7th ETS CONFERENCE 2020

TURF SOLUTIONS for the FUTURE

Ausgewählte Fachbeiträge für die aufgrund der Corona-Pandemie abgesagten 7. ETS-Konferenz in Amsterdam.

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- Ausgabe 02/20: **„Drought, Irrigation and Water consumption“**
- Ausgabe 03/20: **„Disease and Pest Management + Biostimulants“**
- Ausgabe 04/20: **„Maintenance and Nutrition + Impact for the Environment“**

Inhalte Ausgabe 03/20

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Selected papers (Part 2/3) for the 7th ETS Conference 2020, cancelled due to Covid-19

Turfgrass management practices influence fungicide fate on golf course putting greens

Stephens, C.M., J.P. Kerns and T.W. Gannon

Introduction

Fungicide applications are required for disease management on amenity turfgrass systems throughout the United States. Previous research and label recommendations often suggest using pre- and post-application management strategies such as irrigation and delaying mowing events when targeting soil-borne pathogens[1]. Recent research has demonstrated you can remove up to 34% of azoxystrobin with tall fescue clippings following a single mowing event one day after application[2]. However, there limited published research documenting the effects of these practices on fungicide movement on highly maintained golf course putting greens. Determining the influence of post-application management practices on fungicide movement and removal with clippings can help turfgrass managers optimize fungicide application and limit off-target effects. Therefore, the objective of this research is the investigate the influence of post application irrigation and mowing timing on fungicide movement through the soil and fungicide removal with turfgrass clippings.

Materials and Methods

A field experiment was conducted in Raleigh, NC in June 2018. 'A1' creeping bentgrass (*Agrostis stolonifera*) was maintained as a golf course putting green and mowed 6 times week^{-1} at 3.8 cm height of cut prior to study initiation. A single application of pyraclostrobin (0.55 kg a.i. ha^{-1}), triadimefon (3.05 kg a.i. ha^{-1}), and penthiopyrad (1.1 kg a.i. ha^{-1}) was applied and plots received 0.64 cm of post-application irrigation either immediately (0 hour) or 6 hours after the fungicide application. Once the canopy was dry following irrigation treatments, turfgrass clippings were collected at 0, 1, or 3 days after treatment (DAT) using a greens mower set to a height of 0.38 cm. Soil cores were collected using a standard golf course cup cutter at 0, 1, 3, 5, 7 and 14 DAT and subsequently dissected into the remaining above ground vegetation (RAV; verdure+thatch), 0-2.54 cm, 2.54-5.08 cm, and 5.08-7.62 cm subsections. All samples were homogenized using a Fitz Mill with dry ice and fungicide residue was analyzed using high performance liquid chromatography-mass spectrophotometry2. Experimental units were arranged as a randomized complete block design with three replications. Statistical analyses were conducted by analysis of variance using the MIXED procedure in SAS (Version 9.4, ASA Institute Inc., Cary, NC) and means were separated using Fishers Protected least significant difference test at $P < 0.05$.

Results and Discussion

Fungicide recovery ranged from 90-93%, 92-99%, and 92-95% of the percent applied at 0 DAT for pyraclostrobin, triadimefon, and penthiopyrad, respectively (Figure 1, 2 and 3). Only a minor amount of fungicide (0.19-2.31%) was removed with turfgrass clippings regardless of mowing and irrigation treatment. Fungicide was de-

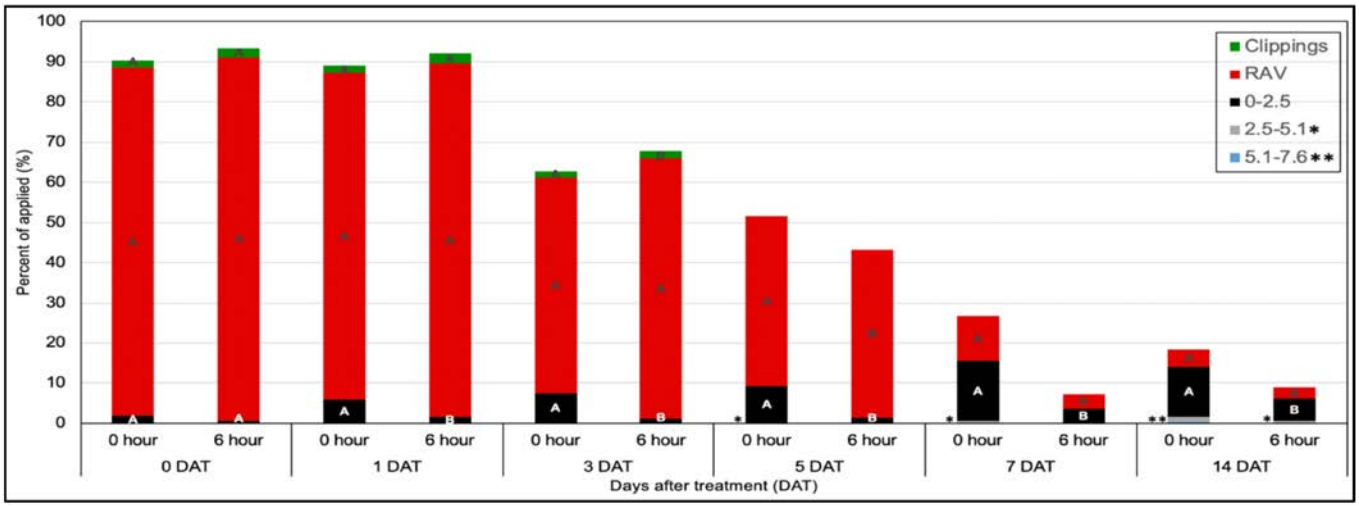

Fig. 1: Environmental fate of pyraclostrobin following different post application irrigation timings. Means denoted with the same letter between irrigation treatments within each day after treatment at each individual depth are not statistically different according to Fishers Protected LSD test at P<0.05. 'RAV' signifies the remaining above ground vegetation and the '*' and '' signify residue detection at the 2.5-5.1 and 5.1-7.6 cm depth, respectively.**

[1] OU, L. and R. LATIN, 2018: Influence of management practices on distribution of fungicides in golf course turf. Agronomy Journal 110:2523.

[2] JEFFRIES, M.D., F.H. YELVERTON, K.A. AHMED and T.W. GANNON, 2016: Persistence in and release of 2,4-D and Azoxystrobin from turfgrass clippings. Journal of Environmental Quality 45:2030-2037.

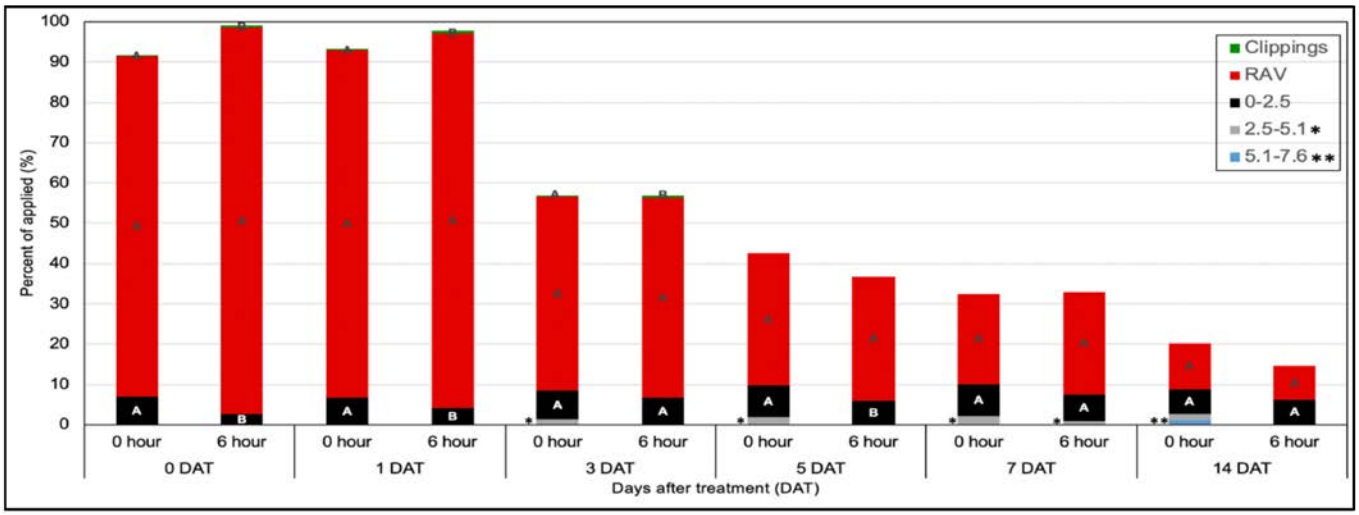

Fig. 2: Environmental fate of triadimefon following different post application irrigation timings. Means denoted with the same letter between irrigation treatments within each day after treatment at each individual depth are not statistically different according to Fishers Protected LSD test at P<0.05. 'RAV' signifies the remaining above ground vegetation and the '*' and '' signify residue detection at the 2.5-5.1 and 5.1-7.6 cm depth, respectively.**

tected in the 5.08 to 7.62 cm depth at 14 DAT only when plots were irrigated immediately for pyraclostrobin, triadimefon, and penthiopyrad. Less penthiopyrad was detected in the RAV and total fungicide recovery was greater through 5 DAT compared to pyraclostrobin and triadimefon. Pyraclostrobin recovery was greater in plots that were irrigated immediately compared to plots that were irrigated 6 hours after treatment on 5, 7, and 14 DAT (Figure 1). This finding may have implications for residual control of this fungicide if irrigating immediately after application. More penthiopyrad was detected in the 0-2.54 cm depth at 1 DAT compared to pyraclostrobin and triadimefon. In general, irrigating immediately, and to a lesser extent delaying mowing events, resulted in greater fungicide movement into deeper depths and less fungicide removed with turfgrass clippings. Post-application management practices can significantly influence fungicide removal with turfgrass clippings and fungicide movement through the soil profile. These findings have implications on optimizing soil-borne disease management with fungicides and limiting off-target environmental effects.

