Trichoderma gamsii as a biological control agent of turfgrass diseases

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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 diseases[1], 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 pesticides[2]. Also, the European legislation is directing the reduction of pesticides[3], 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 hazards[4]. 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 pathogens[5]. *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 soil[6]; isn't pathogenic to plants and may be beneficial for agriculture, protecting the plants from certain diseases[3].

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 oC[7], with an optimum development temperature of 26 oC[7]. In general *Sclerotinia homeocarpa* is active from autumn until spring, when days are hot and humid and the nights with dew[8]. 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.

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Methods

The experiment was carried out in the *Campus* of Gambelas at the University of Algarve, Portugal. Pots, with an area of 56.7 cm^2, 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 10^8 spores mL^{-1}. The fungicide applied was Rovral (Basf, Portugal) being the active substance the ipodrione 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.