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Isolation and characterization of Pythium spp. from South Dakota soils under commerial alfalfa production

Jennifer M. Giles
Dakota State University, jenni.giles@trojans.dsu.edu

Andrew E. Sathoff
Dakota State University, andrew.sathoff@dsu.edu

Isolation and Characterization of *Pythium spp.* from South Dakota soils under commercial alfalfa production

Jennifer Giles , Dr. Andrew Sathoff
Department of Biology, Dakota State University, Madison, SD 57042, USA

Abstract

Alfalfa is a significant crop in South Dakota that provides many different benefits for its growers. South Dakota plants the most acres of alfalfa in the United States. It is used as a protein-rich feed for livestock, a cover crop that protects against soil erosion, and a natural fertilizer because of its ability to fix nitrogen in the soil. However, alfalfa seedlings are susceptible to many diseases. Pythium root and seed rot is one disease known to have devastating effects on alfalfa field establishment and yield. Pythium species are oomycete pathogens that inhabit the soil and remain present and pathogenic as oospores. Pythium diseases of alfalfa cause reduced root systems, plant size, length, and growth rate. Pythium management is centered on fungicidal seed treatments. There have been recent reports of *Pythium* spp. infecting alfalfa across the world in places like Sudan and China, but current research in South Dakota is needed. In our research, we isolated *Pythium* spp. from Lake County South Dakota soils under commercial alfalfa production. We also characterized these isolates with a DNA sequencing analysis and evaluated the isolates for fungicide sensitivity. This summer, we will conduct a statewide Pythium disease survey and assess the collected isolates for fungicide sensitivity and pathogenicity towards various commercial lines of alfalfa. This research will provide growers with the information necessary to Make educated decisions in order to increase yields and maximize their profits.

Objectives

- Assess Lake County fields for soil pathogen *Pythium spp.* That negatively affects alfalfa growth and establishment
- Test fungicide treatments against isolated strains of *Pythium spp*.
- Characterize Pythium isolates through DNA extraction and sequencing

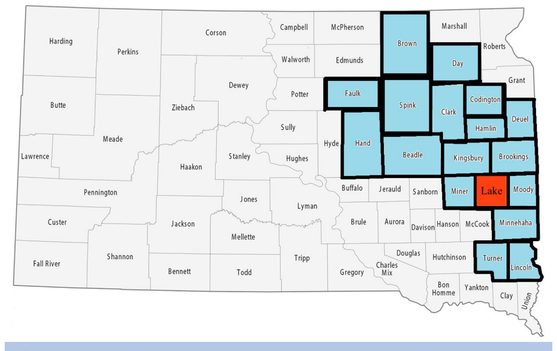

Figure 1. In blue is our grower network where we plan to sample this summer. Lake County is in red where this study took place.

Figure 2. Poor alfalfa stand establishment in Lake Country observed in summer 2020

Methods

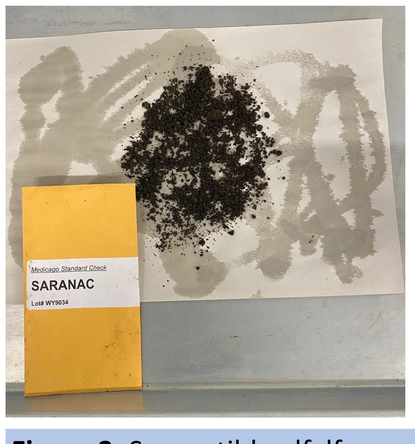

Figure 3. Susceptible alfalfa were planted in Lake County soil with the rolled towel method to bait out the pathogen.[1]

DNA Extraction and Characterization: Fungal isolates from CMA plates were grown at the baiting temperature for 4-7 days in 20 ml of V8 broth. Mycelium was filtered using Miracloth, rinsed, and freeze dried at -80°C. DNA was extracted from the freeze-dried mycelium using a FastDNA Kit. The internal transcribed spacer (ITS) region of DNA was amplified using PCR[2]. Results were confirmed using gel electrophoresis. PCR reactions were purified and quantified with a NanoDrop before being sent for sequencing.

Fungicide Sensitivity: Plugs from different Pythium isolates were placed in the center of CMA plates that have been amended with fungicide at 1.0 µg/mL concentration, plus control plates with no fungicide. The fungicide used was Apron XL (mefenoxam) from Syngenta. Both sets of plates were incubated at room temperature in darkness. Growth was compared after 48 hours.