Authors:

Cameron M. Stephens and
James P. Kerns
Department of Entomology
and Plant Pathology

Travis W. Gannon
Department of Crop and
Soil Science, North Carolina State
University, Raleigh, NC 27695

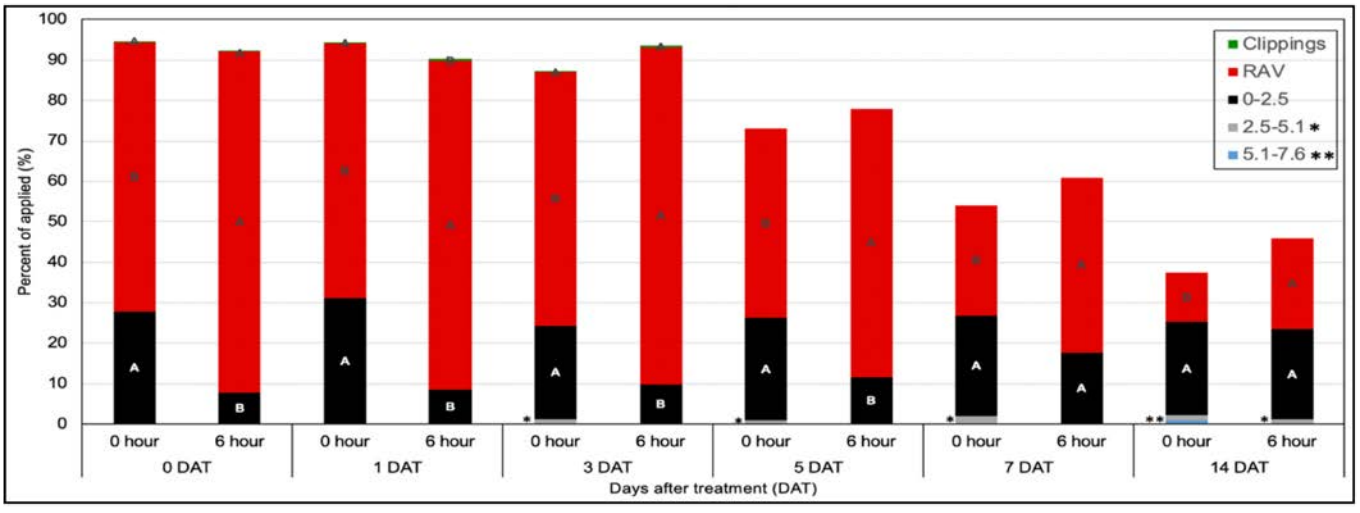

Fig. 3: Environmental fate of penthiopyrad following different post application irrigation timings. Means denoted with the same letter between irrigation treatments within each day after treatment at each individual depth are not statistically different according to Fishers Protected LSD test at P<0.05. 'RAV' signifies the remaining above ground vegetation and the '*' and '' signify residue detection at the 2.5-5.1 and 5.1-7.6 cm depth, respectively.**

Creeping bentgrass summer decline as influenced by climatic conditions and cultural practices

Miller, G.L. and M.A. Brotherton

Introduction

Despite its popularity as a putting surface in North Carolina, creeping bentgrass (*Agrostis stolonifera* L.) is highly susceptible to summer bentgrass decline (SBD) during hot summer months. Summer bentgrass decline is a label applied to the reduction in bentgrass quality as a result of heat-induced stresses.[1] Temperature, the primary contributing factor to SBD, cannot be readily controlled in a golf course setting.[2] Soil properties beneath creeping bentgrass (*Agrostis stolonifera* L.) putting greens are in constant flux. Changes in soil properties are largely reflective of cultural management strategies and can influence overall turfgrass health. Research[3] suggested that cultural management programs may help alleviate secondary stresses that can induce SBD. The objectives of this study were to detail the impacts of N fertility, soil moisture content and hollow- and solid-tine cultivation on creeping bentgrass quality and disease incidence.

Materials and Methods

A study was conducted from October 2008 to December 2010 in Raleigh, NC on two, 2-year old creeping bentgrass putting greens seeded to 'Penn A-1'. The greens were built to Unites States Golf Association (USGA) specifications. Putting greens were mowed five times per week, June through September at 4 mm. The remainder of the year, the greens were mowed at 3.5 mm three to five times per week. Cultural treatments included four N rates (97, 195, 293 and 391 kg ha^{-1} yr^{-1}), four hollow-tine cultivation programs (6.4 mm diameter tines two times yr^{-1}, 9.5 mm diameter tines two and three times yr^{-1} each using 5.1 x 5.1-cm spacing and a control that received no core cultivation), two soil moisture levels (low and high) achieved by daily irrigation to 80% evapotranspiration or approximately weekly irrigation to 40% historical evapotranspiration and summer solid-tine cultivation (spiked or not spiked) with bayonet tines spaced 5.1 x 7.6 cm and penetrated the soil to an 8.9-cm depth. The experiment was arranged in a strip-strip block design. Quadrants of each green were separated by a plastic barrier installed through the depth of the rootzone, which allowed for isolated irrigation and drainage; therefore, enabling the creation of distinct replicated soil moisture levels.

Visual turfgrass quality were taken weekly by the same experienced rater from March through November. Disease incidence were measured several times over a two-year period using established techniques. Soil moisture, soil temperature, and air temperature measurements were continuously monitored. Treatment and interaction effects were determined using PROC MIXED of the Statistical Analysis System (Table 1). Means were separated using the LSMEANS statement and were subjected to least significance test.

Cultural Practice	Quality†
Spiking, S‡	0.284
Moisture, M	0.024
S x M	0.981
Fertility, F	<0.001
F x S	0.210
F x M	<0.001
F x S x M	0.348
Cultivation, C	<0.001
C x S	0.496
C x M	0.858
C x S x M	0.577
C x F	<0.001
C x F x S	0.991
C x F x M	0.577
C x F x S x M	0.977
CV %	14.0

† Units = Quality, scale 1-9;

‡ S = summer solid-tine spiking cultivation, M = soil moisture, F = N fertility, C = hollow-tine cultivation

Tab. 1: Results from analysis of variance of the main effects of spiking, soil water content, N fertility, and hollow-tine coring and their interactions on visual turfgrass quality. ANOVA response represents means of evaluations collected over the two years.

Results and Discussion

It is important to consider weather patterns when evaluating SBD. Compared to 2009, prolonged higher temperatures from June through October in 2010 exposed the turfgrass to greater heat stress. Initially, differences in turfgrass quality were minimal. Prolonged supra-optimal temperature in September and October of 2010 caused large continual decreases in turfgrass quality during that time. From June through October, when SBD typically occurs in the southeast, daily minimum and maximum air temperature were 17.2 and 27.6 °C in 2009 and 19.0 and 30.5 °C in 2010, respectively. In response, the average turfgrass quality decreased from 8.3 in early June 2009 to a low of 6.8 by the end of August. In 2010, SBD was more pronounced as average turfgrass quality decreased from 8.1 in early June to a low of 5.0 in late September (full dataset not shown). From June through October of 2009, mean soil water content was 16.9% and 13.5% for the high and low soil water content treatments, respectively. Mean soil water contents in 2010

[1] CARROW, R.N., 1996: Summer decline of bentgrass greens. Golf Course Manage. 64:51-56.

[2] BEARD, J.B., 1997: Dealing with heat stress on golf course turf. Golf Course Manage. 63:54-59.

[3] FU, J., P.H. DERNOEDEN and J.A. MURPHY, 2009: Creeping bentgrass color and quality, chlorophyll content and thatch-mat accumulation responses to summer coring. Crop Sci. 49:1079-1087.

were 17.5% and 14.6% for the high and low soil water content treatments, respectively. June through October 2009 had four weeks, and in 2010 there were three weeks, when soil water content treatment did not affect ($p < 0.001$) turfgrass quality (data not shown).

Turfgrass that received N at 391 kg ha^{-1} consistently possessed the highest turfgrass quality. Acceptable turfgrass quality (>7) was never achieved with a N rate of 97 kg ha^{-1}. In 2009 turfgrass that received 195 kg ha^{-1} N rate maintained acceptable turfgrass quality until early-August, when it fell below the acceptable quality threshold until mid-September. In 2010, turfgrass that received a 195 kg ha^{-1} N rate maintained acceptable quality until mid-July. Turfgrass that received annual N rates of 293 and 391 kg ha^{-1} followed a similar pattern except those programs provided acceptable turfgrass quality until mid-August. In 2009, turfgrass that received annual N rates of 97 and 293 kg ha^{-1} demonstrated higher turfgrass quality under all the high than under the low soil water content treatments. In 2010, differences in turfgrass quality existed between the two soil water content treatments under all N fertility programs except those plots that received N rates of 391 kg ha^{-1}. Results suggest higher N rates combined with low soil water content can attain similar turfgrass quality as lower N rates and increased soil water content. Differences in turfgrass quality existed among hollow-tine core cultivation regimes on some of the rating dates (data not presented). The majority of those dates were within 3-4 weeks following core aerification with the greatest reduction in turfgrass quality in plots cultivated with the largest diameter tines. Dollar spot incidence was seen throughout 2009, but only a mild outbreak was noted in early 2010 (data not shown).

Results from this research suggest N fertility and irrigation management can aide in maintaining acceptable quality putting greens over the length of a summer in the southeast USA. Nitrogen rates greater than 195 kg ha^{-1} yr^{-1} were needed to maintain acceptable turfgrass quality. Adequate water in the soil profile, particularly the surface 5 cm, played a large role in providing better creeping bentgrass quality. Interactions between soil water content and N fertility affected creeping bentgrass quality. A low soil water content could to a certain extent be compensated by a high nitrogen rate. A 391 kg ha^{-1} N rate moderated the reduction in turfgrass quality from the most aggressive core cultivation program (9.5 mm tines, 3 times per year on 5.1 cm spacing) compared to that seen with lower rates. Results from this study may allow golf course superintendents some flexibility in their management strategy regarding annual N rates and irrigation programs.

Authors:

Grady L. Miller* and
Mark A. Brotherton
Crop & Soil Sciences, North Carolina State University, Campus Box 7620, Raleigh, NC 27695.

* Corresponding author:
grady_miller@ncsu.edu

Hohe Auszeichnung der Crop Science Society of America für Rasenexperten Bernd Leinauer, NMSU

Quelle: NMSU, Bearbeitung: Dr. Klaus Müller-Beck

Der Rasenexperte, Prof. Dr. Bernd Leinauer, von der New Mexico State University erhielt die Auszeichnung als Fellow der Crop Science Society of America. ***(Foto: K.G. Müller-Beck)***

Professor Dr. Bernd Leinauer von der New Mexico State University (NMSU) wurde für das Jahr 2020 zum Fellow der Crop Science Society of America nominiert und gewählt.

Der Preis wird für herausragende Beiträge zur Pflanzenwissenschaft in den Bereichen der Ausbildung, der nationalen und internationalen Beratungen sowie der Forschungsaktivitäten verliehen. Die Ernennung zum „CSSA-Fellow“ ist die höchste Anerkennung, die von der Crop Science Society of America verliehen wird. Etwa 0,3 Prozent der CSSA-Mitglieder kommen in den Genuss, als Fellow gewählt zu werden.

Die Deutsche Rasengesellschaft e.V. ist voll Freude über die Auszeichnung des langjährigen DRG-Mitgliedes und gratuliert Bernd Leinauer zu dieser erneuten Würdigung seiner herausragenden Forschungs- und Beratungsarbeit.

In einem laufenden Projekt untersuchen die Forscher der NMSU derzeit die Auswirkungen von Tensiden auf die Wassereinsparung und die Bodengesundheit bei Rasenflächen.

B. Leinauer ist seit dem Jahre 2000 an der NMSU als Rasen-Spezialist am College of Agricultural, Consumer, and Environmental Sciences Cooperative Extension Service tätig.

„Als Extension-Spezialist fühle ich mich besonders geehrt, diese Auszeichnung zu erhalten, weil sie meine Beratungsbemühungen und mein Engagement zur Unterstützung der Rasenindustrie sowie unsere Forschungsaktivitäten im Bereich der kommunalen Wassereinsparung anerkennt“, sagte Leinauer. „Ich möchte auch meinem außergewöhnlichen Team danken, weil eine solche Leistung das Ergebnis jahrelanger harter Arbeit des gesamten Rasenteams ist und sie verdienen die gleiche Anerkennung.“

Die Auszeichnung ist besonders hoch einzuschätzen, weil Prof. Leinauer bereits 2017 als „Fellow der American Society of Agronomy“ ausgezeichnet wurde. Als Fellow von zwei renommierten Gesellschaften gewürdigt zu werden, ist eine herausragende Ehrung, die nur sehr wenigen Wissenschaftlern zu Teil wird.

