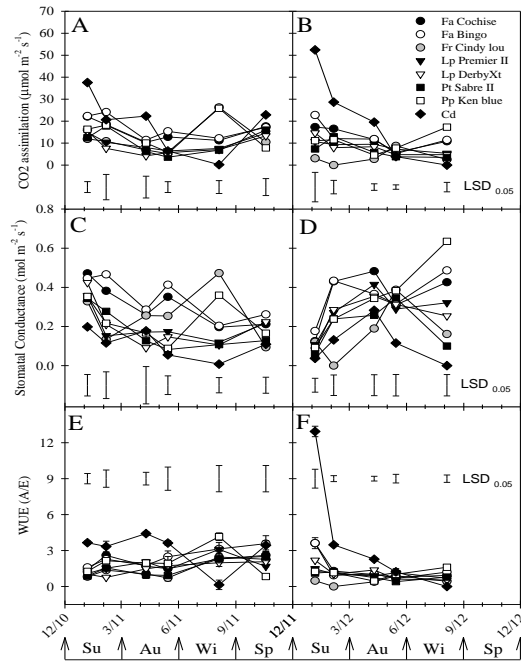


Figure 1. Seasonal physiological (photosynthesis, stomata conductance and water use efficiency) behavior in 6 turfgrass species over 2 years (closed circle=*Festuca arundinacea* Cochise, open circle=*Festuca arundinacea* Bingo, gray circle=*Festuca rubra rubra*, closed triangle=*Lolium perenne* Premier II, open triangle=*Lolium perenne* Derby Xtreme, closed square=*Poa trivialis* Sabre II, open square=*Poa pratensis* Kenblue and closed diamond=*Cynodon dactylon*). A. Seasonal photosynthesis, B. Stomata conductance and C. Water Use Efficiency. SU= Summer; AU=Autumn; WI=Winter; SP=Spring.

Table 1. Total Soil Organic Carbon (SOC) (ton SOC*ha⁻¹) by turfgrass cover, including three soil depths. The data were collected during three years in Pirque Metropolitan Region, Chile. Significant differences between total mean SOC by season for different turfgrass species and bare soil were determined using Fisher's LSD (p≤0.05).

Turfgrass cultivar/cover	Spring 2010	Autumn 2011	Spring 2011	Autumn 2012
Total SOC ton ha ⁻¹				
No cover/bare soil	1.7 c	1.9 a	1.8 b	0.9 b
<i>Poa pratensis</i> cv Kenblue	2.1 bc	2.3 a	2.2 ab	2.4 a
<i>Poa trivialis</i> cv Sabre II	2.0 a	1.8 a	2.3 ab	2.2 a
<i>Lolium perenne</i> DerbyXtreme	2.1 bc	2.2 a	2.2 ab	2.6 a
<i>Lolium perenne</i> PremierII	2.4 abc	1.9 a	2.5 ab	2.4 a
<i>Festuca rubra rubra</i> cv Cindy Lou	2.1 c	2.3 a	2.1 a	2.3 a
<i>Festuca arundinacea</i> cv Cochise	2.6 a	2.2 a	2.5 ab	2.5 a
<i>Festuca arundinacea</i> Bingo	1.7 abc	2.2 a	2.6 ab	2.7 a
<i>Cynodon dactylon</i> cv common	2.0 abc	2.1 a	2.4 ab	2.3 a
<i>Cynodon dactylon</i> cv <i>Cynodon transvaalensis</i> cv Tifway II	1.9 abc	2.1 a	1.9 ab	2.5 a

TURFGRASS NUTRITION AND PHISIOLOGY

POSTER PRESENTATIONS

GERMINATION OF TALL FESCUE CARYOPSES EXPOSED FOR LONG-TERM AT LOW TEMPERATURES

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Introduction

Tall fescue (*Festuca arundinacea* Schreb.), a cool-season bunch-type grass, is a wise choice for stockpiling because it maintains its nutritional value longer into winter than many other forage crops ¹. Its native range encompasses a geographical area that stretches from the Mediterranean coasts to the high altitudes of the Alps and the northern latitudes of continental Europe. This wide distribution is possible thanks to its ability to survive in a wide range of soils, climates, and management conditions ². In response to temperature changes, the allohexaploid genome of this species changes the numbers of interspersed DNA repeats when seedlings are exposed to 10°C as opposed to 30°C ³. Such genome plasticity allows this species to achieve optimal growth dynamics at different developmental stages and in different environments, which may explain, at least in part, the ability of this species to survive over such a wide geographical area. The major factors limiting tall fescue are climatic and geographic, rather than edaphic or biotic ⁴. A number of studies have been conducted on how tall fescue germination, growth and development is affected by limiting temperatures. Knowledge of the response to sugar starvation and adaptation mechanisms in plants is a basic research interest as well as having important agronomic implications. In this paper, we assess the influence temperature has on tall fescue seedling establishment under nutritional stress. To achieve this objective, we carried out a time-course experiment to verify the ability of the caryopses to complete germination under prolonged cold temperatures in complete absence of light and nutrients. We also analyzed the temperature-driven changes at physiological and molecular level. Examination of photosynthetic tissues in plants grown for 400 d under cold conditions hinted at the ability to rapidly restore photosynthetic activity upon illumination. We then examined whether there exists an alternative stress acclimation process over the long term that involves a decrease in storage compound hydrolysis, a drastic reduction of growth and amylases stabilization by mobilization of calcium molecules.

Methods

Caryopses of *Festuca arundinacea* Schreb., cv. Arminda, were sterilized in sodium hypochlorite solution (5% active chlorine) for 10 min. After surface sterilization, they were rinsed ten times in sterile water and placed in Petri dishes containing sterile bi-distilled water. Growth behavior and physiological parameters were recorded in growth chamber under two different temperature regimes, 23°C or 4°C, both in the dark. Temperature monitoring was carried out with a data logger (Campbell Scientific, Logan, UT, USA), and relative humidity was monitored with a hygromograph in each chamber. Three independent, repeated experiments were performed for each experimental condition. All treatments were performed in parallel. For each combination of treatment and target time (21 and 400 d, at 23 and 4°C, respectively, as reported in figures), samples were collected, and immediately processed or ground in liquid nitrogen and stored at –80°C for further molecular and biochemical analyses.

