Pathogenicity evaluations of newly identified ectotrophic root-infecting fungi on ultradwarf bermudagrass

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Introduction

Ultradwarf bermudagrasses are used throughout the southern United States for golf course putting greens (Fig. 1A). These grasses face many adverse conditions in late summer and early fall months and consequently decline in aesthetics, playability, and vigor (Fig. 1B). Root systems of affected plants are typically brown to black in color, diminutive in size, and frequently colonized with dark, runner hyphae (Fig. 1C), which is characteristic of ectotrophic root-infecting (ERI) fungi. Lobed and simple hyphopodia and growth cessation structures are often observed in association with roots of declining plants (Fig. 1D-F). Research at Mississippi State University (MSU) led to the identification of eight ERI fungal species, six of which were novel. The species were identified as *Gaeumannomyces graminis* var. *graminis*, *G. paulograminis* sp. nov., *Magnaporthiopsis incrustans*, *M. hawaiiensis* sp. nov., *M. cynodontis* sp. nov., *M. taurocanis* sp. nov., *Candidacolonium cynodontis* Gen. nov. sp. nov., and *Pseudophialophora cynodontis* sp. nov (Fig 2). The purpose of this study was to test the pathogenicity of ERI fungi that were isolated from affected roots.

Materials and Methods

Fungal Isolates

Representative isolates from each of the eight fungal species were selected and grown on potato dextrose agar (PDA) under 24 hour fluorescent light. Cultures were left until complete colonization of PDA occurred.

Plant Material

'Champion' and 'MiniVerde' ultradwarf bermudagrass plugs (Fig. 3A) were collected and allowed to produce an abundance of aerial stoloniferous plant material (Fig. 3B). Stolons were gathered and plants, containing five nodes and four internodes, were selected (Fig. 3C).

Treatment Application and Inoculation

The experiment was conducted in a split-plot design with three replications. Ultradwarf bermudagrass cultivars were whole plots and fungal isolates were split-plots. Sterilized sand was placed in three inch diameter pots (Fig. 3D), fully-colonized PDA was added on top of the sand (Fig. 3E), five plants were placed directly in contact with the fungal inoculum (Fig. 3F), pots were capped off with sterilized sand (Fig. 3G), and placed in a growth chamber (Fig. 3H) for 8 weeks. Growth chamber environmental conditions that were used in the study are summarized in Table 1.

Data Collection

Plants were gathered from growth chambers and roots were collected, cleaned, and scanned with WinRhizo. Scanned images were subject to pixel color analysis to determine percent disease of root systems.

Results and Discussion

Pathogenicity of MSU-ERI fungal isolates on ultradwarf bermudagrass, based on pixel color analysis, are shown in Fig. 4. Fungal isolates MSU2 and MSU7 are virulent; MSU 3 and MSU9 are moderately virulent; MSU 1, MSU4, MSU5, MSU6, and MSU8 are weekly virulent; and MSU10 is avirulent. MSU10 (Fig. 5J) was an untreated control. MSU3 (Fig 5C), *M. incrustans*, has been tested in previous studies where it was found to be weekly virulent to avirulent. MSU1 (Fig. 5A), *G. graminis* var. *graminis*, is the only isolate included in the study that was previously known to incite root rot of bermudagrass, causing bermudagrass decline. As evident in Fig. 5B and G and Fig. 6B and D, isolates MSU2 and MSU7 caused significant discoloration of root, stolon, and foliar plant material. Not only did many of the stolons of those isolates fail to produce roots, but the ones that did produce roots were discolored. Fig. 6A shows an untreated control plant that did not produce any root material; however, the stolon appears healthy.

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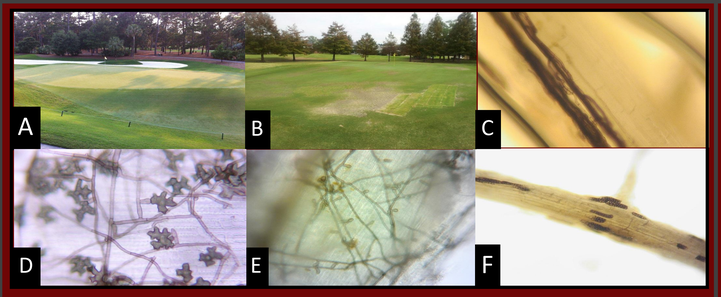

Figure 1. (A) MiniVerde ultradwarf bermudagrass at the number 1 green of The PLAYERS Club at Sawgrass in Ponte Vedra Beach, FL., (B) field symptoms of decline on ultradwarf bermudagrass putting green, (C) dark, runner hyphae on ultradwarf bermudagrass root, (D) lobed hyphopodia on roots of ultradwarf bermudagrass showing symptoms of decline, (E) simple hyphopodia on roots of affected ultradwarf bermudagrass, (F) growth cessation structures on roots of ultradwarf bermudagrass exhibiting decline symptoms

Table 1. Daytime and nighttime environmental conditions for growth chambers used in pathogenicity evaluations.

