2021 Georgia Golf Environmental Foundation

First report (January 2022-December 2022)

Project Title: Remote Sensing and Biological Pesticides to Enhance Rhizoctonia Control of Warm Season Turfgrass in Georgia

Principal Investigator(s): Bochra Bahri[1], Alfredo Martinez[1] and James Buck[1]

Address: [1]Department of Plant Pathology
University of Georgia-Griffin Campus
1109 Experiment Street
Griffin, GA 30223-1797

Staff that worked on the project: Shukti Chowdhury (PhD student), Bikash Ghimire (Post-doctoral associate) and Brian Vermeer (Research Assistant)

1. Project objectives and evolution

Rhizoctonia large patch, caused by the fungus *Rhizoctonia solani*, is a destructive disease of warm season turfgrasses in Georgia. Several fungicides are commercially available for managing the disease but their efficacy is either unknown or they provide inconsistent control. Early disease detection and identification of the natural infection rate of *R. solani* necessary to cause large patch disease are critical for effective control with fungicide applications. In fact, fungicides are rendered ineffective with late detection. Furthermore, environmental stewardship, overreliance on chemical control, and increasing concerns about pesticide resistance has led turfgrass managers to examine alternative practices to reduce plant disease. Several biological agents are promising for the control of *R. solani*; however, the efficiency of these biological agents in controlling Rhizoctonia large patch in turfgrass are unknown. Therefore, the objectives of our research are: 1) to develop a remote sensing tool for early *R. solani* detection and estimate the infection levels of *R. solani* required for large patch disease to develop, 2) investigate the potential use of biofungicides to reduce *R. solani* in vitro, 3) evaluate in the growth chamber the microbial efficacy of biofungicides for the control of Rhizoctonia larch patch in zoysiagrass, and 4) evaluate the microbial efficacy of biofungicides for the control of Rhizoctonia large patch of zoysiagrass in field trials in Georgia and its comparison to fungicide Heritage 50WG (azoxystrobin).

Initially the biofungicide targeted was *Muscodor albus* QST 20799, an antimicrobial biofumigant that controls *R. solani* in sugar beet and eggplant via mycofumigation. This endophytic fungus was developed by Marron Bio Innovations, a global leader in biological crop solutions. However, Dr. Amit Vasavada, Vice President of research and development and chief technology officer of Marron Bio Innovations and an expert in biofungicides, recommended us to instead test Regalia® and Stargus®, two new promising biopesticides due to the fact that *Muscodor albus* QST 20799 is currently not commercially available. Regalia® is a biofungicide that increases crop performance by stimulating plant defense systems. Its active ingredient is *Reynoutria sachalinensis* and it was labelled against several diseases including Rhizoctonia, Pythium, and Anthracnose on several fruit crops and vegetables. The active ingredient in Stargus® is *Bacillus amyloliquefaciens* F727 and it is labelled against downy mildew, *Botrytis* spp., *Fusarium* spp. and other fungal and bacterial diseases in a large number of commercial specialty crops. We have chosen to work on three biofungicides: *Reynoutria sachalinensis* (Regalia®), *Bacillus amyloliquefaciens* F727 (Stargus®) and *B. subtilis* QST713 (Rhapsody®) and compare their efficacy to several fungicides, in vitro and in growth chamber and field trials. A selection of six fungicides were tested: azoxystrobin (Heritage®), fludioxonil (Medallion®), fluxapyroxad (Xzemplar®), mefentrifuconazole (Maxtima®), penthiopyrad (Velista®), boscalid (Emerald®) and propiconazole (Banner Maxx®).

Our goal is to provide to the turfgrass industry practical management solutions for all diseases. To that end, we have included dollar spot, the most economically destructive fungal disease of turfgrass caused by *Clarireedia* spp. (formerly *Sclerotinia homoeocarpa*), to our studies.