¹ Dierking, R.M. and R.L. Kallenbach. 2012. Mediterranean and Continental tall fescue: II. Effects of cold, nonfreezing temperatures on leaf extension, proline, fructan, and abscisic acid. *Crop Sci.* 52: 460-469. doi:10.2135/cropsci2011.03.0160.

² Hopkins, A.A., M.C. Saha and Z.Y. Wang. 2009. Breeding, Genetics, and Cultivars. In: H. A. Fribourg, D. B. Hannaway and C. P. West, editors, *Tall Fescue for the Twenty-first Century*. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. p. 339-366.

³ Ceccarelli, M., M. Esposto, C. Roscini, V. Sarri, M. Frediani, M. Gelati, et al. 2002. Genome plasticity in *Festuca arundinacea*: direct response to temperature changes by redundancy modulation of interspersed DNA repeats. *Theor. Appl. Genet.* 104: 901-907. doi:10.1007/s00122-001-0862-4.

⁴ Burns, J.C. and D.S. Chamblee. 1979. Adaptation. In: R. C. Buckner and L. P. Bush, editors, *Tall Fescue*. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. p. 9-30.

Chemicals analysis: kits for protein extraction were purchased from Bio-Rad Laboratories (Hercules, Ca, USA), kits and gels for electrophoresis were purchased from Amersham Biosciences (Uppsala, Sweden), and all the other reagents used were purchased from Sigma (St. Louis, Mo, USA). Histological analysis: Abscisic acid quantification by radioimmunoassay (RIA); Gibberellic acid bioassay; Starch analysis; Recovery experiments were carried out to evaluate loss during extraction. Two tests were performed for each metabolite by adding a known amount of authentic standards to the samples before proceeding with the extraction. The concentrations of the standards added were similar to those estimated to be present in the tissues in preliminary experiments. The percentage of recovery ranged between 96 and 104% depending on the sugar. The quantity of soluble carbohydrates was corrected on the basis of the recovery percentages for each sample, and expressed as $\mu\text{moles hexose equivalents g}^{-1} \text{FW}$. Post recovery analysis: protein extraction for enzyme activity; zymogram of total amylolytic activity; amylolytic activity in *Festuca arundinacea* mixed with *Hordeum vulgare*. Statistical analysis: Statistical analysis of biometric and physiologic traits was performed using one-way analysis of variance (ANOVA). All computations were performed with R 3.2.3⁵.

Results and Discussion

Tall fescue (*Festuca arundinacea* Schreb.) caryopses grown at 4°C in the dark for an extended period in complete absence of nutrients, showed an unexpected ability to survive. Seedlings grown at 4°C for 210 days were morphologically identical to seedlings grown at 23°C for 21 days. After 400 days, seedlings grown at 4°C were able to differentiate plastids to chloroplast in just a few days once transferred back to the light and 23°C. Tall fescue exposed to prolonged cold showed marked anatomical changes: cell wall thickening, undifferentiated plastids, more root hairs and less xylem lignification. Physiological modifications were observed, in particular related to sugar content, GA and ABA levels and amylolytic enzymes pattern. The phytohormones profiles exhibited under different temperatures were comparable when normalized to the physiological state exhibited. Both the onset and the completion of germination were linked to GA and ABA levels, as well as to the ratio between these two hormones. All plants showed a sharp decline in carbohydrate content, with a consequent onset of gradual sugar starvation. This explained the slowed then full arrest in growth under both treatment regimes. The analysis of amylolytic activity showed the stabilization of several isoforms, with Ca^{2+} playing a central role. The present work functionally characterizes tall fescue germination in response to combined abiotic stresses. In this work, tall fescue is further validated as a model species for physiological studies of thermal adaptation as it expresses strong persistency and high tolerance to cold and nutritional stress. Convergence of starvation signals and hormone signals meet in crosstalk to regulate α -amylase activity upon germination in tall fescue in the cold, with stable amylase isoforms being induced by prolonged exposure.

⁵ R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

HIGH-TEMPERATURE EFFECTS ON SEED GERMINATION OF FOURTEEN KENTUCKY BLUEGRASS CULTIVARS

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Introduction

Kentucky bluegrass (*Poa pratensis* L.) is a perennial cool-season grass commonly used for establishing sport and ornamental turfgrasses in transition zones. This species is rather difficult to establish because seeds take up to three weeks to germinate. Considered best adapted to areas where temperatures are moderate it is usually sowed during late summer or at most in early spring, because of slower establishment in comparison with other cool-season grasses¹. September is the ideal time for sowing Kentucky bluegrass in northern Italy because generally soil temperature and moisture are stable and high enough of a good germination, moreover there is a very little competition from weeds. Spring sowing is more problematic especially because soil temperatures vary significantly across the season and may increase up to 30 °C. Due to climate changes spring temperatures not favorable for germination of cool season species are expected to occur more frequently than in the past. This scenario is confirmed by numerous studies which predict for Italy an increase summer days and tropical nights^{2,3}. Few studies investigated the effect of high temperatures on germination of Kentucky bluegrass. Larsen et al.⁴ observed that germination percentage of cultivars Andantea and Brodway assessed in laboratory was lower at 25 °C than 10 °C and pointed out that the optimum temperature for germination is lower than 25 °C. Other studies on Kentucky bluegrass observed a drastic decrease of germination capacity at high temperatures⁵, while Van't Klooster⁶ in a growth chamber experiment found significant differences in temperature germination response among Kentucky bluegrass cultivars.

With the aim to evaluate the effect of high temperatures on seed germination among the most popular cultivars of Kentucky bluegrass in northern Italy, a laboratory study was conducted in conditioning chamber comparing fourteen cultivars under five temperature regimes.