„Bernd hat ein sehr erfolgreiches Forschungs- und Extension-Programm entwickelt, das ihn und sein Team als einen der führenden Forscher und Beratungs-Experten auf dem gesamten Sektor der Wasserversorgung von Rasenflächen etabliert hat“, sagte Rolston St. Hilaire, Abteilungsleiter der Pflanzen- und Umweltwissenschaften der NMSU. „Die Abteilung für Pflanzenwissenschaften freut sich, dass er für seine zahlreichen Beiträge für die Rasenindustrie anerkannt wird.“

Prof. Bernd Leinauer ist international als Autorität für Wassermanagement-Strategien zur Reduzierung des Trinkwasserverbrauchs auf Rasenflächen anerkannt. Seine Forschungsarbeiten beinhalten die Untersuchung von Rasenflächen mit geringem Wasserverbrauch, kälte- und salztoleranten Rasenflächen, die Maximierung der Effizienz von Bewässerungssystemen, die Bewässerung mit salzhaltigem Wasser sowie die Unterflurbewässerung.

In über 90 „peer-reviewed” Publikationen hat Leinauer seine Forschungsergebnisse in den letzten Jahren veröffentlicht.

Neben seiner Arbeit für die NMSU hat er auch die Position des Stiftungslehrstuhls für Rasen an der Universität Wageningen in den Niederlanden inne.

Die Auszeichnung wird Prof. B. Leinauer im November virtuell erhalten, da das „National Agronomy Meeting“, das vom 8. bis 11. November in Phoenix stattfinden sollte, in ein Online-Format umgewandelt wird.

Use of cork residues to control turfgrass diseases

Coelho, L., L. Dionísio, M. Reis and C. Guerrero

Introduction

The worldwide increase in agricultural and industrial production has created environmental problems. Economic and environmental benefits can be gathered solving a problem of the agroindustry by applying their sub products to soil. The compromise to decrease the use of pesticides and fertilizers, which may be hazardous, has provided opportunities for the development of new sustainable crop management practices. From several strategies to enhance the use of organic matter in agriculture, one has been the use of composts of different mixtures of raw materials, from different agroindustry processes or the use of these raw material (agroindustry residues) directly without any treatment. The incorporation of these products to the soil and its application to the crops proved to be an interesting pathway to apply effective beneficial microorganisms for the crops and for the ecosystems globally. This strategy showed to achieve reasonable crops yields and suppressive effects on phytopathogenic microorganisms. Several microorganisms have been associated to cork throughout tree life and in the end products[1], such as *Trichoderma pseudoconingii, T. viride, Endothiella gyrosa, Mucor hiemalis, Rhysopus* sp., *Penicillium* sp., *Cytospora* sp., *Dichomera* sp., *Acremonium* sp., *Glyocladium* sp., *Botrytis silvatica*, and *Pestalotia* sp. Considering the antagonistic potential of some of these microorganisms[2], a study was carried out at the University of Algarve to identify the presence of beneficial microorganisms in cork residues and to evaluate, in vitro, their antagonistic effect against several fungi turfgrass diseases.

Material and Methods

Physical, chemical and microbiological characteristics of residues from cork transformation industry (NOVACORTIÇA, SA, Portugal), were performed according to methodologies described by COELHO et al.[3]. For this study, an extract from cork residues was prepared in a Ringer solution at a dilution of 10^{-1}, followed by decimal dilutions. Potato dextrose agar medium (PDA) was used to isolate and quantify fungi populations in the extract; Plate Count Agar (PCA) for heterotrophic bacteria and PCA medium at half the manufacturer's recommended concentration (1/2 PCA) for actinomycetes. Culture media were surface inoculated with 100 µL of a serial of dilutions of the cork extract and incubated at 25 °C ± 1 °C for 24 hours, in the dark[2]. The isolation and identification of the fungi was done by microscopy and by molecular biology techniques. DNA was extracted from mycelium grown in PDA medium. The obtained DNA samples were subjected to Polymerase Chain Reaction (PCR) using the primers ITS1 and ITS4[4], and the product obtained was sequenced. The antagonistic capacity of the isolated *Trichoderma harzianum* was evaluated according to the method of direct confrontation, described by Magan and Lacey[5] and its inhibition rate was tested against turfgrass pathogenic fungi: *Rhizoctonia solani, Clarireedia* spp., *Sclerotium rolfsii, Alternaria alternata, Fusarium oxysporum* and *Colletotrichum* spp. The confront direct tests were carried out in Petri dishes with PDA, using two 6.5 mm diameter discs: one with the pathogen mycelium and the other with the antagonist mycelium, 3 cm apart, which had grown for 7 days. Experiment was carried out at 25 ± 2 ºC and in the dark. To determine the percentage of inhibition (PI), each tested fungi was grown alone in PDA where a 6.5 mm discs with its mycelium was placed in the center of the culture medium. The ratio of the growth zones of each fungus were measured daily. All the assays were run in triplicate. Inhibition rate were analyzed by multifactorial analysis of variance (ANOVA) and compared using the Duncan New Multiple-Range Test, using IBM SPSS Statistics ver. 25 (IBM Corp., 1989-2017, U.S.A.).

pH	CE (dS cm^{-1})	DM (%)	OM (%)	RD	BD	AC (%)	EaW (%)	RW (%)	DAW (%)
6.02	0.2	54.1	94.7	1.48	0.229	19.6	24.2	5.0	35.7

CE, electrical conductivity; DM, dry matter; OM, organic matter; RD, real density; BD, bulk density; AC, air capacity; EAW, easily available water; RW, reserve water; DAW, difficult available water.

Tab. 1: Physical and chemical properties of the cork residue.

[1] SANTOS, M.N., M.H. BRAGANÇA and P.P. CASIMIRO, 2005: Microrganismos Associados à Cortiça em Diferentes Fases da sua Fileira. Silva Lusitana, 13(1): 75-93.

[2] COELHO, L., M. REIS, C. GUERRREO and L. DIONÍSIO, 2020: Use of organic composts to suppress bentgrass diseases in Agrostis stolonifera. Biological Control Biological Control. Doi: 10.1016/j.biocontrol.2019.104154

[3] COELHO, L., M. REIS and L. DIONÍSIO, 2013: Variation in microbial populations during composting of agro-industrial wastes. Applied Microbiology and Biotechnology 97: 4179-4186.

[4] WHITE, T.J., T. BRUNS, S. LEE and J. TAYLOR, 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. IN: SNINSKY, J.J. and T.J. WHITE (Eds.): PCR protocols: A guide to methods and applications. New York, New York, Academic Press, p. 315-322.

[5] MAGAN, N. and J. LACEY, 1984: Effect of water activity, temperature and substrate on interaction between field and storage fungi. Transactions of the British Mycological Society, vol. 82, n. 1, p. 83-93. https://doi.org/10.1016/S0007-1536(84)80214-4

Results and Discussion

The cork residue had a high organic matter content, as recommended for agricultural use[6]. The pH is lightly acid and the electrical conductivity is compatible with the agricultural use[7]. According to[8], cork residues presents suitable properties (Table 1) to be used as plant growing media.

Fungi	Bacteria	Actino-mycetes
1.50 x 10^7	1.43 x 10^6	1.57 x 10^6

Table 2: Microorganisms' populations (CFU.g cork^{-1}) quantified in the cork residues.

Cork residues showed high microorganisms' populations (Table 2), namely fungi, such as: *Penicillium* spp., *Aspergillus* spp., *Mucor* spp. and *Trichoderma harzianum*.

Trichoderma harzianum isolated from the cork residues was tested by direct confrontation technique. For the diseases studied, the inhibition rate was higher than 50%, except for *A. alternata* (35.4%). The inhibition rate was higher for *Clarireedia* spp. and *R. solani*, with values above 60% (Figure 1).

T. harzianum showed the fastest growth rate until day 2. On day 3, both *T. harzianum* and *Clarireedia* spp. mycelia occupied all the surface area of the culture media (Figure 2). However, despite *Clarireedia'*s high growth rate, *T. harzianum* was able to inhibit its growth.

Since the fungi isolated from the cork residues had a positive effect on turf diseases control in vitro, further work is being planned to study the effect of the cork extract on turfgrass diseases under field conditions.

Authors:
L. Coelho, L. Dionísio, M. Reis and C. Guerrero*,
Universidade do Algarve, Faculdade de Ciências e Tecnologia and MED Mediterranean Institute for Agriculture, Environment and Development, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

* Corresponding author:
cguerre@ualg.pt

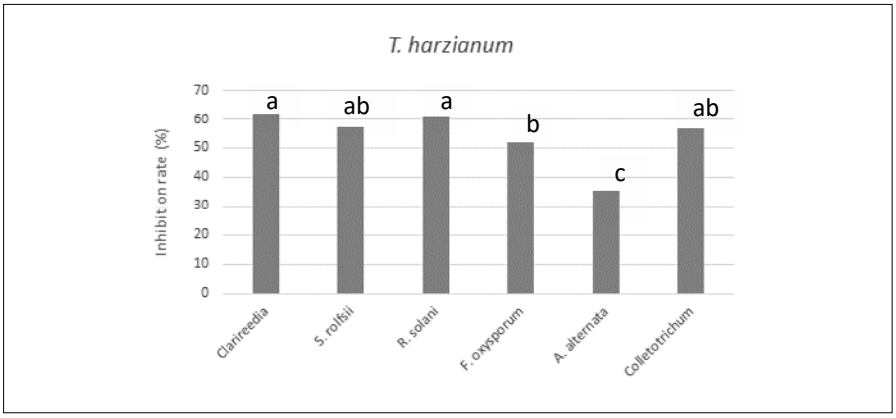

Fig. 1: Inhibition rate by direct confrontation between *Trichoderma harzianum* and the tested phytopathogenic fungi. Bars with the same letter have no statistically significant differences for p<0,05 (Duncan test).

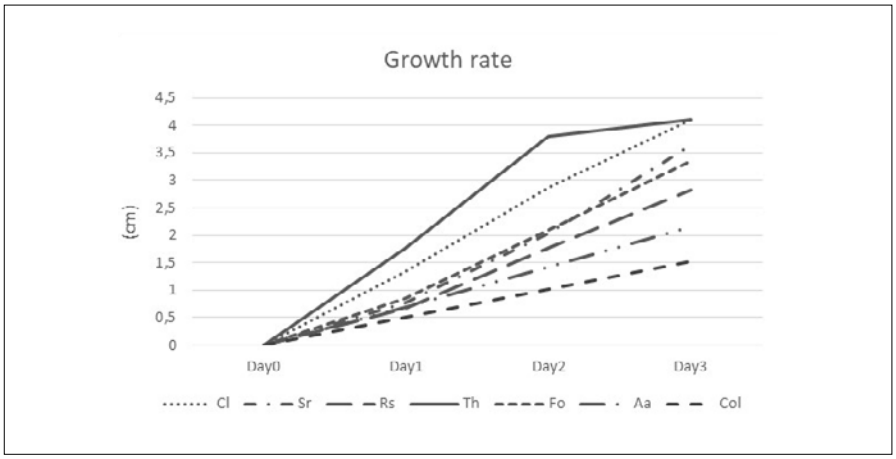

Fig. 2: Growth rate of *Trichoderma harzianum* and the tested phytopathogenic fungi. Cl, *Clarireedia* spp.; Sr, *Sclerotium rolfsii*; Rs, *Rhizoctonia solani*; Ta, *Trichoderma harzianum*; Fo, *Fusarium oxysporium*; Aa, *Alternaria alternata*; Col, *Colletotrichum* spp.