2. Material and Methods of *In vitro* trials:

Isolates used (all stored at -20°C on grain)**:**
RsZeon: *Rhizoctonia solani* isolated that was isolated from Zoysiagrass variety Zeon on the UGA Griffin Campus in 2019.
RsMeyer: *Rhizoctonia solani* isolated from Zoysiagrass variety Meyer from the Golf Course (Rivermont) at Fulton county, Georgia in 2021.
DS8**:** *Clarireedia monteithiana* isolated from seashore paspalum on the UGA Griffin Campus in 2019.

***In vitro* trials:**
Trial 1 (Table1)
The design used for the *in vitro* trials was a Completely Randomized Design (CRD). Each treatment had 5 replications with each replication represented by one Petri plate. The laboratory experiment has been conducted using 'Dual culture technique'. Potato dextrose agar (PDA) media was sterilized in an autoclave at 121°C for 20 minutes then 20 ml was poured into sterilized Petri plates (90 mm diameter). Culture discs (7 day old) of the pathogen were cut separately with sterilized cork bores (9 mm) then transferred aseptically at one periphery of the Petri plate containing the medium. On the other periphery, 175 µl of the fungicides and biofungicides were poured. The pathogen alone on PDA served as the control. The inoculated Petri plates were transferred to an incubator (25 ± 1°C) and colony growth (diameter) of the fungus was measured in each Petri plate after 4 days. The experiment was repeated twice for each isolate tested.

Trial 2 (Table2)
Ten different combinations of bio- and synthetic fungicides including Rhapsody (*B. subtilis* QST713), Regalia (*R. sachalinensis* extr.*)*, and Banner Maxx (propiconazole) at varying label rates (25:75%, 50:50%, and 75:25%) along with non-fungicide amended control were assayed to identify the best treatment combination that could further be tested in the growth chamber and field experiments (Table S2). *In vitro* experimental setup, data collection techniques, and parameters followed as described above.

Table 1: Rates and active ingredients of the treatments tested *in vitro* in Trial 1

#	Fungicides and biofungicides	Active ingredients	Rates/gallon per 1000 square feet
1	Rhapsody	QST 713 strain of *Bacillus subtilis* (1×10^9 cfu/g)	10 fl oz
2	Regalia	Extract of *Reynoutria sachalinensis*	1.5 fl oz
3	Stargus	*Bacillus amyloliquefaciens* strain F727 (1×10^9 cfu/ml)	1 fl oz
4	Heritage	Azoxystrobin	0.4 oz
5	Medallion	Fludioxonil	2 fl oz
6	Xzemplar	Fluxapyroxad	0.21 fl oz

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7	Maxtima	Mefentrifluconazole	0.6 fl oz
8	Velista	Penthiopyrad	0.5 oz
9	Banner Maxx	Propiconazole	2 fl oz
10	Emerald	Boscalid	0.15 oz

Table 2: Rates and active ingredients of the treatments tested *in vitro* in Trial 2

Treatment combination tested	**Rate/gallon water (fl oz)**
100% propiconazole	2.00 fl oz prop
100% *Bacillus subtilis* QST713	10.00 fl oz BS
25% *Bacillus subtilis* QST713 + 75% propiconazole	2.50 fl oz Bs + 1.50 fl oz Prop
25% *Reynoutria sachalinensis* extr. + 75% propiconazole	0.375 fl oz Rs + 1.50 fl oz Prop
50% *Bacillus subtilis* QST713 + 50% propiconazole	5.00 fl oz Bs + 1.00 fl oz Prop
50% *Reynoutria sachalinensis* extr. + 50% propiconazole	0.75 fl oz Rs + 1.00 fl oz Prop
75% *Reynoutria sachalinensis* extr. + 25% propiconazole	1.125 fl oz Rs + 0.50 fl oz Prop
75% *Bacillus subtilis* QST713 + 25% propiconazole	7.50 fl oz Bs + 0.50 fl oz Rs
50% *Bacillus subtilis* QST713 + 50% *Reynoutria sachalinensis* extr.	5.00 fl oz Prop + 0.75 fl oz Rs
100% *Reynoutria sachalinensis* extr.	1.50 fl oz Rs

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Prop = propiconazole; Bs = *Bacillus subtilis* QST713; Rs = *Reynoutria sachalinensis* extr.
Petri-plates under in vitro assay were incubated in PDA media for 4 days in 12-hour photoperiod at 25°C.