Methods

A study was conducted at the laboratory of the Agricultural Research Council in Tavazzano (Lodi), Italy, to define the germination rate of fourteen Kentucky bluegrass cultivars at five levels of alternating temperatures. The experiment was conducted from June 2014 to May 2015 in conditioning chambers (mod. CRIOCABIN 2004, Cavallo srl, Milan). The Kentucky bluegrass cultivars tested were: Right, JumpStart, Moonlight Slt, Nublu Plus, Blue Sapphire, Brooklown, Marauder, Platini, Sobra, Barimpala, Mercury, Balin, Geronimo and Evora. Fifty seeds for each cultivar were placed on filter paper saturated with deionized water (ml 4.0) in glass Petri dishes (11 cm diameter). Additional deionized water was added weekly as needed to keep the filter paper moist. An 8h day photoperiod was adopted. The levels of alternating temperatures tested were: 20/30, 23/33, 26/36, 29/39, 32/42 °C. Irradiance was provided with cold white fluorescent tubes at 880 lux / square meter during the high temperature period. The Petri dishes were arranged in a completely randomized design and replicated six times. The seed was prechilled at a temperature of 5 °C for 5 days in accord with ISTA rules.

The final germination percentage (FGP) was calculated by counting the number of germinated seeds every 7 days for a total of 28 days. Seed vigor was analyzed by the calculation of the Germination Rate Index as

¹ Larsen, S. U. and Bo Martin B. 2005. Differences in thermal time requirement for germination of three turfgrass species. *Crop Science* 45 (5): 2030-2037.

² Toreti, A. and Desiato F. 2008. Changes in temperature extremes over Italy in the last 44 years. *International Journal of climatology* 28: 733-745.

³ Zollo, A.L., Rillo V., Bucchignani E., Montesarchio M. and Mercogliano P. 2015. Extreme temperature and precipitation events over Italy: assessment of high-resolution simulations with COSMO-CLM and future scenarios. *International Journal of Climatology* DOI: 0.1002/joc.4401.

⁴ Larsen, S. U., Baily C., Come D. and Corbineau F. 2004. Use of hydrothermal time model to analyse interacting effects of water and temperature on germination of three grass species. *Seed Science Research* 14: 35-50.

⁵ Aamlid, T.S. and Arntsen D. 1998. Effects of light and temperature on seed germination of *Poa pratensis* from high latitudes. *Acta Agriculturae Scandinavica, Section B, Soil and Plant Science* 48:4, 239-247.

⁶ Van 't Klooster, G. 2007. Response of grass seed germination relative to soil temperature. *Greenkeeper International*, April, 16-17.

recommended by Maguire⁷ as well as of the Corrected Germination Rate Index. Germination Rate Index (GRI) was calculated using the formula: $GRI = [(G7/7) + (G14/14) + \dots + (Gx/x)]$ where Gx is the number of normal seedlings germinated from the last count on day (x) and x represents the corresponding day of the count (e.g. 7, 14, 21, 28)⁸. The Corrected Germination Rate Index (CGRI) was also calculated using the formula: $CGRI = \left(\frac{GRI}{FGP}\right) * 100$ where FGP is the final germination percentage. The data of FGP, GRI, and CGRI were transformed as required and were subjected to ANOVA using SAS Proc Mixed (version 9.2; SAS Institute, Cary, NC) followed by multiple comparison of means using Fisher's protected LSD test at the 0.05 probability level.

Results and discussion

The treatment of 29/39°C was the highest temperature regime where germination was observed. At alternating temperature of 32/42° C no germination was observed for all the tested cultivars. Under temperature treatments of 20/30, 23/33, and 26/36 great germination was observed and differences among cultivars were negligible. In figures 1 are shown cumulative germination percentages of the fourteen cultivars under temperature treatment of 29/39°C. Such image clearly displays the large difference among cultivars for their ability to germinate. Under this temperature 'Jumpstar' displayed the highest final germination percentage followed by 'Brooklawn' and 'Blue Sapphire' while 'Nuble Plus', 'Evora' and 'Sobra' showed a very low germination (Fig. 2). It is interesting to note that 'Nuble Plus' revealed the highest germination percentage under the 20/30 °C regime (data not shown).

The highest GRI and CGRI occurred at temperature treatment of 23/33° C. Among cultivar tested 'Balin' displayed the highest CGRI, while Mercury and Marauder, followed by Barimpala, had the lowest (data not shown). Observing the correlations between germination percentage at 14 and 28 days for each temperature treatment, we noted an increase of R^2 with temperature treatments. Correlation coefficient ranged from 0.309 to 0.970 for 20/30° C and 29/39 °C respectively. The increase of R^2 is due to the increase of germination percentage during the first two weeks with the increase of temperatures.

Our results corroborate those of Van't Klooster's (2007)⁶ who reported good germination at high temperatures of some Kentucky bluegrass cultivars. However, Van't Klooster's (2007)⁶ reported high germination capacity at high temperatures of 'Barimpala', while in the present study this cultivar showed poor germination at temperature of 29/39°C (Fig. 2).

The results of this study displayed the high diversity in high temperature germination response among Kentucky bluegrass cultivars. Therefore, in the coming years, cultivar selection based on germination rate at high temperatures should be essential for allowing a rapid and regular establishment of Kentucky bluegrass turfgrasses. However, a field study will be essential in understanding the influence of temperature germination response on speed of establishment.

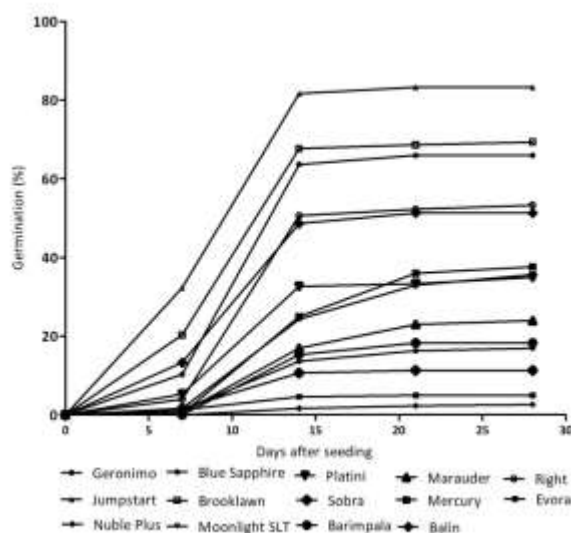


Fig.1. Cumulative germination percentage of fourteen Kentucky bluegrass cultivars in conditioning chamber under alternating temperatures 29/39 °C.