[6] FERREIRA, J., J. CONCEIÇÃO, A. STRECHT, J. RIBEIRO, A. SOEIRO and G. COTRIM, 2002: Manual de agricultura biológica – Fertilização e proteção das plantas para uma agricultura sustentável. Agrobio. 3ª Edição. Lisboa. pp. 435.

[7] BRINTON, W., 2000: Compost quality standards & guidelines – Final Report. Woods End Research Laboratory, Inc. Available at http://compost.css.cornell.edu/Brinton.pdf. Access in January, 2019.

[8] ABAD, M., P. NOGUERA and C. CARRIÓN, 2004: Los substratos en los cultivos sin suelo. In: Abad et al., M.U., 2004: Tratado de cultivo sin suelo. Ediciones Mundi-Prensa. 3ª Edição. pp. 113-158.

In vitro screening of turfgrass species and cultivars for resistance to Dollar spot isolates of different origin

Espevig, T., K. Normann, M. Usoltseva, K. Entwistle, J.A. Crouch and T.S. Aamlid

Introduction

Dollar spot caused by fungal species in the genus *Clarireedia*[1] (formerly *Sclerotinia homoeocarpa*) is one of the economically most important turfgrass diseases worldwide. Dollar spot was first officially documented in Scandinavia in 2013[2]. On some golf courses and in some years the damage from dollar spot in Scandinavia can be up to 70-80% dead turf on greens and fairways. In Scandinavia we have at least two species of *Clarireedia*[3] spp.. There is no available information on resistance to dollar spot in turfgrass species and cultivars that are used on Scandinavian golf courses (http://www.scanturf.org/). Therefore, the purpose of this study was to screen the most widely used turfgrass species and cultivars for resistance to the Scandinavian dollar spot isolates in vitro.

Materials and Methods

The experiment was conducted in winter-spring 2018 at the NIBIO Turfgrass Research Centre Landvik. Eight hundred and eighty glass test tubes (150 mm x 20 mm in diameter) were filled with 12 g dry Green Mix (80% sand and 20% garden compost v/v) and 1 ml water, covered with a test tube cap and autoclaved. Prior to sowing, seeds were surface sterilized in 70% ethanol for 30 seconds, rinsed with sterile water and dried at 25 °C for 24 h. Nine turfgrass species (a total of 20 cultivars) were seeded at a rate of 150, 52, 115, 50 and 105 seeds per tube for bentgrasses (*Agrostis stolonifera, A. capillaris* and *A. canina*), red fescues (*Festuca rubra* spp. *commutata, F. rubra* spp. *litoralis* and *F. rubra* spp. *rubra*), Kentucky bluegrass (*Poa pratensis*), perennial ryegrass (*Lolium perenne*) and annual bluegrass (*Poa annua*), respectively, and 0.75 ml sterile water was added to each tube after sowing. Due to differences in germination time and growth rate, the cultivars of Kentucky bluegrass, bentgrasses, red fescue, annual bluegrass and ryegrass were seeded 22 d, 17 d, 15 d, 10 d and 7 d, respectively, prior to the inoculation. After sowing, the tubes were re-covered with test tube caps and maintained at 21 °C (day, 16-h light of 250 µmol m-1 s-1) and 16 °C (night). When plants were 5-7 cm high, they were inoculated using dollar spot fungi as follows. Ten isolates of Clarireedia from Denmark, Norway, Sweden, the USA and the UK (Table 1) were grown on 50% PDA at 24 °C for 2 days. A total of 800 tubes were inoculated with one fungal plug (5 mm diameter) taken from the colony edge. Eighty control tubes (20 cultivars x 4 reps) were inoculated with 50% PDA plugs without fungus. After inoculation, the tubes were capped and maintained at 18 °C (day, 16-h light of 250 µmol m-1 s-1) and 14 °C (night). All tubes received 1.5 ml sterile water 25 d after inoculation. Dollar spot disease levels were reassessed 34 days post-inoculation using a resistance scale from 1 to 9, where 1=0-12.4%, 2=12.5-24.9%, 3=25-37.4%, 4=37.5-49.9%, 5=50-62.4%, 6=62.5-74.9%, 7=75-87.4%, 8=87.5-99.9%, 9=100% healthy plants=no disease. The experiment was conducted according to a two-factorial randomized complete block design (RCBD) with four replicates. Data were analysed using the SAS procedure PROC ANOVA (SAS Institute, version 9.4). Fisher's LSD at 5% probability level was used to identify significant differences among the treatments.

Results and Discussion

The average resistance of the 20 tested turfgrass cultivars to the 10 dollar spot isolates varied significantly (Table 1). The most aggressive *Clarireedia* isolates included one from the UK (*Clarireedia* sp. 17.12) and two from the USA (*C. jacksonii* MB01 and *C. monteithiana* SH44), while the weakest *Clarireedia* isolate was from Norway (*Clarireedia* sp. 14.12). *Clarireedia* isolates from Denmark and Sweden were in the middle. The aggressiveness among *C. jacksonii* isolates MB01, SH44 and 14.15 varied significantly. It appears that *C. jacksonii* isolates from USA were more aggressive than those from Sweden and Norway. However, based on the current data, we cannot conclude that aggressiveness in *Clarireedia* spp. is species-specific, as the aggressiveness of *C. jacksonii* isolates varied in different turfgrass species and varieties as indicated by a significant interaction CULTIVAR x ISOLATE (p=0.02). This is a question that requires further investigation.

Both cultivars of perennial ryegrass, 'Fabian' and 'Bargold', and both cultivars of slender creeping red fescue (*F. rubra* ssp. *litoralis*), 'Nigella' and 'Cezanne', were the most resistant (Table 2). There was great variation among the cultivars of Chewings fescue (*F. r.* spp. *commutata*) and of colonial bentgrass (*A. capillaris*). Here, Chewings fescue 'Musica' and colonial bentgrass 'Jorvik' were the least resistant cultivars, while Chewings fescue 'Bargreen II' and 'Lystig' and colonial bentgrass 'Greenspeed' exhibited better resistance. In general, creeping bentgrass (*A. stolonifera*) cultivars had resistance between 3 and 5, and there was no significant difference between, for example, 'Crystal Blue', 'Luminary' and 'Declaration'. Based on the USA field trials (NTEP, National Turfgrass Evaluation Program), it was surprising that 'Independence' scored 0.4 points higher than 'Declaration', but the difference was nonetheless small and not significant. On average for the five Nordic isolates, velvet bentgrass (*A. canina*) had significantly higher resistance than creeping bentgrass (6.2 vs. 4.1, data not shown). Annual bluegrass had the same level of resistance as creeping bentgrass (Table 2). Both cultivars of Kentucky bluegrass scored 5 or higher.

We would like to emphasize that ranking of cultivars after screening in glass tubes provides a general indication of resistance, but that the ranking will not necessarily be the same as in local field trials. Screening in glass

[1] SALGADO-SALAZAR, C., L.A. BEIRN, L. ISMAIEL, M.J. BOEHM, I. CARBONE, A.I. PUTMAN, L.P. TREDWAY, B.B. CLARKE and J.A. CROUCH, 2018: Clarireedia: A new fungal genus comprising four pathogenic species responsible for dollar spot disease of turfgrass. Fungal Biol. 122:761-773.

[2] ESPEVIG, T., M.B. BRURBERG and A. KVALBEIN, 2015: First report of dollar spot, caused by Sclerotinia homoeocarpa, of creeping bentgrass in Norway. Plant Disease 99:287.

[3] ESPEVIG, T., K. NORMANN and M. USOLTSEVA, 2018: Risiko for myntflekk på norske golfbaner. Gressforum 3:8-11 (In Norwegian).

Isolate no.	Country of origin	Host grasses	Species	GenBank number of ITS sequence	Resistance, scale 1-9, 9=no disease (main effect isolates)	
17.12	UK	*Poa pratensis*	TBD[1]	-	3.2	e[2]
MB01	USA	*Agrostis stolonifera*	*Clarireedia jacksonii*	KF545290	3.3	de
SH44	USA	*Agrostis stolonifera*	*Clarireedia jacksonii*	KF545299	3.8	cd
14.112	Sweden	*Poa annua*	TBD	-	4.3	c
14.10	Denmark	*Poa annua*	TBD	-	4.8	b
14.16	Sweden	*Festuca rubra spp.*	TBD	-	5.1	b
RB19	USA	*Cynodon dactylon x transvaalensis*	*Clarireedia montethiana*	KF545306	5.1	b
17.11	UK	*Festuca rubra spp.*	TBD	-	5.3	b
14.15	Sweden	*Festuca rubra spp.*	*Clarireedia jacksonii*	-	5.3	b
14.12	Norway	*Agrostis stolonifera*	TBD	KJ775860	7.3	a

[1] TBD, Clarireedia species identification to be determined.

[2] The same letter indicates no difference among the means based on Fisher protected LSD test (=0.05).

Tab. 1: Dollar spot isolates used in the study and average resistance of 20 turfgrass cultivars to these isolates (main effect ISOLATE, p <.0001, $LSD_{0.05}$ = 0.8).

tubes is primarily a basis for selection of cultivars for further testing. Dollar spot is a growing problem in Scandinavia, especially in Denmark and southern Sweden. Thus, the resistance of turfgrass species and cultivars should be tested under field conditions. However, because the disease is not well known in Scandinavia and it is not desirable to spread it, we are reluctant to inoculate with dollar spot, e.g. in the SCANGREEN variety trials. At least, we prefer to run experiments under controlled conditions first.

Turfgrass species	Cultivar	Resistance, scale 1-9, 9=no disease	
Lolium perenne	Fabian	7.3	a[1]
Lolium perenne	Bargold	7.2	a
Festuca rubra spp. litoralis	Nigella	7.0	a
Festuca rubra spp. litioralis	Cezanne	6.6	ab
Festuca rubra spp. commutata	Bargreen II	6.1	bc
Festuca rubra spp. rubra	Frigg	5.8	cd
Poa pratensis	Limousine	5.7	cd
Festuca rubra spp. commutata	Lystig	5.2	de
Agristis canina	Avalon	5.1	de
Poa pratensis	Julius	5.0	de
Agrostis capillaris	Greenspeed	4.5	ef
Agrostis canina	Villa	4.5	ef
Poa annua	Two Put	4.0	fg
Agrostis stolonifera	Independence	3.9	fgh
Agrostis stolonifera	Declaration	3.5	igh
Agrostis capillaris	Leirin	3.2	ijh
Agrostis stolonifera	Crystal Blue	3.1	ij
Agrostis stolonifera	Luminary	2.9	ij
Agrostis capillaris	Jorvik	2.5	kj
Festuca rubra spp. commutata	Musica	2.0	k

[1] The same letter indicates no difference among the means based on Fisher protected LSD test (=0.05).

Tab. 2: Resistance of turfgrass cultivars on average for 10 dollar spot isolates used in this study (main effect CULTIVAR, p <.0001, $LSD_{0.05}$ = 0.5).