3. Results of *In vitro* trials

Bacillus subtilis QST713 (Rhapsody) and propiconazole (Banner Maxx) were found to significantly reduce *Rhizoctonia solani* RsZeon and RsMeyer and *Clarireedia monteithiana* DS8 growth up to 100% in Trial 1 (Figures 1, 2 and 3).
Ten treatments comprising *Bacillus subtilis* QST713 and propiconazole were further tested, either alone or in combination in Trial 2. *Bacillus subtilis* QST713 alone or a combination of 75% *B. subtilis* QST713 + 25% propiconazole showed to be as efficient as 100% propiconazole for controlling *Rhizoctonia solani* RsZeon and RsMeyer and *Clarireedia monteithiana* DS8 in Trial 2 (Figures 4, 5 and 6).

In conclusion, our study suggests that biofungicides, particularly *Bacillus subtilis* QST713 (Rhapsody), could hold promise to complement synthetic fungicides in an efficacious and environmentally friendly disease management program.

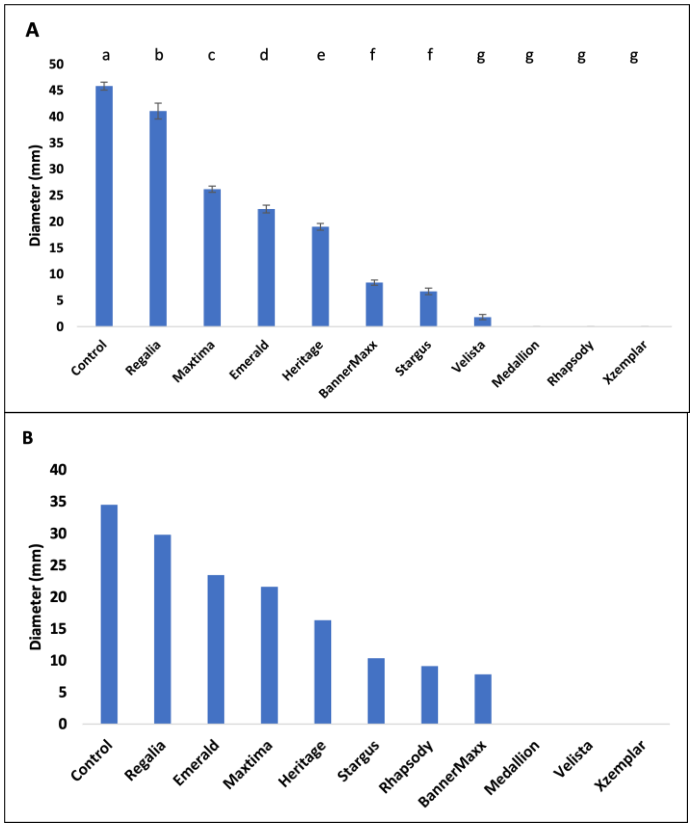

Figure 1: Diameter of mycelial growth of *R. solani* isolate RsZeon for the 11 treatments of Trial 1 tested in vitro on Petri plates amended with PDA after 4 days of inoculation at 25°C. A: Experiment 1; B: Experiment 2