	GC†1	GC2	GC3	Mean‡
Daytime				
Air Temperature (°C)	33.96	33.55	33.38	33.63
Soil Temperature (°C)	29.51	30.48	29.89	29.96
Relative Humidity (%)	70.72	71.29	63.67	68.56
Dew Point (°C)	27.83	27.57	25.50	26.97
PAR§ (µmol x m^{-2} x sec^{-1})	274.60	240.95	245.75	253.77
Nighttime				
Air Temperature (°C)	27.37	26.48	30.35	28.07
Soil Temperature (°C)	26.94	27.22	30.64	28.27
Relative Humidity (%)	92.88	93.43	92.79	93.03
Dew Point (°C)	26.10	25.32	29.03	26.82

† Growth Chamber
‡ Mean, expressed as the average of GC1, GC2, and GC3
§ Photosynthetically Active Radiation, measured with LightScout® Quantum Light Sensor

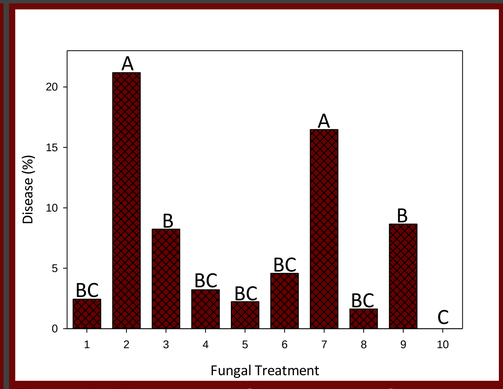

Figure 4. Mean percent disease, measured as percent discoloration of roots following inoculation with fungal isolates 1 – 10.

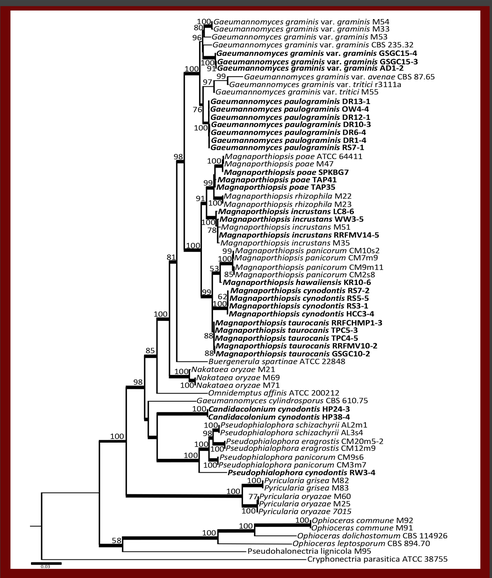

Figure 2. Maximum likelihood phylogram based on the concatenated ITS, LSU, SSU, MCM7, RPB1, and TEF1 sequence datasets. Boldfaced branches indicate BI posterior probabilities ≥ 0.95 and ML bootstrap values ≥ 50% are presented above internodes.

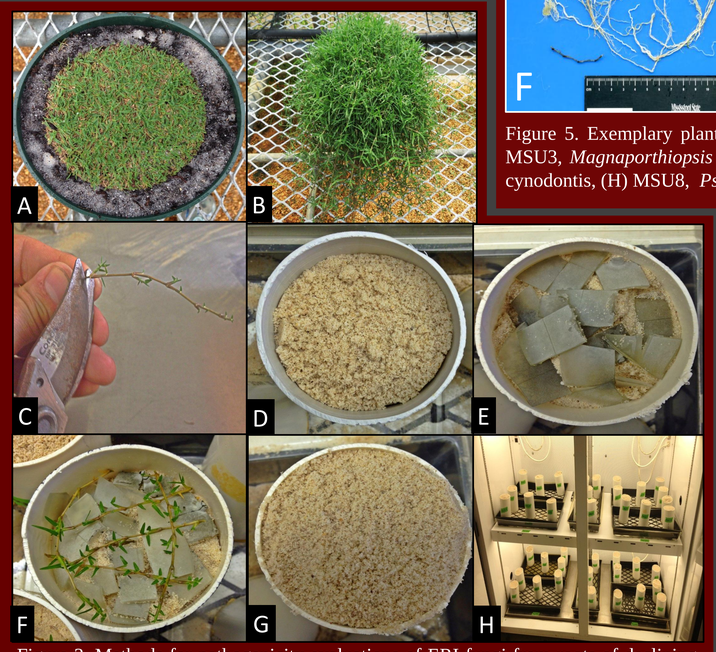

Figure 3. Methods for pathogenicity evaluations of ERI fungi from roots of declining ultradwarf bermudagrass plants. (A) four inch plug of Champion ultradwarf bermudagrass collected from research plot, (B) stoloniferous plant material extending from turf plugs, (C) collection of plant material, consisting of five nodes and four internodes, (D) sterilized sand filled to within two centimeters of the top of three inch diameter pots (E) PDA, fully colonized with fungal material, placed directly on top of sterilized sand (F) plant material placed directly in contact with inoculum source (G) sterilized sand placed on top of plant material for moisture retention, and (H) placement of inoculation containers in growth chamber