The interaction CULTIVAR x ISOLATE in the study was significant. Thus, the data from this study is preliminary and needs further analysis. Moreover, the experiment was repeated in winter-spring 2019, and the 2-yr data will be analysed and published at the earliest opportunity.

Acknowledgements

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Authors:

Tatsiana Espevig and
Trygve S. Aamlid
NIBIO – Norwegian Institute of Bioeconomy Research,

Karin Normann,
Asbjørn Nyholt ApS (Denmark),

Marina Usoltseva,
Botanisk Analysgrupp (Sweden),

Kate Entwistle,
The Turf Disease Centre (UK),

Jo Anne Crouch,
Mycology & Nematology Genetic Diversity and Biology Lab, US Department of Agriculture, Agricultural Research Service (USA).

Antagonist and soil additive to control *Microdochium nivale* disease

Rosenbusch, J., A. Floss and W. Praemassing

Fig. 1: Field trial at Osnabrueck Golf Club.

Introduction

Regarding to Integrated Pest Management (IPM) the use of chemical pesticides on public spaces as on golf courses has to be restricted to a minimum use. *Microdochium nivale* is one of the most economically important fungal pathogens in Germany during the winter months causing Microdochium patch on turf[1]. Due to some experiences liquid fertilisers can have the potential to reduce fungal turfgrass diseases[2]. A study has been carried out comparing two liquid products (antagonist and soil additive) with regard to the symptomatic infestation caused by *M. nivale* on a green at Osnabruecker Golf Club (north-west Germany).

Materials and Methods

Osnabruecker Golf Club is situated on a hill and surrounded by an old and dense tree population. The field trial was located on the 8th green (Figure 1) and was selected because of its annually returning intensive *M. nivale* infestation. The 1-factorial experiment took place between October 2018 and April 2019 and was laid out according to completely randomised block design with four replications. The following four treatments were used: (1) Microdoc Turf (M) – product of Intrachem Bio Deutschland. A liquid combination of iron fertiliser, wetting agent and adhesive which is supposed to provide resistance for turfgrass during critical vegetation phases[3]. Nutrient composition: 10.5% N total nitrogen (10.5% methylene urea), 0.5% K_2O water-soluble potassium oxide, 5.5% Fe iron complex, 1.4% trace elements (Mg, Mn, Cu, B, Zn, S, Mo). Spray cocktails of 250 ml (25 ml M + 125 ml water) per plot were applied in four-week intervals; (2) Trichostar® (T) – product of Intrachem Bio Deutschland. A liquid soil additive containing the antagonist *Trichoderma harzianum* T58. The product should promote root development and reduce the abiotic stress factor[4]. Spray cocktails of 250 ml (3.125 ml T + 246.875 ml water) per plot had to be prepared at least 12 hours before application with tepid water (25-30 °C) and were applied in four-week intervals; (3) Microdoc Turf + Trichostar® (MT) –- combination of treatments 1 and 2. The products were applied every four weeks in accordance with treatments 1 and 2, with two-week intervals between the different products; (4) Control (N) –- untreated. The applications were carried out by using a hydraulic hand pump sprayer. The trial was not fertilised in any other way during the trial period. The infestation was recorded as percentage of plot area covered by Microdochium patch symptoms (disease %). It was also scored as an index: At the trial beginning, the aspect of each plot was assessed by using the 0-9 scoring system. There was no disease % at that time. On the later assessments, index scores were made depending on disease %, maximal to the best aspect score of the first assessment (8), to evaluate disease-caused aspect deterioration starting from the pre-experimental state (0% disease = index score 8 to > 6.5% disease = index score 0). There was a statistical evaluation of the results at the end of the trial (one-way ANOVA).

Results and Discussion

Figure 2 shows the average results across all assessment dates. No results were significantly different. But there was a trend that M achieved the best results with an index score of 3.2 and a disease % of 5.6%. The control N showed the second best results with an index score of 2.2 and a disease % of 8.1%. The combination MT achieved

[1] NONN, H., 2002: Rasenkrankheiten in den Wintermonaten. In: Deutsche Rasengesellschaft e.V. – URL: https://www.rasengesellschaft.de/rasenthema-detailansicht/rasenthema-dezember-2002.html. (German)

[2] SCHNOTZ, G. and M. HUNT, 2012: Gesund durch den Winter – gezielte Duengung zur Unterstützung der Graeser. In: Deutsche Rasengesellschaft e.V. – URL: https://www.rasengesellschaft.de/rasenthema-detailansicht/dezember-2012-427.html. (German)

[3] INTRACHEM BIO DEUTSCHLAND, 2019 a: Microdoc Turf – EG-Fluessigduenger zur Naehrstoffoptimierung für Herbst bis Fruehjahr – URL: https://www.intrachem-bio.de/produkte/microdoc-turf/. (German)

[4] INTRACHEM BIO DEUTSCHLAND, 2019 b: Trichostar® – Mikrobielle Aktivitaet für Herbst und Winter – URL: https://www.intrachem-bio.de/produkte/trichostar/. (German)

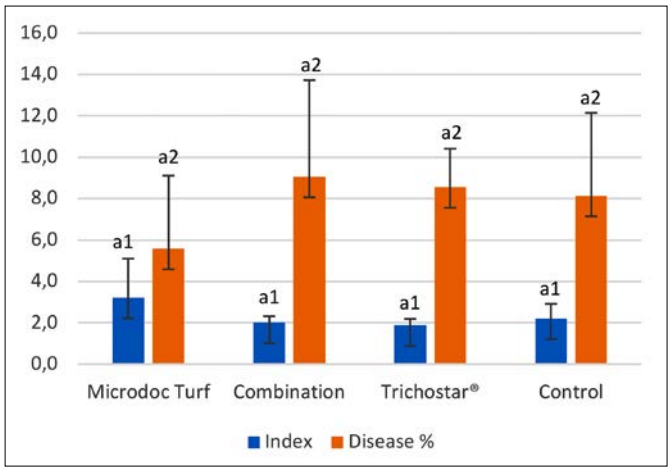

Fig. 2: Average results over the entire experiment for each variant for parameters index (blue) and disease % (orange). No significant differences (p = <0.05, ANOVA Dunett, Tukey-HSD, Games-Howell).

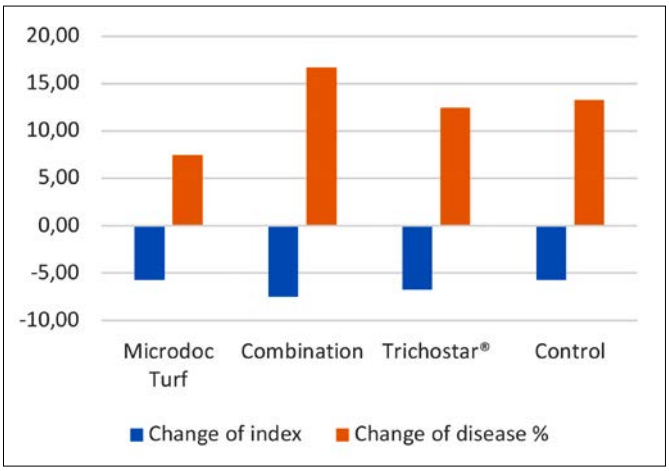

Fig. 3: Changes in the results of index (blue) and disease % (orange) over the entire duration of the experiment from Oct 4, 2018 to Apr 5, 2019.

the third best index score with 2.0, but with 9.1% the worst disease %. Product T received the lowest index score at 1.9 and showed the third highest disease % at 8.6%. Figure 3 shows the differences between first and last assessments. It clearly shows up, that control N has the same change in index scores as variant M. It also clearifies, that variant T represents the second least infested variant at the end of the trial. However, the values of the combination (16.75%) and the control (13.25%) are only slightly worse. According to Figure 2 variant MT can be identified by trend with lowest control effect against snow mold disease. This is confirmed with many assessments during the trial period (Figure 5). Figures 4 and 5 illustrate the development of index and disease % over the whole experiment period. The fundamental superiority of variant M compared to the other variants can be due to its nutrient content. Relationships between nitrogen application, low soil pH (e.g. by applying ferrous sulphate) and less damage caused by *M. nivale* have already been determined[5]. All in all, the difference of disease % from the best variant M to the unfertilised control N is only 2.5%.

Authors:
Jan Rosenbusch, Andre Floss and Wolfgang Praemassing,
Faculty of Agricultural Sciences and Landscape Architecture at University of Applied Sciences Osnabrueck.

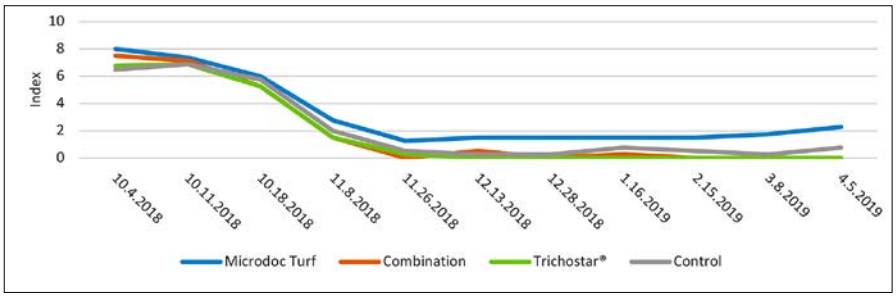

Fig. 4: Development of the results for parameter index over the entire duration of the experiment from Oct 4, 2018 to Apr 5, 2019.

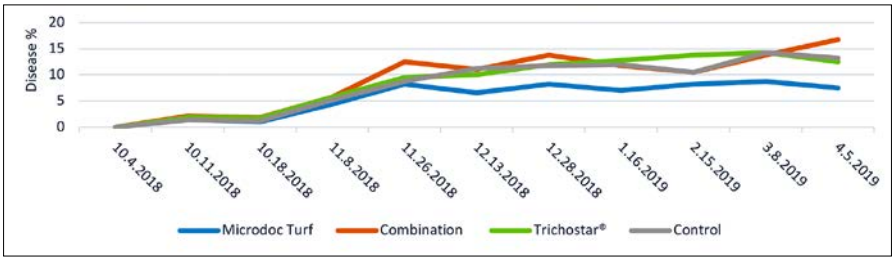

Fig. 5: Development of the results for parameter disease % over the entire duration of the experiment from Oct 4, 2018 to Apr 5, 2019.

[5] PORTMESS, R.E., J.A. GRANT, F.S. ROSSI, 2009: Reducing Chemical Use on Golf Course Turf: Redefining IPM. New York State Integrated Pest Management Program – Publication No. 617.

Development of a logistic regression model for the prediction of Microdochium patch

Koch, P., D. Smith, C. Mattox, B. McDonald, E. Braithwaite, A. Kowalewski, M. Sheridan and E. Nangle

Introduction

Microdochium patch caused by *Microdochium nivale* is the most economically important disease of golf course turfgrass grown in cool, wet environments such as the U.S. Pacific Northwest, the United Kingdom, Ireland, and much of northern Europe and Scandinavia. The disease often requires numerous fungicide applications for acceptable control, however increasing restrictions on synthetic fungicide use in many parts of the world are making management of Microdochium patch more difficult. A logistic model that successful predicts the development of dollar spot (*Clarireedia jacksonii*) was recently developed and has provided excellent disease control with reduced fungicide inputs.[1] A similar logistic model for the prediction of Microdochium patch development may offer the same benefits for managing this important disease. The objectives of this research were to determine the environmental factors most important in Microdochium patch development and use those factors to create an accurate logistic model to predict disease development.