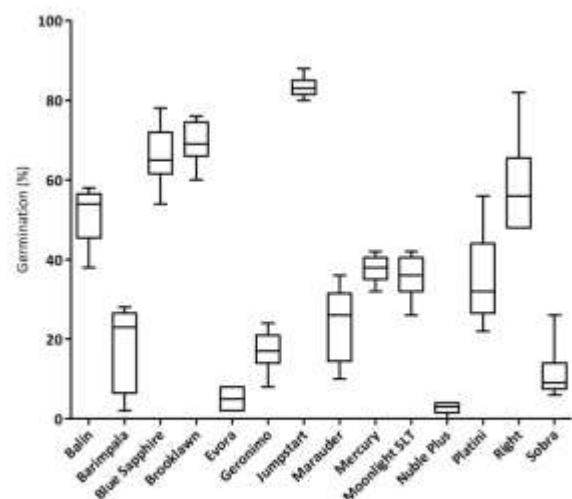


Fig.2. Box plots (minimum, first quartile, median, third quartile, and maximum) of final germination percentage after 28 days in conditioning chamber under alternating temperatures 29/39 °C of fourteen Kentucky bluegrass cultivars under.

⁷ Maguire, J.D. 1962. Speed germination-aid in selection and evaluation for seedling emergence and vigour. *Crop Science* 2: 176–177.

⁸ Esehie H. 1994. Interaction of salinity and temperature on the germination of sorghum. *Journal of Agronomy and Crop Science* 172: 194–199.

ESTIMATED NET ECOSYSTEM (NEE) ON TURFGRASS AT DIFFERENT MANAGEMENT INTENSITIES IN A GOLF COURSE IN THE PROVINCE OF VERONA

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Introduction

The carbon (C) sequestration potential of turf grass systems has been investigated and demonstrated in several studies^{1,2,3,4,5}. The role of these ecosystems in continental and Mediterranean climates, however, are not yet clearly understood due to environmental limiting factors and because management intensities can strongly influence the overall C budget.

The aim of this present study is to improve the understanding of the mechanisms underlying C fluxes in a turf grass ecosystem and to assess its C sequestration potential by estimating the annual C budget. NEE of turf grass was calculated in its seasonal variation over one year, and compared between areas characterized by different degrees of maintenance.

Material and Methods

The C sequestration potential of the turf grass was investigated in a golf course near Verona (Italy), adopting a small-chamber enclosure approach⁶. The measurements of gas exchanges between biosphere and atmosphere, allowed us to estimate the Net Ecosystem Exchange (NEE), as a function of different management intensities.

The intensity of management seems to have had influence on its C balance.

This study needs further research to understand which maintenance variables are determinant on turf grass C sequestration.

The study was conducted from August 2012 to September 2013 at the Verona Golf Club. The area used for the surveys was the 18th hole of the golf course, extended for 18,270 m², positioned close to the club house and the technical recovery.

The course of the 18th hole features different playing areas, characterized by different species composition and management intensities. In descending order from the high maintained playing areas, we define green, tees, collar, fairway, semi-rough, mount and rough.

The degree of management for each playing area was determined by collecting data regarding cultural operations during the surveys period. The data was provided by the superintendent of the Verona Golf Club, who monitored all the cultural operations effectuated in the different playing areas during the period of the present study.

Within the course, 20 NEE measurement points were marked with iron plates positioned in different playing areas. These areas have been then grouped in 3 categories according to their degree of maintenance: high intensity (HI), including tees, green and collar, medium intensity (MI), including fairway and semi-rough, and low intensity (LI), including rough and mount. HI, MI and LI had respectively 4, 12 and 4 measurement points. The number of measuring points repeated for playing areas was proportional to the width of their area within the hole.

¹ Chapin III F.S. and Matson P.P.A. 2011. Principles of terrestrial ecosystem ecology, Springer, pp. 529.

² Pataki D.E. 2006. Controls on C and N cycling in a southern California urban turfgrass ecosystem. *Soil Carbon and California's Terrestrial Ecosystems* 2005221.

³ Zhou X., Wang X., Tong L., Zhang H., Lu F., Zheng F., Hou P., Song W. and Ouyang Z. 2012. Soil warming effect on net ecosystem exchange of carbon dioxide during the transition from winter carbon source to spring carbon sink in a temperate urban lawn. *Journal of Environmental Sciences* 24: 2104-2112.

⁴ Allaire S.E., Dufour-L'Arrivée C., Lafond J.A., Lalancette R. and Brodeur J. 2008. Carbon dioxide emissions by urban turfgrass areas. *Canadian Journal of Soil Science* 88: 529-532.

⁵ Livesley S.J., Dougherty B.J., Smith A.J., Navaud D., Wylie L.J. and Arndt S.K. 2010. Soil-atmosphere exchange of carbon dioxide, methane and nitrous oxide in urban garden systems: impact of irrigation, fertilizer and mulch. *Urban Ecosystems* 13: 273-293.

⁶ Baldocchi D.D., Hincks B.B. and Meyers T.P. 1988. Measuring biosphere-atmosphere exchanges of biologically related gases with micrometeorological methods. *Ecology*: 1331-1340.

Results and Discussion

Whole-canopy gas exchange measurements were performed with a portable IRGA (EGM-4, PP Systems, UK) equipped with a canopy chamber (CPY-2, PP Systems, UK) characterized by the following measures:

- 14.5 cm height x 14.6 cm diameter;
- 167 cm² of exposed area;
- 2425 cm³ of volume.

In the spring and autumn, diurnal values of NEE were similar both in the maxima and in the minima, the latter representing the highest diurnal uptake rates observed in the whole period. Night time respiration was however higher in spring and summer than in autumn. The maximum and minimum diurnal values during summer were closer to zero compared to spring and autumn. During the winter, the diurnal values of NEE were always negative while the nocturnal respiration obviously showed the lowest values.

During the day, the relationship between CO₂ flux ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$) was well described by linear functions.

All the maintenance categories areas showed similar NEE trends, however the mean daily CO₂ fluxes were different according to the intensity of maintenance and the season.

The intensity of turf management seems to have an influence on its C balance. Carbon dioxide emissions from high intensity surfaces were lower compared to low intensity ones, however this could be the result of methodological errors and need further research to understand which maintenance variables are determinant on turf grass C sequestration.