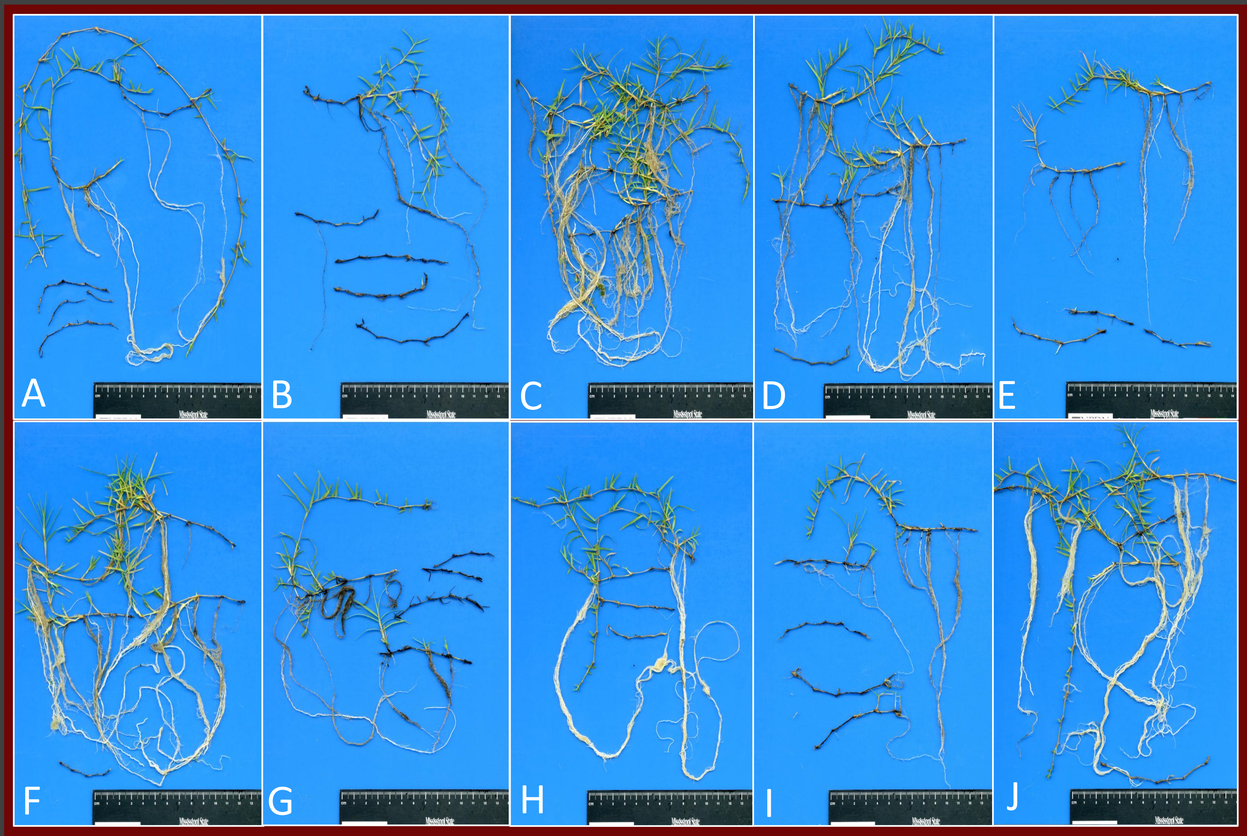

Figure 5. Exemplary plants from pathogenicity evaluations. (A) MSU1, *Gaeumannomyces graminis* var. *graminis*, (B) MSU2, *G. paulograminis*, (C) MSU3, *Magnaporthiopsis incrustans*, (D) MSU4, *M. hawaiiensis*, (E) MSU5, *M. cynodontis*, (F) MSU6, *M. taurocanis*, (G) MSU7, *Candidacolonium* cynodontis, (H) MSU8, *Pseudophialophora cynodontis*, (I) MSU9, Combination of fungal species listed in B through I, and (J) MSU10, untreated control.

Figure 6. Symptomatology of plant material from pathogenicity evaluations with ERI fungi isolated from roots of declining ultradwarf bermudagrass. (A) untreated control (B) *Candidacolonium* cynodontis, (C) *Gaeumannomyces graminis* var. *graminis*, (D) *M. hawaiiensis*, (E) *Magnaporthiopsis cynodontis*