Materials and Methods

Field trials were conducted during the winters of 2016-2017, 2017-2018, and 2018-2019 in Corvallis, OR, USA by Oregon State University and in Blessington, Ireland by the Irish Sportsturf Institute. The trials at both sites were conducted on stands of primarily annual bluegrass (*Poa annua*) maintained under fairway conditions. The first two winters were used to identify the environmental factors most important in Microdochium patch development and develop an initial predictive model. The experimental area consisted of two treatments, a fungicide-treated positive control and a non-treated negative control, in a randomized complete block design with six replications. Individual plot size was 1.2 x 1.5 m. Environmental conditions were measured from October 1st through March 31st each year using weather stations at both sites that measured hourly air temperature, relative humidity, rainfall, soil temperature, and dew point. Disease development was assessed daily over the same time period using Cannopeo digital image analysis software with a red ratio of 1.010, blue ratio of 0.950, and noise reduction of 1 to differentiate diseased from healthy turf. The weather and disease data were then used to create a logistic regression model in SAS (PROC LOGISTIC).

The model created in the first two winters was tested in the field at Oregon State University in 2018-2019 as well as in Ballan-Mire, France. Five treatments consisting of a non-treated control, a calendar-based fungicide program, and model thresholds of 50%, 75%, and 90% were evaluated using a randomized complete block design with four replications and a plot size of 1.2 x 1.5 m. Weather conditions were recorded hourly throughout the winter disease severity was assessed weekly using the same methods as outlined above. The number of fungicide applications used during each treatment was also assessed to determine any potential fungicide savings of the model over a calendar-based.

Results and Conclusion

The weather and disease results from the first two winters in Oregon and Ireland indicated that the most accurate

Treatment	Calendar	50% threshold	70% threshold	90% threshold
Dates of fungicide application	4 Oct 18	8 Oct 18	11 Oct 18	9 Nov 18
	1 Nov 18	5 Nov 18	8 Nov 18	7 Dec 18
	29 Nov 18	3 Dec 18	6 Dec 18	4 Jan 19
	27 Dec 18	31 Dec 18	3 Jan 19	1 Feb 19
	24 Jan 19	28 Jan 19	31 Jan 19	1 Mar 19
	21 Feb 19	25 Feb 19	28 Feb 19	X
	21 Mar 19	26 Mar 19	26 Mar 19	X
	19 Apr 19	22 Apr 19	X	X
Total Number of Applications	8	8	7	5

Tab. 1: Dates and total number of fungicide applications under each treatment Oregon State University in 2018-2019 using the Microdochium Patch predictive model.

[1] SMITH, D.L., J.P. KERNS, N.R. WALKER, A.F. PAYNE, B. HORVATH, J.C. INGAUGIATO, J.E. KAMINSKI, M. TOMASO-PETERSON, P.L. KOCH, 2018: Development and validation of a weather-based warning system to advise fungicide applications to control dollar spot on turfgrass. PLoS ONE 13(3): e0194216.

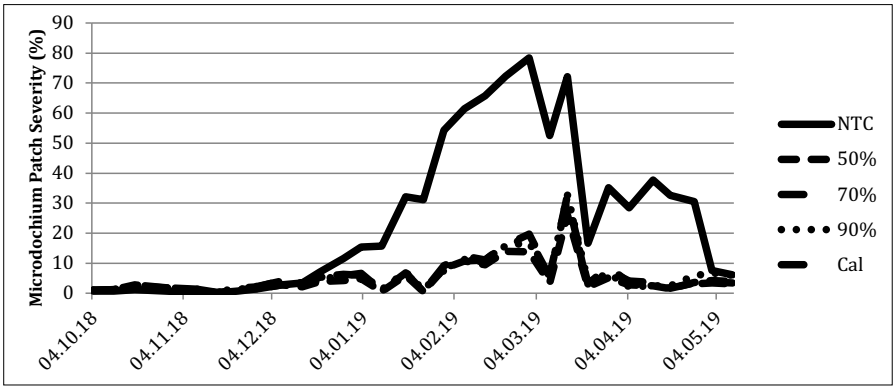

Fig. 1: Microdochium patch development in 2018-2019 at Oregon State University in Corvallis, OR, USA on a non-treated control (NTC), three thresholds using the Microdochium patch predictive model, and a calendar-based fungicide program (Cal).

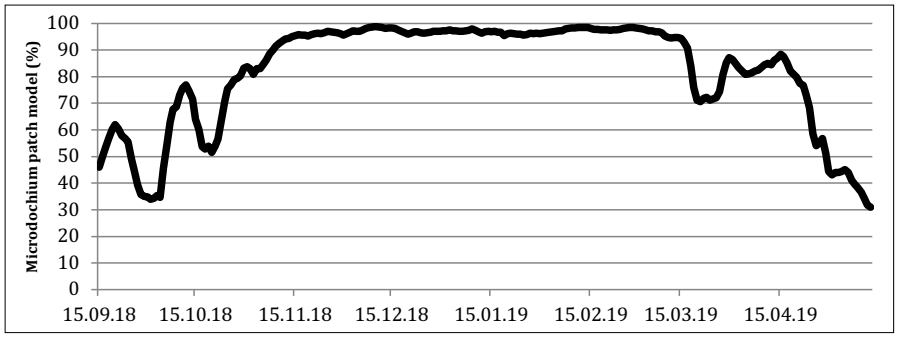

Fig. 2: Microdochium patch prediction model results in 2018-2019 at Oregon State University. in Corvallis, OR, USA.

Microdochium patch model as predicted by the SAS statistical program used a 10-day moving average of relative humidity and air temperature. Use of the model at Oregon State resulted in fewer fungicide applications at the 70% and 90% thresholds (Table 1). Disease control using the model was similar to that of a calendar-based program, though disease at the 90% threshold was marginally higher and would be considered unacceptable by many standards (Figure 1). The biggest conclusion from the first year of field-testing the model is that at the Oregon (Figure 2), French, (data not shown), and Irish (data not shown) locations the model over-predicted the onset and severity of disease relative to the actual development of disease. The authors are currently investigating additional environmental factors such as rainfall and adding more data to the logistic model to improve the accuracy for future testing.

Authors:

P. Koch and D. Smith,
University of Wisconsin – Madison, Madison, WI, USA,

C. Mattox, B. McDonald, E. Braithwaite and A. Kowalewski,
Oregon State University, Corvallis, OR, USA,

M. Sheridan,
Irish Sportsturf Institute, Blessington, Ireland,

E. Nangle,
The Ohio State University, Wooster, OH, USA

Effect of a biostimulant on late season bermudagrass implantation

De Luca, V. and D. Gómez de Barreda

Introduction

Seeded bermudagrass (*Cynodon dactylon* L.) cultivars are known for quick, easy, and economical turfgrass establishment[1] and their use have been established as a common practice in Europe. In transition zone, sowing date for bermudagrass germination and establishment is crucial in order to achieve a dense turf sward before temperatures drop in autumn, being the most suitable maximum and minimum growing range temperatures between 26 to 35 °C and 15 to 21 °C[2], respectively. Traditionally, late spring or early summer plantings have been recommended for bermudagrass establishment because the environmental conditions3. The use of biostimulants could be helpful to establish bermudagrass when sowing takes place after the recommended period. The objective of the study was to determine if a biostimulant application on a seeded bermudagrass could improve implantation when sowing is performed late in the season.

Materials and Methods

A field study was conducted in the Manises Royal Golf Course in Valencia, Spain (39°28'00"N 0°22'30"W). Bermudagrass (cv. 'Princess 77') was sowed on 22 July at 8 g·m^{-2} in eight 1 m^2 elemental plots, with or without the application of a biostimulant. This process was repeated four times in a 2 week-interval on 4 August, 18 August, 1 September and 16 September (40 elemental plots in total). The biostimulant was a root enhancer composed of 8.0% free amino acids, (5.57% glutamic acid, 1.20% alanine, 0.71% aspartic acid, 0.11% valine, and other 13 amino acids at 0.41%); 6% total nitrogen derived from 3% organic [amino acids from vegetal origin, corn (*Zea mays* L.)], and 3% ammoniacal nitrogen (NH4-N); micronutrients [0.4% iron (Fe), and 0.4% zinc (Zn)]; and 2.5% polysaccharides. For each sowing date, the biostimulant was applied 3 times starting on the day of sowing, and in a 14 day-interval, at 20 L ha^{-1}, diluted in 4.000 L ha^{-1} of water. Applications were made using a CO_2-pressurized sprayer calibrated to deliver 325 L ha^{-1} with a single flat-fan nozzle (9504 EVS flat-fan; TeeJet Spraying Systems) at 206 kPa. Turf was mowed weekly starting 1 month after every sowing, and irrigation was performed 3 times per week. Bermudagrass growth was evaluated weekly during 2 months for each sowing date and it was determined by measuring turf height 5 times per plot using a 1 mm accuracy rule. Visual percentage of bermudagrass green coverage was evaluated weekly until 4 November. The experimental design was a randomized complete block with two factors (biostimulant treatment and sowing date) and four replications. All statistical analysis were made with Statgraphics Centurion XVI where Fisher's protected LSD test was used at the 0.05 probability level to identify significant differences.

Temperature (°C)	July	August	September	October	November
Maximum	29.3	29.0	26.0	22.7	20.3
Minimum	23.6	23.0	18.3	15.8	11.5

Tab. 1: Maximum and minimum averaged temperatures in the period of the experiment. ***(Source: Agencia Estatal de Meteorología, in www.datosclima.es.)***

Results and Discussion

Air temperature (Table 1) on the first and second sowing dates (22 July and 4 August) was adequate for bermudagrass growing according to McCarty (2018)[2]. Maximum turf growth achieved when sowing on 22 July was 14.6 cm, achieved 5 weeks after sowing (WAS), while the maximum growth achieved on the 4 August sowing was 7.9 cm 8 WAS (Figure 1). Bermudagrass did not grow more than 4.8 cm on the other sowing

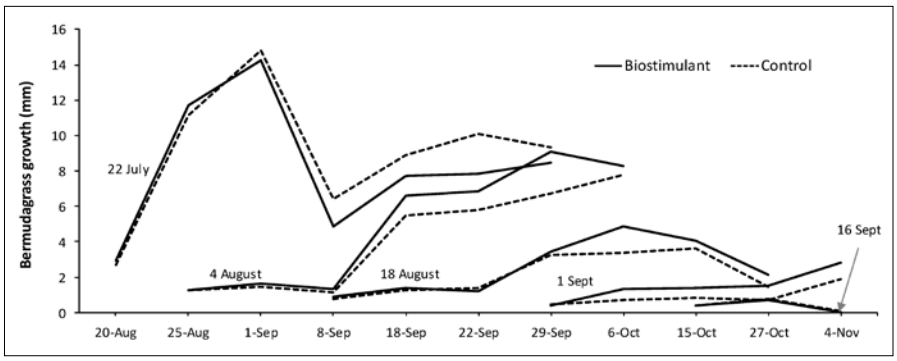

Fig. 1: Bermudagrass growth (mm). Sowing dates are indicated over their corresponding evolution lines.

[1] PATTON, A.J., G.A. HARDEBECK, D.W. WILLIAMS and Z.J. REICHER, 2004: Establishment of bermudagrass and zoysiagrass by seed. Crop Sci. 44:2160-2167.