CONTRIBUTION OF GRASS-CLIPPINGS TO TURFGRASS FERTILIZATION UNDER FOUR N LEVELS

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Introduction

Returning grass clippings on turfgrass is a sustainable practice with many benefits. In addition to reducing the workload associated with lawn mowing, clippings are a source of nutrients for turfgrass, reducing the need for fertilizers. However, the reported contribution of clippings to turfgrass fertilization is highly variable (from 30 to 50%) making it difficult for turfgrass managers to adapt their fertilization practices. It has been shown that increasing N fertilization increases both yield and tissue N concentration of grass clippings¹, but very little information is available on P and K content of grass clippings.

Another presumed benefit of returning grass clippings is an increase in soil water conservation. By releasing water during their decomposition, protecting the soil from direct sunlight and increasing soil organic matter, fresh grass clippings could increase soil moisture content. However, to our knowledge, no published research has measured the impact of returning grass clippings on soil water content.

The objective of this study was to quantify the yield and leaf mineral content of Kentucky bluegrass (*Poa pratensis* L.) clippings under four different N levels (0, 0.48, 0.96 and 1.44 kg N 100 m⁻² yr⁻¹) and to measure the impact of returning grass clippings on soil water content.

Methods

The experimental site was established in July 2011 on the experimental farm located on Université Laval campus in Québec city (46°46'47"N, 71°17'07"W). Vegetation was first removed using a sod cutter, and Kentucky bluegrass (*Poa pratensis* L.) was sodded on the exposed soil (St-Nicolas schist loam). Experimental design was a 2 X 4 factorial experiment in a randomized complete block design with 4 m² plots (2 m x 2 m) replicated four times. Treatments consisted of two clippings management strategies (clippings returned or clippings removed) and four nitrogen fertilization levels (0, 0.48, 0.96 and 1.44 kg N 100 m⁻² yr⁻¹). Fertilizer application was undertaken by hand on each plot, split into four applications each year (May, June, August and September) starting in May 2012. Plots were not irrigated and water supply depended only on natural precipitations.

Following the first fertilizer application and throughout the growing season (May to October), turfgrass was mowed to a height of 8 cm once it reached a height of 12 cm, which corresponded to about 7 to 10 days of growth. This resulted on 21 mowing dates in 2012, 23 mowing dates in 2013 and 22 mowing dates in 2014. All plots were mowed using a motorized mulching mower, on which a collection bag was attached before mowing plots when clippings were removed. One pass of a 40-cm wide helicoidal hand mower was made in each plot where clippings were returned prior to mowing in order to collect a leaf sample. Leaves were then dried during 48 hours at 60 °C, weighted to determine clippings dry weight and ground to a fine powder. For nitrogen, subsamples of 100 mg of leaf material were first digested using a mixture of sulfuric and selenious acids, and N content in samples was then measured by colorimetry. Another 300 mg sample of leaf material was collected, and calcinated at 500 °C during 16 hours. Phosphorus content was then measured by colorimetry while potassium content was determined using a atomic absorption spectrophotometry.

Volumetric soil moisture content was measured using a FieldScout TDR-300 portable moisture meter (Spectrum technologies, Plainview, IL, USA). A total of ten readings per plot were taken weekly between June and October using 7.5 cm rods.

Data were analyzed using linear mixed models with year and collection week treated as repeated measures, nitrogen fertilization levels and collection week as fixed effects and blocks as random effect. All statistical analyses were performed with SAS software version 9.4 (SAS Institute, Cary, NC, USA). Means were compared with the least significant difference (LSD) method with a significance level of 5%.

¹ Kopp, K. L. & K. Guillard. 2002. Clipping management and nitrogen fertilization of turfgrass: growth, nitrogen utilization, and quality. *Crop Science*, 42: 1225–1231.

Results and discussion

Clippings contribution to fertilization

Increasing N fertilization rate had a significant positive effect on clipping dry yield for all four treatments. Clipping dry weight increased significantly with N levels, and ranged from 21 kg 100 m⁻² yr⁻¹ for unfertilized plots to 59.5 kg 100 m⁻² yr⁻¹ for plots that received the highest N rate (i. e. 1.44 kg N 100 m⁻² yr⁻¹).

Nitrogen fertilization also influenced significantly N and P content in turfgrass clippings, but not K content. Throughout the year, increasing the amount of N applied with the fertilizer increased N content in clippings even if the magnitude of the effect varied between weeks (Interaction “Week X Nitrogen level” significant). For phosphorus, there was also a significant “Week X Nitrogen level” interaction, but generally, leaf P content decreased as the rate of applied N increased. This is likely the result of a dilution effect: since no supplemental P was provided with the fertilizer, P absorption by the plant remained stable, but an increase in plant biomass resulted in a lower relative leaf P content.

Total contribution of turfgrass clippings to fertilization was likely to be more affected by clippings weight than by clippings mineral content. The amount of N returned to turf through clippings increased with the fertilizer level for all weeks when samples were collected (Figure 1). Increasing N fertilization levels increased the amount of, N P and K returned to turfgrass by clippings, with the exception of the increase from 0.48 to 0.96 kg N 100 m⁻² yr⁻¹, which did not result in a significant difference in the quantity of P and K returned by clippings. The total quantity of N in clippings was higher than the amounts applied by the fertilizer, indicating a recycling of N in the soil-plant system.