Results

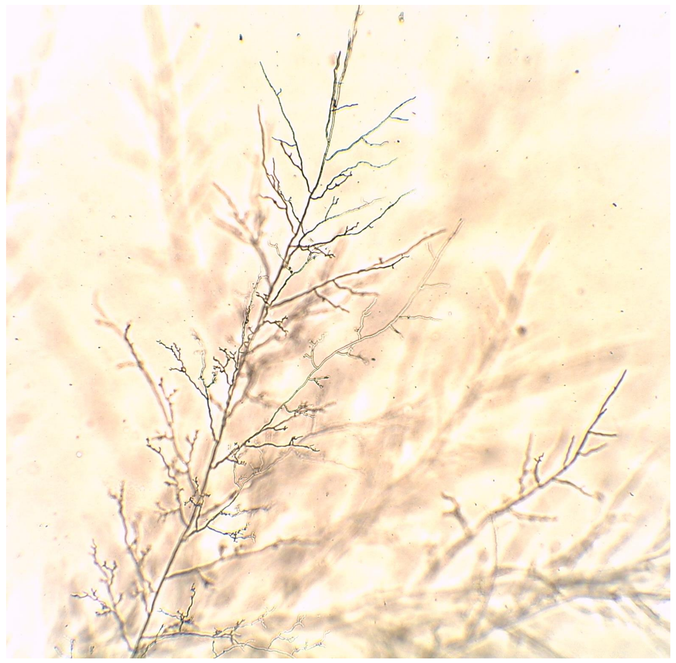

Figure 4. Hyphal morphology from seedlings on 1.5% WA at 10x magnification.

Baited alfalfa seedlings were grown on water agar to produce mycelial growth. Pythium hyphae branch off in a multidirectional manner (Fig 2) and lacks a Y-shaped junction that is often found in other fungal alfalfa root rotting pathogens. DNA was obtained from pure cultures and amplified using PCR. ITS barcoding primers were used to amplify Pythium DNA.[2] Results from gel electrophoresis showed banding at the 250 bp region, indicating the presence of Pythium in our soil samples. These PCR products were sent for further DNA sequencing analysis.

Pythium root and seed rot of alfalfa can be treated with fungicides included in alfalfa seed treatments. Our Pythium isolates displayed varying sensitivity towards fungicide Apron XL at 1.0 µg/mL concentration shown in (Fig 3). Apron XL has been continuously used as a seed treatment for decades, and South Dakota Pythium isolates are becoming resistant to it.

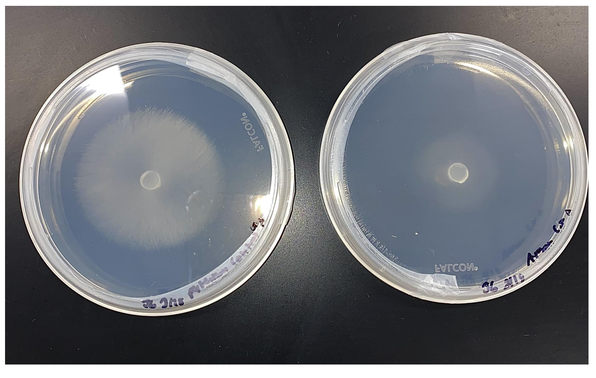

Figure 5. Plugs from Pythium isolates showed varying sensitivity to fungicide Apron XL.

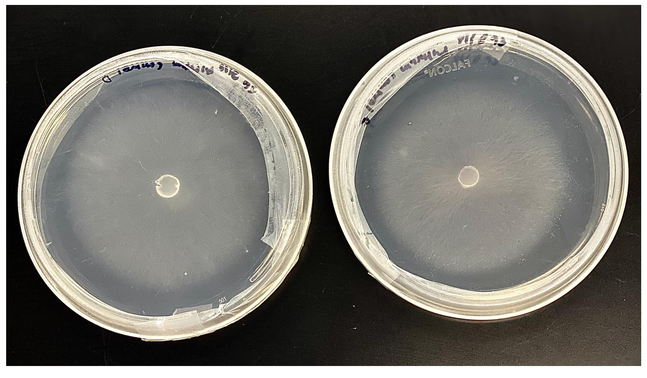

Figure 6. Plugs from Pythium isolates showed little sensitivity to fungicide Apron XL.

Conclusions & Future Directions

- Pythium isolates are likely causing alfalfa field growth and establishment issues in Lake County and other fields across South Dakota.
- Fungicides are the best way to manage Pythium in commercial alfalfa fields, but new treatments are needed to combat pathogen resistance
- Providing farmers with information on the pathogens in their fields will increase yields and alfalfa productivity throughout the state.
- This summer, we will continue this research by conducting a disease survey and pathogenicity testing on alfalfa fields across Eastern South Dakota.
- This research is funded by Mustang Seeds, a South Dakota owned company that is working to provide local research to their customers.

References

1.. Berg LE, Miller SS, Dornbusch MR, Samac DA, 2017. Seed Rot and Damping-off of Alfalfa in Minnesota Caused by *Pythium* and *Fusarium* Species. *Plant Disease* **101**, 1860-7

2. White TJ, Bruns T, Lee S, Taylor J, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Page 350 in: *PCR Protocols: A Guide to Methods and Applications.* MA Innis, DH Gelfand, JJ Sninsky, TJ White eds. Academic Press Inc., New York, NY.