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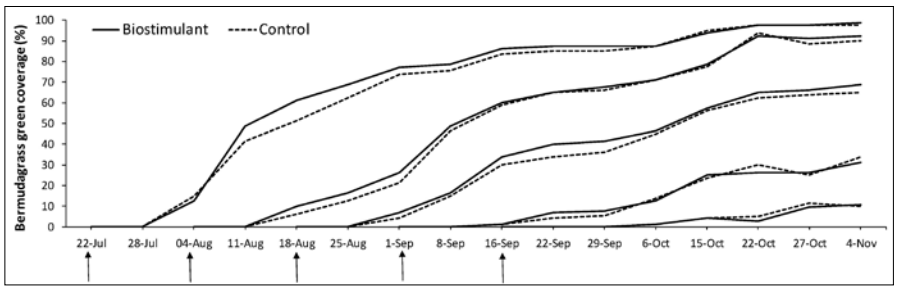

Fig. 2: Bermudagrass green plot coverage in percentage (%) in the different sowing dates, which are indicated with black arrows.

dates. These results confirm that a delay in the sowing date in mid summer reduces bermudagrass growth.

Sowing date was also decisive to achieve full green coverage. Princess-77 bermudagrass sowing could be delayed until 4 August to achieve more than 90% of green coverage in October, which corresponds to 11 WAS. More specifically, the 22 July and 4 August sowing dates resulted in turf covering almost the whole plot (98.1% and 91.3%, respectively) 3.5 and 3 months after each sowing (Figure 2). The rest of sowing dates were not acceptable, the 18 August sowing coverage only reached 66.9% at the end of the experiment, and when sowing took place in September (1st and 16th) coinciding with the decrease of temperatures (Table 1), turf coverage only reached 32.5% and 10.3%, respectively. Musser and Perkins (1969)[4] also reported that late sowing dates generally do not provide sufficient time for warm-season grasses to achieve adequate establishment. No differences were observed between treated and untreated turf nor in growth neither in coverage in none of the sowing dates, so it can be said that the use of the tested biostimulant did not enhance coverage although it seemed to promote a slight effect on turf coverage but not statistically significant.

Conclusions

Under the present climatic conditions, the latest sowing date that is recommended is 4 August. The tested biostimulant did not help bermudagrass to establish earlier in any of the studied sowing dates at least under the conducted management. This experiment should be repeated under other summer conditions or different type of biostimulant application.

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Authors:

V. De Luca and
D. Gómez de Barreda,
Universitat Politècnica de València,
Camino de Vera, 46022,
Valencia, Spain

Seaweed (*Ascophyllum nodosum*) extraction method produces chemically different formulations with contrasting effects on turfgrass rooting

Owen, A.G., T.I. Williams and D. Hiltz

Introduction

Seaweed extracts have been applied to turfgrass surfaces, particularly golf greens, as part of a maintenance programme for many years[1]. There are numerous commercial products available to the turf manager containing seaweeds from a range of sources and utilising multiple extraction techniques. KAHN[2] comprehensively reviewed the benefits of applying seaweed extracts to plants, illustrating a wide range of potential benefits; including increased plant rooting and improved abiotic and biotic stress tolerance, important characteristics for the contemporary sports turf manager. KAHN also emphasised that seaweed extracts are complex, contain many different compounds; macro and micro nutrients, amino acids, vitamins, plant hormones and a range of simple and complex carbohydrates and the mode of action for generating the in-plant benefits are not often clear, suggesting many components act synergistically. This complexity has led to a one-size fits all approach in the marketing of commercial seaweed extracts which is false. GUINAN[3] demonstrates a clear specificity of response of plants to differing seaweed extracts and advocates a more rigorous fitness-for-purpose approach. It was the objective of this research to examine the effect of a range of extract types from the same source seaweed to turf grass rooting and to determine if this classic turf response to seaweed extract applications was comparable across extract types.

	ANE-01*	ANE-02*	ANE-03*	ANE-04~
pH	11.0	4.6	3.1	5.9
Dry Matter w/w %	9.5	2.9	7.7	10.1
Carbohydrates (%)				
Mannitol	0.475	0.667	0.847	0.606
Alginate	1.425	0.232	0.984	2.520
Fucoidins	0.891	0.232	1.540	1.010
Laminarin	0.285	0.319	0.539	0.404
Nutrients (ppm)				
Calcium	418	170	690	1520
Magnesium	504	310	920	1010
Phosphorus	105	196	1190	120
Potassium	17500	1130	2420	2200

Tab.1: Chemical analysis of *Ascophyllum nodosum* extracts. (*ANE01-03 are extracted samples, ~ANE04 is a micronized suspension).

Materials and Methods

A homogeneous sample of harvested and dried *Ascophyllum nodosum* (L.) le Jol. from Nova Scotia, Canada was extracted utilising four methods to produce samples of aqueous *Ascophyllum nodosum* extract (ANE) for comparison. A ratio of 1:7 solid:liquid with a five hour, constant stirring method was utilised for three extractions. ANE-01; Potassium hydroxide extract utilised to produce an alkaline extraction at pH 12. ANE-02; water at 70 °C utilised to produce a hot water extract. ANE-03; nitric acid / phosphoric acid at 70 °C to produce a hot acid extract at <pH 3.0. ANE-04 was developed differently but using the same ratio; water at < 10 °C added and micronized in a blender to produce a cold-blend water suspension / extract. Each extract was filtered (1 mm mesh) and centrifuged at low speed to remove seaweed debris. Each extract was chemically analysed using ICP-AES, to determine macro and micro nutrient concentrations, plus signature carbohydrates (mannitol, laminarin, fucoidins and alginate) were measured using various high-performance liquid chromatography methods based on those outlined by MANNS[4].

Rooting of seedling *Lolium perenne* (Torsion) was assessed within clear plastic phytotox kit test containers, measuring 155 mm x 210 mm x 8 mm, (Microbiotests, Gent, BE). Seeds were germinated in a petri-dish and transferred at day 7 to the test containers. Four seedlings per container were

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	Total root length (cm)	Mean root diameter (mm)	Total root volume (cm^3)
Control	420 b	0.173 b	0.104 b
ANE-01	608 a	0.181 ab	0.158 a
ANE-02	340 b	0.195 a	0.102 b
ANE-03	209 b	0.186 ab	0.055 b
ANE-04	320 b	0.171 b	0.074 b

Tab. 2: Root characteristics measured by WinRhizo. Four plants per sample unit n=6. Accompanying letters indicate difference P<0.05.

transferred with the establishing root placed onto filter paper dampened with 20 ml of one of each of the four extracts diluted to equivalent field application rates (10L/500L water ha^{-1}). Six test containers were prepared for each extract alongside a control (only water applied). The seedlings in the test containers were then grown-on in a constant temperature cabinet set to provide a 16-hour light period per day at 20 °C. At day 15 test containers were scanned using a flat-bed scanner and the rooting area analysed with the WinRhizo root scanning software. Each container set of 4 plants was treated as a single experimental unit, to reduce root overlap errors. Data was collected for total root length, total root volume and root diameter.

Results and Discussion

Chemical analysis of the extracts reveals distinct differences (Table 1), particularly in terms of extract pH; from 3.1 for ANE-03 to 11.0 for ANE-01, but also for carbohydrates such as alginate (%) which shows a 10-fold difference between ANE-02 and ANE-04. Macronutrient content is generally greater for the micronized suspension ANE-04 than for the extracts, except for potassium, which shows elevated values for ANE-01 because of the KOH extracting solution. The low pH extraction increases the amount of phosphorus, but phosphoric acid was part of the extractant.

Measured rooting characteristics demonstrate significantly different values for the extracts (Table 2). ANE-01 shows significantly greater total root length, being the only extract to promote *Lolium perenne* root length significantly over the control set. Mean root diameter is significantly greater for the hot water extract when compared with the control and ANE-04. Total root volume is significantly greater for ANE-01, being the only extract to promote total root volume over the other extracts and the control.

The results demonstrate clear differences in chemical constituency between various seaweed extracting solutions and techniques from the same base seaweed. The differing pH and chemical extractants solubilize varying amounts of carbohydrates and nutrients from seaweed. Only a few characteristics were measured in this study, but this data clearly supports the supposition that different extractants and extraction methods produce differing chemical profiles of seaweed extracts for a range of constituent compounds found within the same seaweed. However, for the turf manager what is most critical is not the extract chemistry but the response in the plant following application. When we measure seedling rooting response of *Lolium perenne*, ANE-01; an alkaline extraction of the base seaweed, produces significantly greater root length and total root volume. This clearly demonstrates that differing seaweed extracts produce contrasting rooting responses in the turf grass plant, however the causal factor for this cannot be determined. It is likely that various components of the extract act synergistically and very likely that unmeasured specific compounds derived from the extracting process contribute to this effect.

Authors:

Andy G. Owen,
ICL-Specialty Fertilisers,
Waadenburg, The Netherlands,

Tamsin I. Williams,
Department of Biological Sciences,
Royal Holloway University of London,

D. Hiltz,
Acadian Seaplants Limited,
Dartmouth, Nova Scotia, Canada.

Ascophyllum nodosum extract use on plant parasitic nematode abundance and diversity on a golf green

Williams, T.I., A.C. Gange and A.G. Owen

Introduction

Seaweed extracts are often used in integrated turf management programs as a soil fertilizer and conditioner. Many of the seaweed products sold commercially utilise *Ascophyllum nodosum*; a brown bladder wrack found on the shores of the North Atlantic Ocean. As these seaweeds occupy the intertidal range, they have evolved to survive both in and out of the water meaning they contain unique compounds such as fucoidan and alginate. It is this unique profile of compounds that allows seaweed extracts to have a bio-stimulant effect in soils and possibly infer plant protection against pests and diseases. The effect of *A. nodosum* extracts against plant parasitic nematodes (PPN) has not been widely documented in turf grass systems. While previous work has shown that seaweed extracts may reduce the incidence of root knot nematode infection in tomato, it has only been suggested that the same may be seen in turf[1,2]. Much of the previous work has found it hard to translate significant PPN reduction found in laboratory studies to the field[3,4]. The objective of this research was to see if the use of an alkaline extracted *A. nodosum* solution could reduce plant parasitic nematode abundances on a golf green, compared to an untreated control, following some positive results seen in laboratory trials.

Materials and Methods

A 20 m^2 plot was marked out on a golf green consisting of a *Poa annua* and *Agrostis stolonifera* mix (approximately 70:30) on a medium sand loam soil in the south of England. Within the 20 m^2 plot individual 1 m^2 plots were marked, totaling five replicates (1 m^2 plots) per treatment arranged in a randomized block design. To each 1 m^2 an alkaline extracted *Ascophyllum nodosum* solution (Acadian marine plant extract powder, Acadian Seaplants Nova Scotia) was applied at three rates; 0.5 kg/ha, 1 kg/ha (the recommended dosage rate) and 2 kg/ha in 600 L water, control plots received water only application. *A. nodosum* was applied every 21 days over three months (four applications). At the trial start and after final seaweed applications, three soil cores were taken from each 1 m^2 plot and pooled, totaling in approximately 200 g of soil per plot. The soil was then placed in a modified whitehead tray in tap water for 17 hours[5]. The resulting nematode suspension was centrifuged and siphoned down to 10 ml, before killing the nematodes in a 90 °C water bath. To preserve the nematodes, 10 ml of double strength TAF (Triethnaolamine, formaldehyde and sterile distilled water) was added to each suspension. Nematode counts and taxa were recorded from 1 ml aliquots. The proportion of plant parasitic nematodes per sample, Simpson's diversity index and nematode percentage prevalence were calculated. The results were assessed firstly for normality using Shapiro Wilk test and differences in the abundance per 100ml of each nematode taxa, the proportion of PPN's, and the maximum abundance per 100 ml of each taxa between treatments were examined with the Kruskal Wallis test.