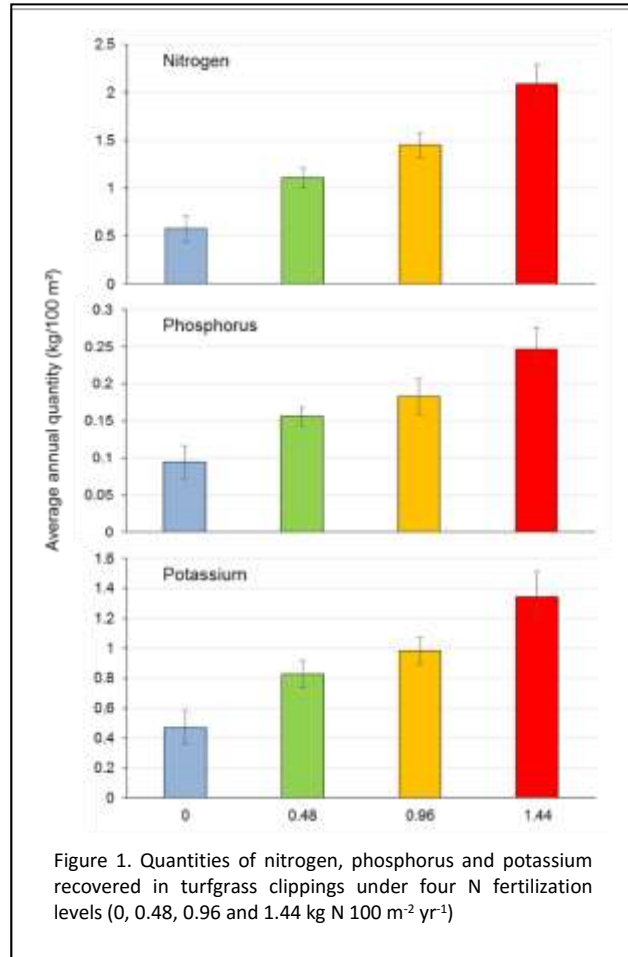


Figure 1. Quantities of nitrogen, phosphorus and potassium recovered in turfgrass clippings under four N fertilization levels (0, 0.48, 0.96 and 1.44 kg N 100 m⁻² yr⁻¹)

Soil volumetric water content

Returning grass clippings resulted in a significant increase in soil volumetric water content. On average, soil underneath the plots where clippings were returned retained approximately 4% more water compared to plots where clippings were removed.

Conclusion

The contribution of grass clippings to turfgrass fertilization varies based on N fertilization levels. This practice has a positive impact on nutrient recycling and, under low fertility, increases soil water content.

BIOGEOCHEMICAL CYCLING OF CARBON AND NITROGEN IN COOL-SEASON TURFGRASS SYSTEMS

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Managed turf areas are both a source and a sink for greenhouse gases (GHGs). Management practices, including turfgrass selection and mowing, influence the amount of carbon (C) and nitrogen (N) stored in the soil, as well as the associated GHG emissions. The objective of this research was to determine the net C and N balance (i.e. the amount of C and N stored less the amount emitted) of managed turfgrass systems with different grasses (species and cultivars) and management practices (mowing frequency and grass clippings management). Data explicitly quantified in this experiment include annual mowing requirements and accompanying GHG emissions, annual dry matter yield, soil C and N accumulation, and GHG flux of tall fescue (*Schedonorus arundinaceus* Schreb.) and Kentucky bluegrass (*Poa pratensis* L.) cultivars with varying growth rates. Leaf, verdure, and root tissue C and N were also determined, along with the corresponding biomass. Estimations of emissions from fertilization, irrigation, and pesticide applications were also included in the net balance calculations.

Kentucky bluegrass and tall fescue had gross total soil C accumulations of 1.408 and 1.629 Mg C ha⁻¹ yr⁻¹, respectively, in the top 5 cm of soil. Returning grass clippings over a 2 yr period increased total soil C to a 5 cm depth by 0.532 Mg C ha⁻¹, which was an increase of about 3% compared to when clippings were collected. The relative increase in total soil N was similar to that of soil C in this experiment. Returning grass clippings for 2 yr resulted in an increase of 0.066 Mg N ha⁻¹ compared to when clippings were collected (1.516 vs. 1.450 Mg N ha⁻¹). Mowing emissions ranged from approximately 28 to 68 kg C equivalent ha⁻¹ yr⁻¹, depending on the species, growth rate, and management practices. The combined emissions from mowing and assumed emissions for fertilization, irrigation, and pesticides did not exceed that of the gross total soil C accumulations. Thus, all of the turfgrasses and management practices in this experiment resulted in a system-wide net C sink, though the magnitude of the sink was variable.

In general, higher-yielding grasses and management practices that increased soil C also increased mowing requirements and thus emissions. Returning grass clippings was found to increase yield, soil and leaf tissue N, and soil C, but it also marginally increased mowing requirements. The results of this experiment support the assertion that managed turfgrass areas can act as a net C sink to help curb the increasing atmospheric GHG concentrations. The C sequestration potential of managed turfgrass is another of the numerous functional and environmental benefits of urban grasslands.

LOSS ON IGNITION: A RAPID AND SIMPLE METHOD TO MONITOR SOIL ORGANIC MATTER CONTENT UNDER DIFFERENT TURFGRASS SPECIES GROWN IN CENTRAL CHILE

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Introduction

Organic matter is a key component in soils because it can contribute to water holding capacity, interact with clay minerals, sorb and desorb natural and synthetic compounds, absorb and release plant nutrients¹. Turfgrasses provide a suitable place environment for diverse and active microbes where organic matter is easily formed and maintained². The loss-on-ignition (LOI) method for the determination of organic matter involves the heated destruction of all organic matter in the soil³. The objective of this study was to determine the effect of eight different turfgrass covers versus no turfgrass cover on organic matter by soil depth content over time.

Methods

In 2010 *Festuca arundinacea* Schreb (cultivars Cochise and Bingo), *Festuca rubra* L. ssp *rubra* (cv.Cindy Lou), *Cynodon dactylon* L.(common cv), *Cynodon dactylon* L. x *Cynodon transvaalensis* Burt Davy (cv.Tifway), *Poa pratensis* L.(cv Kenblue), *Poa trivialis* L (cv. Sabre II), *Lolium perenne* L.(cultivars Derby Xtreme and Premier II) and areas with bare soil were established in a randomized complete block design, 3 blocks. Soil samples from 10 cm, 20 cm and 30 cm depth were collected seasonally (Spring 2010, Autumn 2011, Spring 2011, Autumn 2012). Soil type corresponds to a sandy loam Estero Seco Ultic Haploxerolls (Mollisol) with an original organic matter content of three percent.

Soil organic matter (SOM) was determined using the loss-on-ignition (LOI) method, in which a known weight of dry soil sample is placed in a ceramic crucible and then heated at 400°C overnight. SOM was calculated, as a percentage, between the initial and final sample weights divided by the initial sample weight times 100%. The weights were corrected for moisture content prior to organic matter content calculation. Data were analyzed using Proc GLM by SAS© (SAS©Institute Inc., Cary, NC, 2002). Overall analysis of variance (ANOVA) considered block, season, turfgrass specie soil depth as factors. In addition, a repeated measures ANOVA was used to analyze the SOM by season using depth as the repeated factor. Mean SOM treatments were compared by depth and season using Fisher's LSD ($p < 0.05$).

