Identification of Species Associated with a New Disease of Annual Bluegrass Putting Greens

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INTRODUCTION

Within the last decade, an unusual disease caused by an unidentified pathogen has been observed periodically on golf course putting greens in the northeastern United States. The disease primarily occurs on native soil putting greens with mixed stands of creeping bentgrass (*Agrostis stolonifera* L., CBG) and annual bluegrass (*Poa annua* L., ABG). The disease primarily affects ABG.

In 2016, the appearance of symptoms characteristic of the disease appeared on 13 golf courses. Symptoms on ABG include yellow to orange, circular patches ranging from 8 to 30 cm in diameter (Fig 1). Following incubation, coenocytic mycelium may be observed in the canopy of symptomatic leaves (Fig 2). Leaves look discolored and water soaked and eventually collapse. Initial findings suggest that *Pythium* species may be the cause of this purported new disease that has been referred to as Pythium patch.

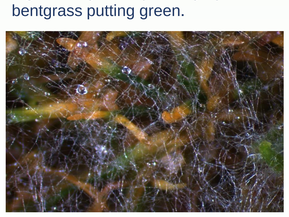
Observations on Pythium Patch

- Disease symptoms on ABG include yellow to orange circular patches
- Symptoms develop on native soil putting greens that have been modified with heavy topdressing
- Symptoms are slow to develop and mimic those of summer patch (*Magnaporthe poae*)
- Anecdotal findings suggest that fosetyl-Al applications are not effective at suppressing the disease

OBJECTIVES

- Identify potential pathogenic species associated with Pythium patch symptoms
- Determine cardinal temperatures for growth of isolates associated with this potential new disease
- Successfully complete Koch's postulates using species associated with symptoms

Figure 1. *Pythium* patch symptoms on a mixed annual bluegrass/creeping bentgrass putting green.



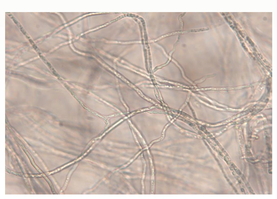


Figure 2. Following incubation, coenocytic mycelium may develop around symptomatic leaf tissues. Mycelium is visible, but not profuse.

MATERIALS & METHODS

Pathogen Isolation and Identification

- A total of 14 isolates were collected from 14 golf courses in five states
- Isolates sequenced using ITS markers
- Morphological characterization of isolates described

Cardinal Temperatures for Growth

- Isolates were incubated on PDA at 6 temperatures (10 to 40C)
- Diameter of colony measured in two directions and averaged after 48 hrs
- Study arranged as a CRD with 4 replications and study repeated twice

Figure 3. Incubation of cultures in growth chambers at 6 temperatures (10 to 40C)

Koch's Postulates

Plant and inoculum preparation

- 'Penn A4' CBG and ABG seeded at 97.6 kg/ha in conetainers
- All plants grown in greenhouse for 60 days
- A total of 6 representative isolates selected
- A single oat grain infested with each isolate used to inoculate plants
- Noninfested oat grains and no inoculum used as controls

Incubation and ratings

- Individually covered plants were incubated at 25C with a 12h day/night cycle
- Mycelium and severity were rated daily based on a 1 to 9 scale where 1 = no mycelium or symptoms and 9 = plants covered in mycelium or dead. Data were analyzed using MIXED procedure on SAS 9.4
- Fungi were re-isolated from symptomatic tissues and DNA sequenced

Figure 4. Preparation of plants for Koch's postulates and symptoms on infested annual bluegrass and creeping bentgrass.

RESULTS

Table 1. List of golf courses and corresponding organism isolated from annual bluegrass on putting greens.

Course	Genus	Species
The Kahkwa Club, PA	*Pythium*	*arrhenomanes*
Manufacturer GCC, PA	*Rhizopus*	*oryzae*
South Hills GC, PA	*Rhizopus*	*oryzae*
Fox Chapel GC, PA	*Pythium*	*arrhenomanes*
West Point GC, NY	*Pythium*	*aristosporum*
Hempstead GC, NY	*Pythium*	*aristosporum*
Villa (seeds)	*Rhizopus*	*oryzae*
Philmont CC, PA	*Pythium*	*aristosporum*
Innis Arden GC, CT	*Pythium*	*aristosporum*
Indiana CC, PA	*Pythium*	*aphanidermatum*
Wee Burn GC, CT	*Pythium*	*arrhenomanes*
Westfield CC, OH	*Pythium*	*aristosporum*
The resort of Glade Springs, WV	*Pythium*	*inflatum*
Berkshire GC, OH	*Pythium*	*aphanidermatum*