Results and Discussion

The main taxa of nematodes found in order of prevalence were *Helicotylenchus, Pratylenchus, Tylenchulus* and *Criconema*. The most prevalent nematode genus *Helicotylenchus* reduced in mean and maximum abundance after applications with all seaweed treatments, compared to the water only control (Table 1, Figure 1), but there was no increased effect with higher application rates. After four applications of seaweed a decrease of 11 percentage points in the mean proportion of PPN's within the total nematode population, was observed when using the recommended rate of *A. nodosum* extract. However these reductions in *Helicotylenchus* abundance when using the recommended rate of alkaline extracted seaweed (1 kg/ha) were found to be non-significant from a Kruskal Wallis test (at p=0.05), due mainly to high variability of numbers within each treatment (Figure 1).

This trial has clearly illustrated that nematode populations are highly variable, even across a single golf green,

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Treatment	Mean proportion of PPN %	*Helicotylenchus* spp		
		Maximum abundance per 100 ml	Mean abundance per 100 ml	Coefficient of variation %
0 kg/ha	22.96	54000	2540	86.0
0.5 kg/ha	21.95	2100	1440	40.4
1.0 kg/ha	12.31	2000	1120	51.1
1.5 kg/ha	23.91	3200	1780	70.6

Tab. 1: The mean proportion of plant parasitic nematodes (PPN) compared to non-PPN in the sample (%). Mean abundance and maximum abundance per 100 ml of *Helicotylenchus spp* found from golf green after four applications of seaweed. The coefficients of variation for *Helicotylenchus spp* after seaweed applications.

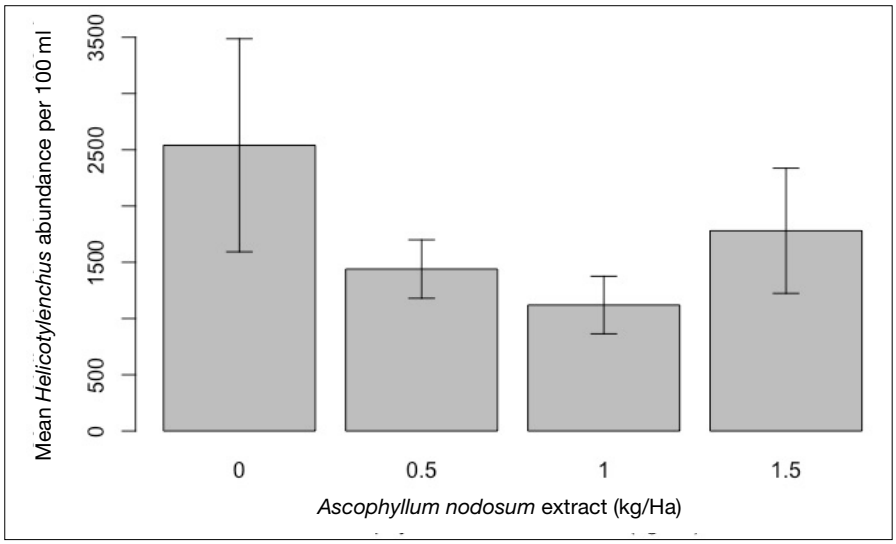

Fig. 1: The mean abundances of *Helicotylenchus spp* found in 1oo ml of a nematode suspension extracted from soil cores from a golf green in the south of England. Each nematode population was taken from three soil cores from each 1 m2 plots after treatment with 0 kg/ha, 0.5 kg/ha, 1 kg/ha and 1.5 kg/ha of an *Ascophyllum nodosum* extract every three weeks for approximately three months. Error bars = standard error of the mean.

despite samples being pooled per plot to overcome the spatial heterogeneity. The trial does demonstrate that seaweeds can be used to reduce this variability that is illustrated when comparing coefficients of variation (CV) between treatment groups. The highest CV *Helicotylenchus* spp was in control plots at 86%, compared to 40.4% when 0.5 kg/ha of seaweed was applied (Table 1).

The findings from this trial support research showing how difficult it is to replicate laboratory results in the field, as the complexity of soil communities are difficult to account for. It appears that with some fine-tuning and further research, *A. nodosum* extracts could be used to reduce variability and numbers of the most prevalent taxa of PPN in nematode populations. Less spatial variability may result in a reduction in visual symptoms for the course manager and potentially improve the use of available nematocides as part of an integrated pest management scheme. It is necessary that more field trials and research is needed, particularly using the recommended rate and higher replication, however the initial results are promising.

Authors:

Tamsin I. Williams and
Alan C. Gange,
Department of Biological Sciences, Royal Holloway, University of London,

Andy G. Owen,
ICL-Specialty Fertilisers,
Waadenburg, The Netherlands.

Biodetection of turfgrass fungal diseases using sniffer dogs

Serrão, M., L. Coelho, L. Dionísio, C. Guerrero and A. Duarte

Introduction

The Directive 2009/128/EC[1] of the European Parliament and of the Council, of 21 October, establishes a framework, for Community action, to achieve the sustainable use of pesticides by reducing the risks and impacts of its use on human health and in the environment. This Directive also promotes the use of integrated pest management, as well approaches or techniques such as non-chemical alternatives to pesticides that enhanced the need to find new techniques for detection, identification and quantification of pathogens in plants or in soil in fields used for agriculture.

Fungi turfgrass pathogens cause economically important destructive diseases and the symptoms of infection are mainly recognized after the pathogen has invaded plant tissues. Precocious detection of infection, precise identification, differentiation, and quantification of the pathogens in plants and/or in soil are essential for the development of strategies to reduce the incidence and spread of the diseases. On golf courses, maintaining a healthy turfgrass and identifying in vivo the presence of turfgrass diseases has become increasingly important in turfgrass management. An on-sight identification of the presence of the disease is normally done when symptoms are present. However, it is important to establish adequate management practices, or techniques, that may gather information of turfgrass health before disease symptoms appear. For fungal diseases, symptoms appear only a few days or even weeks after the fungus infection. On the other hand, fungi emit volatile substances that, although not noticeable by human smell, can be detected by dogs.

Since the use of hunting dogs, 12 000 years ago[2], several different applications for sniffer dogs have emerged, including the most classics and well-known such as explosives, narcotics, people searching and tracking, and the search for corpses[2,3]. However, the continued study of the dog's olfactory ability has determined that its olfactory detection threshold for a given volatile organic compound can be as low as 40 parts per billion to 1.5 parts per trillion[4]. This evidence reinforced the introduction of olfactory detection (biodetection), in different areas of science. In human health, biodetection can be used for early detection of various types of cancer[5,6] and the specific localization of bacteria responsible for hospital-acquired infections[7]. It can be used also in the area of plant protection[8], to which this work refers.

A biodetection of fungal disease has been carried out at the University of Algarve where it has been developed a project for the biodetection of the phytopathogenic fungus *Sclerotium rolfsii*, using a 4-year-old English Springer Spaniel female dog "Julieta". This work is divided into 3 different phases. The first phase, already completed and to which this article refers, had two main objectives: (i) determination of dog's olfactory ability to detect and recognize the fungus odor in inoculated samples; (ii) analyze the ability to differentiate it when compared to control samples. The next two phases will assess the olfactory ability to detect the presence of the fungus in inoculated turfgrass samples and, finally, to detect it in field conditions. Preliminary results validate the potential use of canine biodetection in the early identification of turfgrass pathogens, achieving 100% sensitivity and 100% specificity in the identification of the fungus *Sclerotium rolfsii*.

Materials and Methods

The phytopathogenic fungus was inoculated into 50 mL Falcon tubes containing 25 mL of Potato dextrose agar (PDA). For this purpose, PDA was autoclaved and solidified in an inclined position. In each Falcon, a 6 mm PDA disc containing *Sclerotium rolfsii* mycelium was inoculated.

The whole dog training protocol, from basic obedience to biodetection, uses only clicker positive reinforcement techniques. The first training stage was the direct association of the fungus odor. This 3-week stage, with a total

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Number of sessions	Number of repetitions	True positives	True negatives	False positives	False negatives
10	10	100	400	0	0

Tab. 1: Results of the olfactory capacity of the English Springer Spaniel female dog. Total attempts performed: 100.

Fig. 1: "Julieta" in one of the 100 attempts to face a straight line of 5 equally distanced Falcons. The guide is signaling a positive response of biodetection

of 1000 repetitions, consisted of the establishment of a positive direct odor conditioning, since each repetition in which the dog "smelled" the inoculated sample, she was rewarded with its favorite food. At the same time, the focus position was also trained (the dog remains standing with its muzzle close to the inoculated sample). The second and third training stages consisted of introducing odor discrimination, where the dog gradually learned to discriminate between inoculated samples and empty Falcons up to discrimination between inoculated samples and control samples (1 inoculated sample and 4 control samples). At the end of the training stages, 10 sessions with 10 repetitions each were performed where the dog was faced to a straight line of 5 equally distanced Falcons (Figure 1), to ignore the control samples and focus the inoculated one. In each session new inoculated sample and new control samples were used. All sessions were recorded.

For this work it was registered the number of (1) True positives: the dog correctly focuses the inoculated sample; (2) True negatives: the dog does not focus on the control samples; (3) False positives: the dog focuses on a control sample; (4) False negatives: the dog does not focus on the inoculated sample. Ten sessions of 10 repetitions each, were performed, to obtain the expected results. The position of the inoculated sample in each repetition was randomly determined with the roll of a die. During this random process, the dog and her guide waited in a different room, thus ensuring that the biodetection performance wasn't influenced by her guide.

Results and Discussion

The results in this work evaluated the sensitivity and specificity of the olfactory capacity of the intervening dog in the fungus biodetection. On 100 attempts, a sensitivity and a specificity of 100% (Table 1) were obtained for the biodetection of the fungus *Sclerotium rolfsii* when the 4-year-old English Springer Spaniel female dog was faced to a straight line of 5 equally distanced Falcons (Table 1).

The post-inoculation time of the samples, used in the sessions, varied between 24 and 72H, however there was no difference in the detection response by the female dog Julieta. This is also a noteworthy additional fact, given the potential for prevention that can represent the detection of a fungus with such a short post-inoculation time. Further work is being established to assess the olfactory ability to detect the presence of the fungus in inoculated turfgrass samples and finally to detect it in the field.

Authors:

M. Serrão,
Associação Kokua –
Cães de Ajuda Social
(Social Aid Dogs),
Tavira, Portugal,

L. Coelho, L. Dionísio,
C. Guerrero* and A. Duarte,
Universidade do Algarve,
Faculdade de Ciências e Tecnologia,
MedtiBio, Centre for Mediterranean
Bioresources and Food,
Faro, Portugal.

* E-Mail: cguerre@ualg.pt

TurfgrassSociety.eu