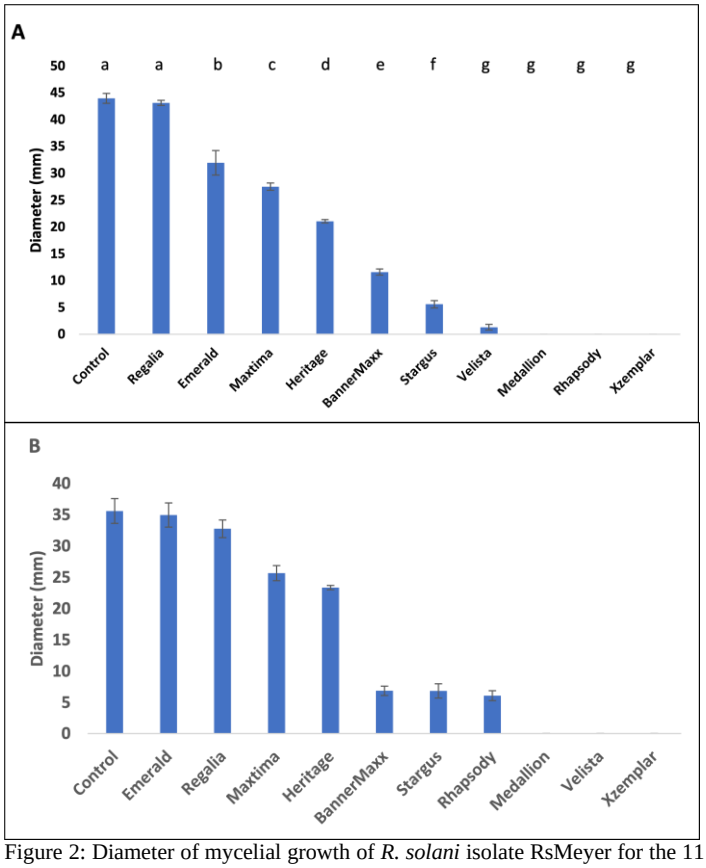

Figure 2: Diameter of mycelial growth of *R. solani* isolate RsMeyer for the 11 treatments of Trial 1 tested in vitro on Petri plates amended with PDA after 4 days of inoculation at 25°C. A: Experiment 1; B: Experiment 2

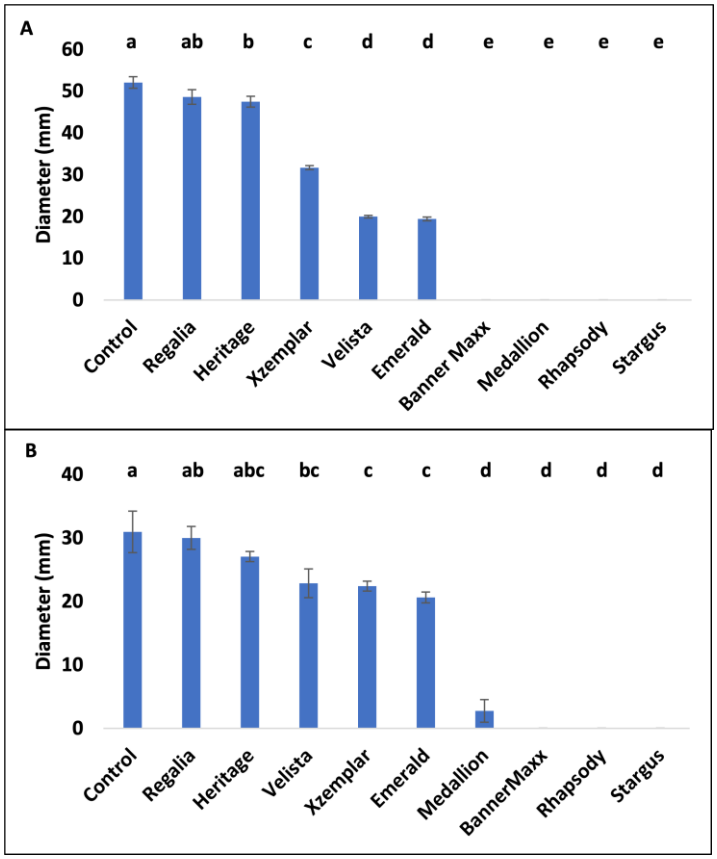

Figure 3: Diameter of mycelial growth of *C. monteithiana* isolate DS8 for the 11 treatments of Trial 1 tested in vitro on Petri plates amended with PDA after 4 days of inoculation at 25°C. A: Experiment 1; B: Experiment 2.

