University of Minnesota

PLANT PATHOLOGY

Does *Fusarium solani* Form an Endophytic Association with Soybean Roots?

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ABSTRACT

Fusarium solani (F. solani) is a soil-borne filamentous fungus which causes seed and seedling rot in soybean (*Glycine max*). Infection during germination can cause severe stand reductions. However, if a soybean seedling is able to survive through the initial infection the plant tends to be larger in size and produces higher yield. The objective of this research is to determine if infection by *F. solani* persists in the soybean roots after initial infection. Seedlings were grown in soil inoculated with uninfested and infested red sorghum for 28 days. Three observation techniques, plating, staining, and quantitative PCR (qPCR) using primers specific for *F. solani*, were conducted on roots after the plants were harvested. When roots were plated on ampicillin rifampicin infused water agar (ARWA) media there was general contamination of the roots with what appears to be *Pythium spp.*, a fungus-like genus which interferes with the growth of filamentous fungi. This contamination may also complicate microscopic assessment of fungal infection of stained roots due to our inability to distinguish hyphae of *F. solani* from those of *Pythium spp*. Use of specific primers allowed us to detect *F. solani* in the sampled roots; however, competition with *Pythium* spp. compromised our results. Use of selective culture media and sterilization of growth media may be necessary to prevent the growth of contaminating *Pythium* spp.

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INTRODUCTION

Fusarium solani is a soil borne fungus that causes seed and seedling rots of soybeans. The pathogen has the potential for causing severe stand reduction, yield loss, and plant mortality. (Nelson, 1999) *F. solani* is considered an important soybean pathogen in Minnesota because of its prevalence and the impact of infection on soybean yield and productivity. (Nelson, 1999) Previous research indicates that infection is favored by conditions that cause plants to stress, particularly during germination and early plant development. (Meyer, 2010) However, this same researcher observed that a soybean plant which survives the initial infection by *F. solani* is generally larger in size and produces greater yield than plants which were not inoculated with *F. solani* (Meyer, 2010; Kurle et al, 2011) suggesting that *F. solani* benefits plant growth. The objectives of this research are: 1) to determine if plants which survive inoculation are infected asymptomatically and retain *F. solani* within the root tissues and 2) to determine the location of the fungus in the root tissue during and after infection.

OBJECTIVES

(1) Determine if plants which survive inoculation are infected asymptomatically and retain *F. solani* within the root tissues
(2) Determine the location of the fungus in the root tissue after infection

MATERIALS AND METHODS

Experiment Details:

- Soybean variety: McCall
- Isolate: *Fusarium solani* isolate #910-2
- Treatments: Soil Only, Soil mixed with uninfested seed of Red Sorghum, Soil mixed with infested seed of Red Sorghum
- Repetitions: 15 repetitions per treatment

Substrate Preparation and Inoculation

- 300 mL substrate and 150 mL DI water were added to a flask and sealed with tin foil
- Flask was then autoclaved at 121C for 20 minutes and left to cool
- Uninfested sorghum was not inoculated with the isolate
- Infested sorghum was prepared by adding 6 5x5mm plugs from a 2-4 week old culture on PDA media and allowed to grow for 4 weeks
- Inoculum concentration was calculated using a haemocytometer

Soil Inoculation and Planting:

- Inoculum (infested or uninfested) was added to soil in a 1:20 ratio
- Seeds were planted in containers and grown for 28 days
- Growth chamber temperature set at 23C, 12 hour daylight cycle

Data Collected:

- Shoot and Root necrosis scores & dry weights
- Stand Count
- Root Plating
  - Cut off lateral roots and surface sterilize tap root
  - Cut tap root into segments and plate on ARWA media
- Root Staining
  - Cut off and surface sterilize lateral roots
  - Using Cotton Blue dye, dye the roots and look under a stereoscope for fungal material

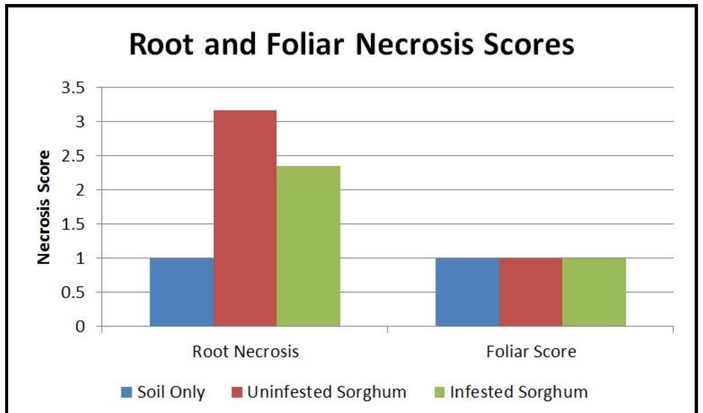


Chart 1. Root necrosis was most severe in the uninfested treatment, and moderately severe in the infested treatment. There was no difference in foliar necrosis scores.