Results and Discussion

Soil depth was a significant factor ($p < 0.0001$) in three seasons out of four included in this study (Table 1). The greatest amount of soil organic matter (SOM) was detected at the 0 to 10 cm soil depth. Considering season as significant factor ($p < 0.01$), higher percentages of SOM was observed at the end of the study for all turfgrasses (Table 1), indicating that a turfgrass ecosystem is able to increase SOM over time as observed by Yao and Shi². Turfgrass species was not a significant factor ($P < 0.05$), however *Festuca arundinacea* 'Cochise', resulted in a highest percentage of SOM at the end of the study (Table 1), which is explained by the high performance of this specie under Chilean climate⁴. SOM is a simple way to calculate soil organic carbon (SOC) and estimate changes over time using different turfgrass species as soil covers. However since changes in SOM and SOC are slow processes, more time is needed to better understand the effect of different turfgrass species on SOM changes over time.

¹ Schumacher, B. 2002. Methods for the determination of total organic carbon in soils and sediments. United States Environmental Protection Agency, Environmental Sciences Division National, Exposure Research Laboratory. P.O Box 93478 Las Vegas, NV 89193-3478.

² Yao, H and Shi, W. 2010. Soil organic matter stabilization in turfgrass ecosystems: importance of microbial processing. Soil Biology and Biochemistry 42: 642-648.

³ Robertson, S. 2011. Direct Estimation of Organic Matter by Loss on Ignition: Methods http://www.sfu.ca/soils/lab_documents.html. Last Accessed: 03/15/2016.

⁴ Acuña, Alejandra A., T. Karl Danneberger and Claudio Pastenes. 2013. Turfgrass adaptation in Central Chile. International Turfgrass Society Research Journal Vol.12: 757-760

Table 1. Mean Soil Organic Matter (SOM), as percentage, by turfgrass specie, soil depth and season of sampling, overall Analysis of Variance (ANOVA) and repeated measures ANOVA by season. Mean SOM treatments were compared by depth and season using Fisher's LSD ($p < 0.05$).

	SPRING 1			AUTUMN 1			SPRING 2			AUTUMN 2		
	Soil Depth			Soil Depth			Soil Depth			Soil Depth		
Turfgrass cover	0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm
<i>Poa trivialis</i>	3.58	3.58	2.75	4.58	3.41	2.43	3	3.53	3.53	3.81	4.1	3.6
<i>Cynodon dactylon</i>	3.98	3.73	5.2	3.81	3.26	2.42	3.77	3.53	3.53	4.56	3.21	2.53
<i>Festuca rubra rubra</i>	4.01	3.59	2.38	3.79	3.47	2.97 a	3.4	3.91	3.91	4.21	3.15	3
<i>Festuca arundinacea</i> cv Bingo	3.87	3.7	2.21	3.66	3.27	2.85 a	4.32	3.18	3.18	5.04	3.61	3.05
No Cover	3.35	2.74	2.72	3.63	3.63	3.20 a	3.08	3.3	3.3	3.4	3.47	3.03
<i>Festuca arundinacea</i> cv Cochise	3.49	3.03	3.03	3.63	3.34	2.86 a	3.8	3.79	3.79	5.27	3.69	3.49
<i>Poa pratensis</i>	3.92	2.86	2.41	3.52	2.78	2.84 a	3.42	3.54	3.54	4.06	3.7	3.24
<i>Lolium perenne</i> cv Premier II	4.05	3.16	2.85	3.38	3.67	2.83 a	4.12	3.92	3.92	4.97	3.88	2.67
<i>Lolium perenne</i> cv Derby Xtreme	3.66	3.18	2.61	3.27	2.89	3.14 a	4.35	3.37	3.37	4.13	4.1	2.84
<i>Cynodon dactylon</i> x <i>Cynodon transvaalensis</i>	3.30	2.68	2.72	3.12	2.29	2.61 a	3.31	3.39	3.39	3.71	3.17	2.55
Least Significant Difference (LSD)	1.13	1.6	2.04	1.37	1.1	0.93	1.62	1.45	1.45	1.57	1.19	1.18
Overall ANOVA												
Source of variation	DF	Pr>F										
Block	2	0.74										
Turfgrass cover	9	0.35										
Soil Depth	2	<0.0001										
Season	3	0.01										
Turfgrass cover*block	18	0.08										
Soil Depth*block	4	0.72										
Soil Depth*Turfgrass cover	18	0.8										
Turfgrass cover*season	27	0.21										
Soil Depth*Turfgrass cover*block	36	0.99										
REPEATED MEASURES ANOVA												
	SPRING 1			AUTUMN 1			SPRING 2			AUTUMN 2		
Source of variation	DF	Pr>F		DF	Pr>F		DF	Pr>F		DF	Pr>F	
Within subjects effects												
Soil Depth	2	0.0081		2	0.0001		2	0.76		2	<0.0001	
Soil Depth*Block	4	0.35		4	0.46		4	0.56		4	0.81	
Soil Depth*Turfgrass cover	18	0.68		18	0.73		18	0.54		18	0.26	
Error	34			30			34			32		
Between subjects effects												
Block	2	0.04		2	0.59		2	0.82		2	0.31	
Turfgrass cover	9	0.16		9	0.49		9	0.95		9	0.58	
Error	17			15			17			16		

EXPERIMENTAL EVALUATION OF GOLF COURSES GRASSLANDS AS A CO₂ SINK

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Introduction

The role of greenhouse gases to trap infrared radiation in the atmosphere and their contribution to Global Warming is a subject of extreme importance. The reduction of emissions and the development of new sinks are the main environmental goals for XXI century.

In the South of Iberian Peninsula, the number of golf courses has increase due to the mild climatic conditions over all the year. They contribute to the economic development of the area, increase the surface of green areas, but can cause different types of environmental problems. In this sense, it is important to evaluate their sustainability under the fragile Mediterranean areas.

In the analyses of ecosystem services, golf courses can be considered as a sink of CO₂ as they include large grass areas and some islands of woodlands. They also act as green areas favouring landscape connectivity.