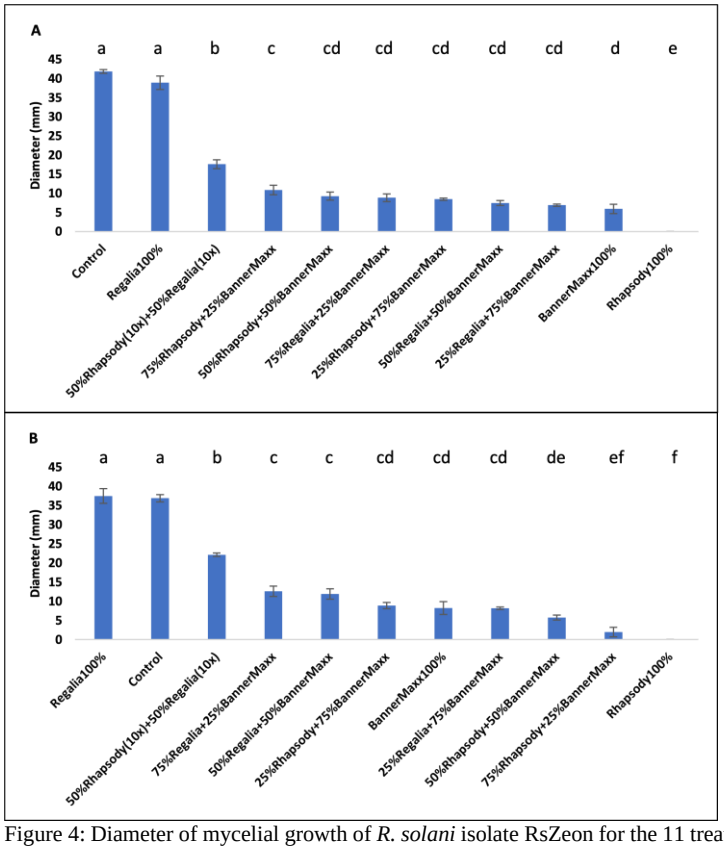

Figure 4: Diameter of mycelial growth of *R. solani* isolate RsZeon for the 11 treatments of Trial 2 tested in vitro on Petri plates amended with PDA after 4 days of inoculation at 25°C. A: Experiment 1; B: Experiment 2

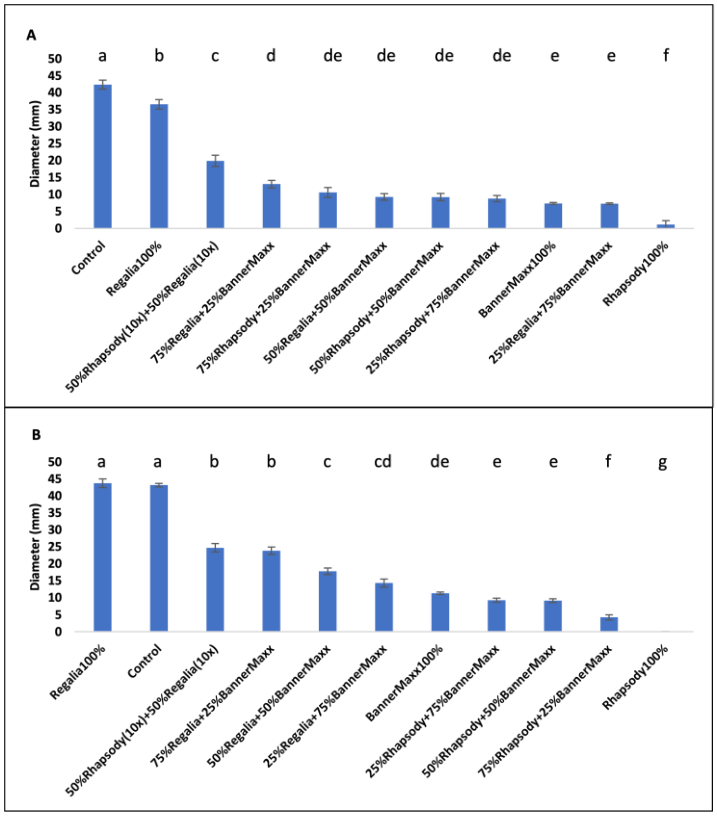

Figure 5: Diameter of mycelial growth of *R. solani* isolate RsMeyer for the 11 treatments of Trial 2 tested in vitro on Petri plates amended with PDA after 4 days of inoculation at 25°C. A: Experiment 1; B: Experiment 2