Species Morphology

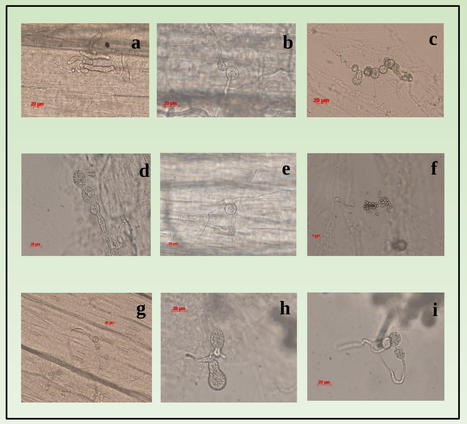
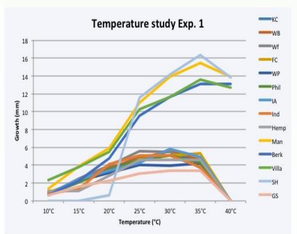


Figure 5. Morphological characteristics of *Pythium* isolated from annual bluegass. Specific morphological structures include *P. aristosporum* lobulate sporangia (a), sphaerical sporangia (b), catenulate sporangia (c and d), oogonium with diclinous antheridium (e), zoopores (f), and plerotic oospores (g). Specific morphological structures of *P. arrhenomanes* include sporangia (h and i).

Temperature Study

- Limited growth of all species evaluated occurred at 10 and 40C
- Maximum growth occurred between 25 and 35C for all isolates
- *P. arrhenomanes*, *P. aristosporum* and *P. inflatum* generally grew the slowest among all isolates
- Faster-growing species included *P. aphanidermatum* and isolates of the non-pathogenic *Rhizopus oryzae*



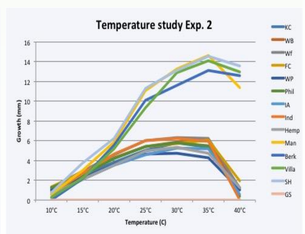


Figure 6. Cardinal temperatures for the growth of various species of *Pythium* and *Rhizopus* when grown on potatoe dextrose agar. Isolates include *P. aristosporum* [Innis Arden (IA), Hempstead (Hemp), Westfield Group (Wf), West Point (WP), and Philmont (Phil)], *P. arrhenomanes* [Wee Burn (WB), The Kakhwa Club (KC), and Fox Chapel (FC)], *P. aphanidermatum* [Berkshire (Berk), and Indiana (Ind)], *R. oryzae* [Villa, Manufacturer (Man), and South Hills (SH)] and *P. inflatum* [Glade Spring (GS)]

RESULTS

Koch's Postulates

- Disease symptoms were observed from all organisms on both species evaluated except for *R. oryzae*
- All isolates of *P. arrhenomanes* and *P. aristosporum* caused disease symptoms
- *Pythium* species infected both turfgrass species, but disease severity was generally higher on ABG

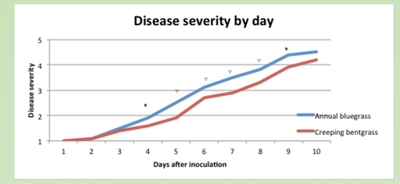


Figure 7. *Pythium* severity on creeping bentgrass (red line) and annual bluegrass (blue line). Means with * indicate significantly different main effect of grass species at $P \leq 0.05$ according to Fisher's Protected least significant difference test. ▽ indicates a species by isolate interaction was present.

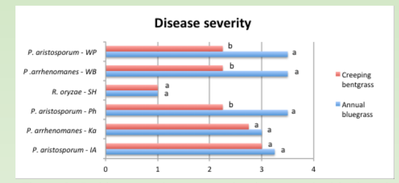


Figure 8. *Pythium* severity on creeping bentgras (red bar) and annual bluegrass (blue bar) for each isolate after 5 days of incubation. Means with the same letter are not significantly different at $P \leq 0.05$ according to Fisher's Protected least significant difference test.

CONCLUSIONS

- Several slow-growing isolates of *Pythium* may be involved in this disease complex
- The species grow over a wide range of temperatures with maximum growth between 25 and 35C
- *P. arrhenomanes* and *P. aristosporum* appear to impact ABG more severely than CBG, which may explain the selective nature of the disease in the field

FUTURE WORK

- Continue to identify the scope of Pythium patch on golf courses
- Conduct and confirm Koch's postulates in the field
- Identify the relationship between fosetyl-Al and the *Pythium* species identified in this study
- Elucidate potential interactions between *Pythium* species associated with Pythium patch and nematodes
- Identify cultural and chemical management options for the disease

ACKNOWLEDGEMENTS

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