



7th ETS CONFERENCE 2020

TURF SOLUTIONS for the FUTURE



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

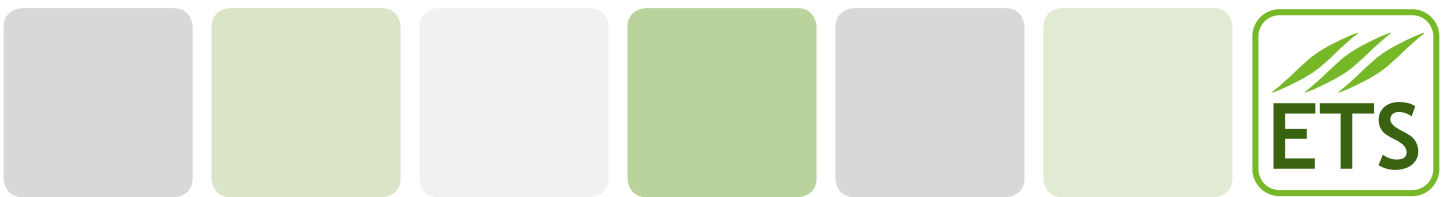
In Abstimmung mit dem ETS-Board und unter fachlicher Leitung der Deutschen Rasengesellschaft e.V. veröffentlicht die Köllen Druck + Verlag GmbH ausgewählte und „peer-reviewed 2-page-paper“ der ETS-Tagung.

In drei Ausgaben der Zeitschrift „**RASEN – European Journal of Turfgrass Science**“ erscheinen Fachbeiträge zu folgenden Schwerpunkt-Themen:

- 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**“

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Biodetection of turfgrass fungal diseases using sniffer dogs

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

Introduction

The Directive 2009/128/EC¹ 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 ago², 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 trillion⁴. 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 infections⁷. It can be used also in the area of plant protection⁸, 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

¹ DIRECTIVE 2009/128/EC: Official Journal of the European Union, L 309, pp. 71-84.

² FURTON, K.G. and J.M. LAWRENCE, 2001: The scientific foundation and efficacy of the use of canines as chemical detectors for explosives. *Talanta*, 54(3), 487-500.

³ LAZAROWSKI, L. and D.C. DORMAN, 2014: Explosives detection by military working dogs: Olfactory generalization from components to mixtures. *Applied Animal Behaviour Science*, 151, 84-93.

⁴ CONCHA, A.R., C.M. GUEST, R. HARRIS, T.W. PIKE, A. FEUGIER, H. ZULCH and D.S. Mills, 2019: Canine Olfactory Thresholds to Amyl Acetate in a Biomedical Detection Scenario. *Frontiers in Veterinary Science*, 5.

⁵ GUIRAO MONTES, Á., L. MOLINS, L. LÓPEZ-RODÓ, I. RAMÓN RODRÍGUEZ, G. SUNYER DEQUIGIOVANNI, N. VIÑOLAS SEGARRA, R.M. MARRADES SICART, ... and Á. AGUSTÍ GARCÍA-NAVARRO, 2017: Lung cancer diagnosis by trained dogs¹. *European Journal of Cardio-Thoracic Surgery*, 52(6), 1206-1210.

⁶ TAVERNA, G., L. TIDU, F. GRIZZI, V. TORRI, A. MANDRESSI, P. SARDELLA, ... and P. GRAZIOTTI, 2015: Investigative Urology Olfactory System of Highly Trained Dogs Detects Prostate Cancer in Urine Samples. *Journal of Urology*, 193, 1382-1387.

⁷ CHARLES, M.K., Y. WANG, T. ZURBERG, J. KINNA and E. BRYCE, 2019: Detecting *Clostridioides (Clostridium) difficile* using canine teams: What does the nose know? *Infection Prevention in Practice*, 1(1), 100005.

⁸ ANGLADA, L.P., M.D. ÀNGELS and C. TORRAS, 2016: Detection of *Verticillium dahliae* in Olive Groves Using Canine Detection Units. *Agricultural Sciences*, 7(7), 225-229.

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