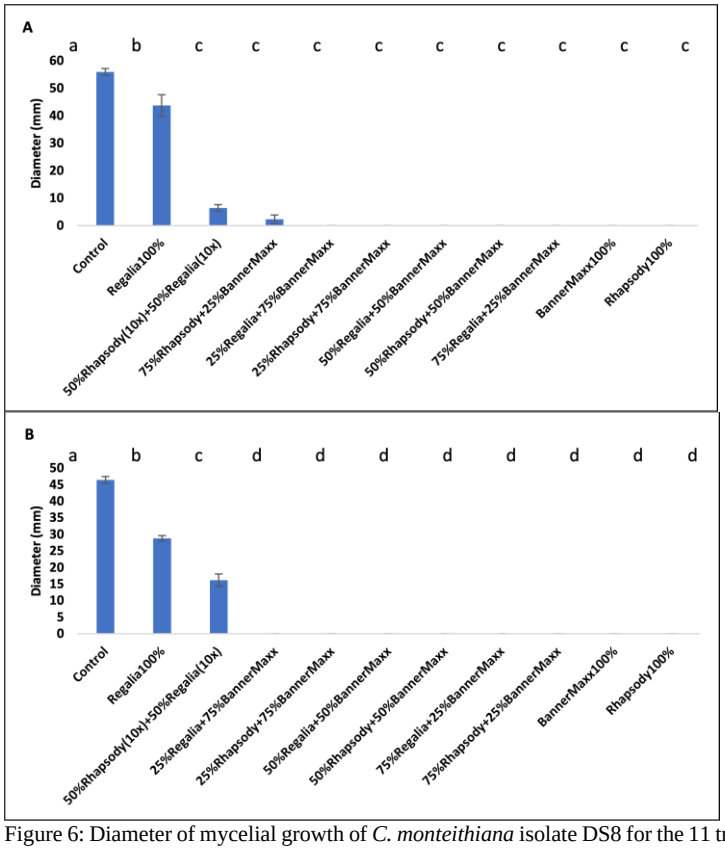

Figure 6: Diameter of mycelial growth of *C. monteithiana* isolate DS8 for the 11 treatments of Trial 2 tested in vitro on Petri plates amended with PDA after 4 days of inoculation at 25°C. A: Experiment 1; B: Experiment 2.

4. Preliminary Growth chamber and field trials

Seven fungicide spray programs comprising *Bacillus subtilis* QST713 and propiconazole were tested, either alone or in a tank mix in a reduced rate, on *R. solani* infected zosiagrass 'El toro' and dollar spot infected bermudagrass 'TifTuf' in growth chamber and field environments. So far, two trials were performed against dollar spot in growth chamber and field environments during summer and fall 2022. One trial against *R. solani* in the field was performed in fall 2022; the second field trial will be carried out in spring 2023 as well as the two growth chamber trials.

The full growth chamber and field trials results will be presented in the second report at the end of the project. Briefly, the stand-alone application of the biofungicide *B. subtilis* QST713 every seven days was the most effective and equally efficacious as propiconazole, suppressing dollar spot severity up to 34 and 75% and AUDPC up to 43 and 75% in growth chamber and field experiments, respectively, while resulting acceptable turf quality (>7.0) in the field.

In addition, we are currently initiating objective 1 on developing a remote sensing tool for early *R. solani* detection and estimate the infection levels of *R. solani* required for large patch disease to develop. All the field plots have also been photographed using a light box and data analysis is currently ongoing.

5. Deliverables

The dollar spot results of this project were submitted to the journal *Frontiers in Plant Sciences* on January 30, 2023 under the title "Sensitivity of *Clarireedia* spp. to Benzimidazoles and Dimethyl Inhibitors Fungicides and Efficacy of Biofungicides on Dollar Spot of Warm Season Turfgrass." The results on *R. solani* will be submitted to the journal Plant Disease by the end of 2023.
The output of the project was also presented at the Field day on August 3, 2022 in Griffin, GA (oral presentation) and will be presented at the 2023 GAPP in Savannah on March 7, 2023 (oral presentation).