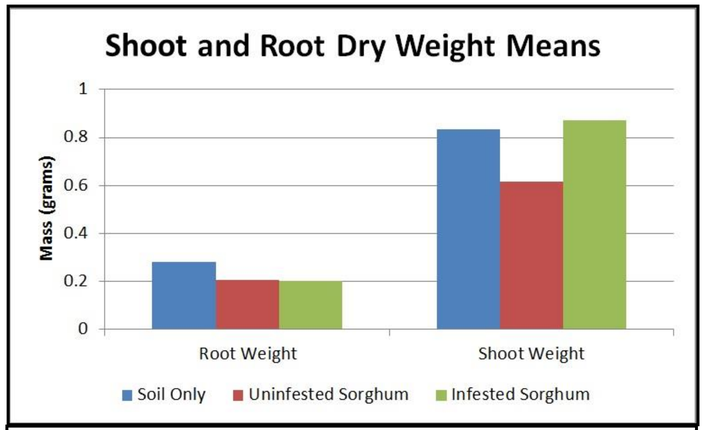
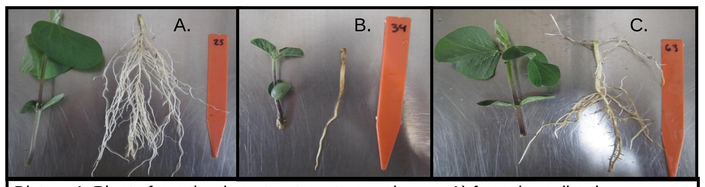
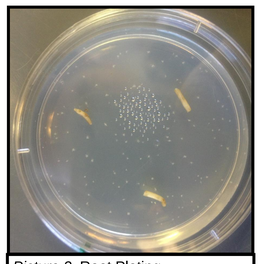


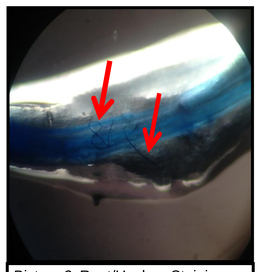
Chart 2. Root weight was impacted almost equally by having uninfested and infested sorghum mixed into the soil. Uninfested sorghum severely decreased shoot biomass whereas infested sorghum increased shoot biomass.



Picture 1. Plants from the three treatments are shown: A) from the soil only treatment, B) from the soil and uninfested red sorghum treatment, and C) from the soil and infested red sorghum treatment.



Picture 2. Root Plating



Picture 3. Root/Hyphae Staining

RESULTS

- **Plant Growth**
  - Uninfested red sorghum caused more severe root necrosis than infested red sorghum.
  - Inoculation with either uninfested and infested red sorghum decreased root biomass by 26 and 28% respectively.
  - Inoculation with uninfested substrate resulted in a 26% decrease in shoot biomass. Inoculation with infested substrate increased above ground biomass 4%.
- **Root Plating**
  - Isolates from plated roots were morphologically identified as *Pythium sp.,* with some isolates which could be *Fusarium sp*. Isolates were gathered from all three treatments.
- **Root Staining**
  - Fungal hyphae were present within root tissues from the infested treatment.

CONCLUSIONS

- **Plant Growth –** Uninfested sorghum had more of an impact upon plant development than the infested treatment which indicates that the substrate has some influence on disease evaluation.
- **Root Plating –** Due to the presence of *Pythium sp.* in samples from all treatments it can be concluded that the growing media was contaminated. Utilization of selective media which inhibits *Pythium sp.* would have helped in culturing *F. solani* if it was present in the roots.
- **Root Staining –** Contamination by *Pythium spp*. made visual assessment of infection unreliable
- **DNA Extraction –** Molecular techniques are the most reliable method of confirming the presence of *F. solani* in the root systems due to the specificity associated with the primers used for PCR.

FUTURE RESEARCH QUESTIONS

(1) What was the source of the contamination seen when the roots were plated and stained?
(2) Can use of selective media or rigorously applied sterile techniques ensure infection by only *F. solani*?
(3) Does infection by *F. solani* alone enhance soybean plant growth?

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