In order to evaluate the capacity of the golf grasses to trap carbon, we have developed an experimental approach, growing grasses under different concentration of carbon dioxide. Plant performance was evaluated through the application of physiological measurements of gas exchange, biomass estimation, climatic data and the use of ecological models to calculate carbon sinks.

Methods

Our experimental material was a set of two grass species collected from golf courses in the Algarve and grown in the General Greenhouse Service of the University of Seville. Grasses were cultivated in 100 pots (12cm diameter and 9 cm depth) under two different CO₂ atmosphere and four different water regimes 100%, 75%, 50% y 25% of soil field capacity. We selected two different carbon dioxide concentrations: 400 ppm, which corresponds to present atmospheric concentration and 700 ppm, which would occur in an enriched CO₂ atmosphere. Two common species were selected: *Agrostis stolonifera* (agrostis) commonly used in greens and tees and *Cynodon dactylon* (Bermuda) commonly used in fairways and rough.

To estimate the annual capacity of grasses as a sink of carbon we use an Infrared Gas Analyser LI-6400. Gas exchange is useful to characterize leaf-level CO₂ and H₂O flux, stomatal conductance and water use efficiency (calculated as the ratio between photosynthetic assimilation and transpiration). This system allows controlling CO₂ concentration at a fixed level, temperature 20±3°C and relative humidity around 50%. To evaluate quantic yield we have carried out light curves from 0 to 2000 μmols.m⁻².s⁻¹ (measuring values were 0, 50, 200, 750, 1000 and 2000 μmols.m⁻².s⁻¹). These data have been used to estimate sink capacity of the species through the application of ecological models.

Aerial and underground biomass data of the different grasses has been obtained at the end of the experiment in order to evaluate the total capacity of species to trap carbon.

CLIMA subsystem from the regional government of Andalucía has been used to obtain climatic information.

To estimate the capacity of the species to fix atmospheric CO₂ we have used a model that integrates physiological, biomass and climatic data¹.

Results and Discussion

Light saturation curves represent the photosynthetic capacity of the species under different light regimes; experimental values have been adjusted to Edwards-Walker model of photosynthetic metabolism to obtain the different parameters of photosynthetic response. In agrostis photosynthetic capacity saturates at irradiance values of 500 μmols.m⁻².s⁻¹, while bermuda increases its capacity of assimilate carbon until 2000 μmols.m⁻².s⁻¹. The different parameters of the model are also shown in the graphic as the red table inside the plot:

¹ Muñoz Vallés, S., Cambrollé, J., Figueroa-Luque, E., Luque, T., Niell, F.X., Figueroa, M.E., 2013. An approach to the evaluation and management of natural carbon sinks: From plant species to urban green systems. *Urban Forestry & Urban Greening* 12(4), 450–453.

m1 = maximum photosynthesis, which is close to 3 in agrostis and 4.5 $\mu\text{mols CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in bermuda. m2 = light compensation point, or the light intensity in which net CO_2 balance is zero, this value ranges near 8 in agrostis to 36.5 $\mu\text{mols}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in bermuda. Finally, m3 means the irradiance value in which species reaches $\frac{1}{2}$ of maximal photosynthetic capacity, this value is 31 in agrostis and 91 in bermuda.

With these results we can conclude that agrostis presents lower photosynthetic capacity than cynodon, but this species adjusts well under shaded environments. While bermuda is better adapted to open areas, with elevated light intensity reaching higher values of CO_2 even under elevated irradiance.

Under full light intensity bermuda also presented higher values of dry weight than agrostis, especially because this species accumulated higher underground biomass, but both species didn't present higher performance when they are growing under an enriched CO_2 atmosphere.

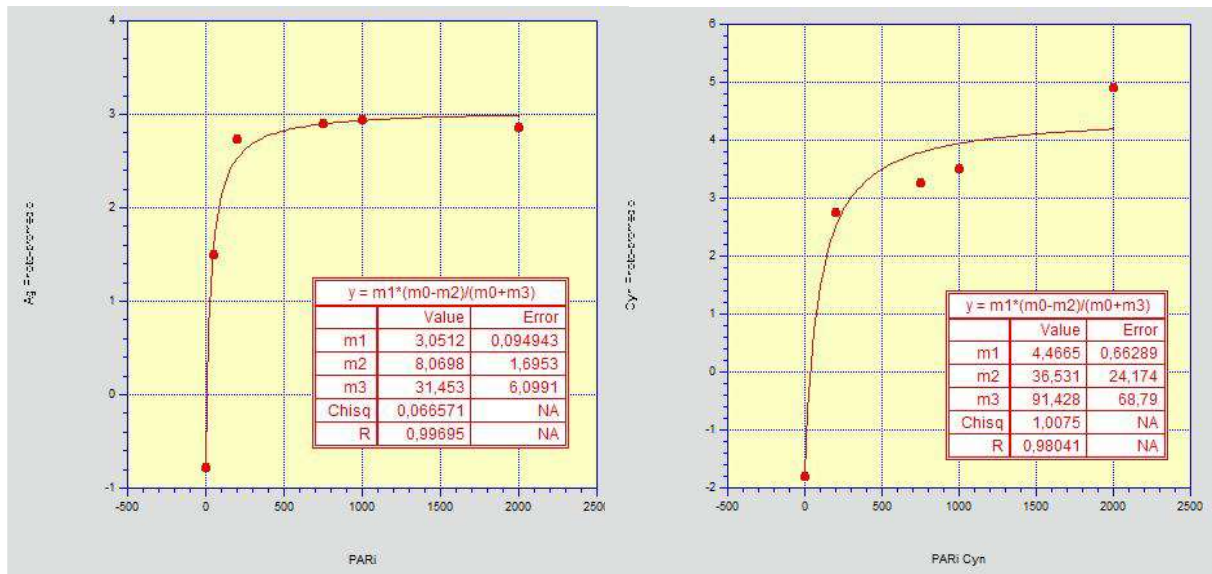


Figure 1. Light saturation curves for agrostis (left) and bermuda (right). In this graphics CO_2 assimilation in the Y-axis is represented under different levels of irradiance intensity in the X-axis. Experimental data have been adjusted to Edwards-Walker model, and the parameters of the model are included in the plot (see text)