

# SPURIOUS SYMPTOMS CAUSED BY INOCULUM CARRIERS USED IN PLANT DISEASE RESISTANCE EVALUATION

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## ABSTRACT

Symptom development resulting from inoculation and infection with plant pathogens is necessary in order to evaluate plants for disease resistance. The standard technique for infecting soybean (*Glycine max*) with *Fusarium solani*, a root rot pathogen, involves inoculation of growth media with infested seed as an inoculum substrate carrying the pathogen. It has been reported that some uninfested substrates can have adverse effects upon shoot growth and root development. These effects include lesion-like necrosis similar to that caused by soybean root rot pathogens. The objective of our research is to determine if uninfested inoculum substrate affects soybean shoot or root growth and root symptom development. Soybean seeds were planted in media inoculated with seed of red sorghum, white sorghum, or sudan grass that had been infested with *Fusarium solani* or remained uninfested. After 14 days of growth, foliar and root necrosis scores, shoot and root dry weights, and stand counts were evaluated. When the effect of uninfested seed was compared with that of infested seed, the uninfested treatment caused more severe root rot symptoms and decreased shoot and root biomass by 20% and 15% respectively. Our results indicate that the uninfested inoculum substrate can cause both spurious growth reduction and symptom development that may mislead the researcher conducting soybean variety evaluations for disease resistance.

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## INTRODUCTION

Evaluation of plant resistance to infection by plant pathogens is often accomplished by inoculation with cultures of the pathogen growing on a nutrient substrate. Inoculations with the root rot pathogen, *Fusarium solani* (*F. solani*), have been conducted using a variety of different substrates such as rice kernels (Navi & Yang, 2008), sorghum seed (Roy, 1997 and Huang, 1998), cornmeal (Gray, 1996), and oat grains (Scherer, 1996). Production of *F. solani* on a grain substrate is similar to the situation in a field setting where fungi colonize and persist on crop residues. It has been reported that some uninfested substrates can have adverse effects upon shoot growth and root development. These effects include reduced growth and lesion-like necrosis resembling that caused by root rot pathogens. Although the effect of uninfested substrate upon plant growth has been reported it is usually not considered when experiments are conducted. The objective of this research project is to (1) confirm that uninfested seed can have an effect on plant growth and root development and (2) evaluate the effect on root development of a number of commonly used seed substrates.

## OBJECTIVES

- (1) Confirm that uninfested substrate has an impact upon plant growth.
- (2) Evaluate differences in symptom severity seen when red sorghum, white sorghum, and sudan grass are used as an inoculum substrate.

## MATERIALS AND METHODS

### Experiment Details:

- **Soybean variety:** McCall
- **Isolate:** *Fusarium solani* isolate #910-2
- **Substrates:** Red Sorghum, White Sorghum, Sudan Grass
- **Treatments:** Soil Only, Soil mixed with uninfested seed of Red Sorghum, White Sorghum, and Sudan Grass, Soil mixed with infested seed of Red Sorghum, White Sorghum, and Sudan Grass
- **Repetitions:** 25 repetitions per treatment for each substrate

### 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 overnight
- Uninfested sorghum was not inoculated with the isolate
- Infested sorghum was prepared by adding 6 5x5mm plugs from a 2-4 week old culture grown on PDA media and allowed to grow for 4 weeks
- Inoculum concentration was calculated using a haemocytometer

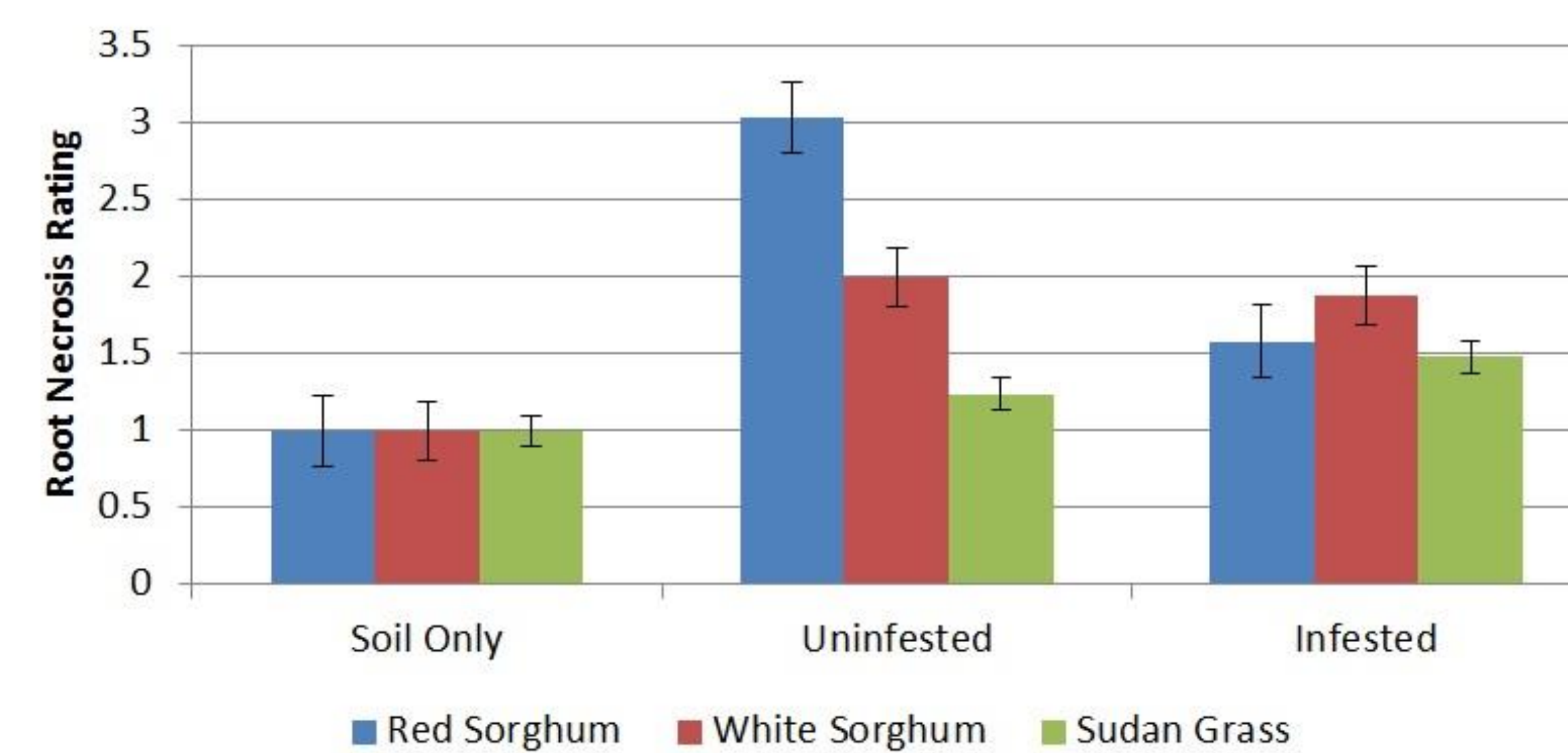
### Soil Inoculation and Planting:

- Inoculum (either infested or uninfested) was added to soil in a 1:20 ratio
- Seeds were planted in containers and grown for 14 days
- Growth chamber temperature was set at 23C, 12 hour daylight cycle

### Data Collected:

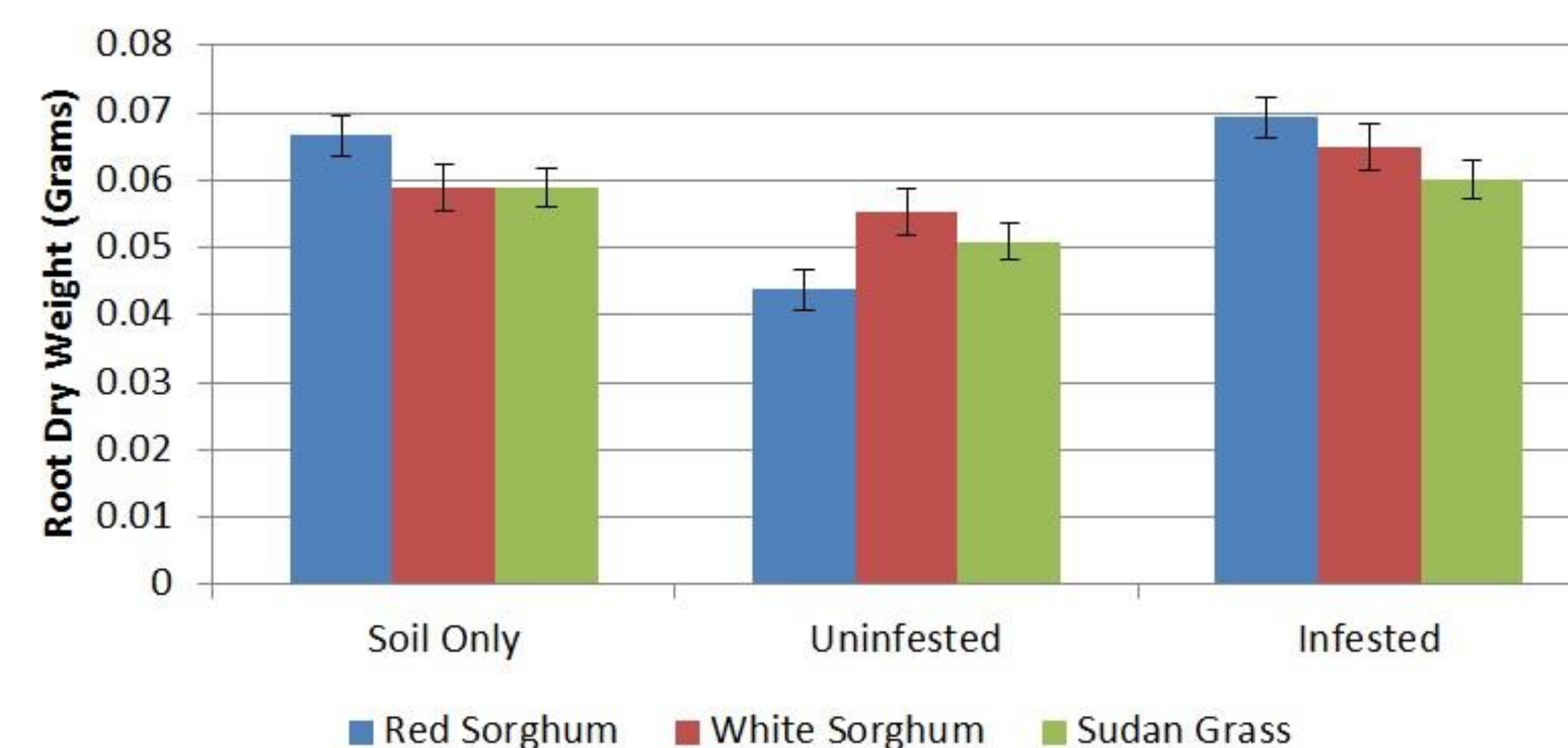
- Foliar and Root necrosis scores
- Shoot and Root dry weights
- Stand Count

## Substrate Root Necrosis Comparison



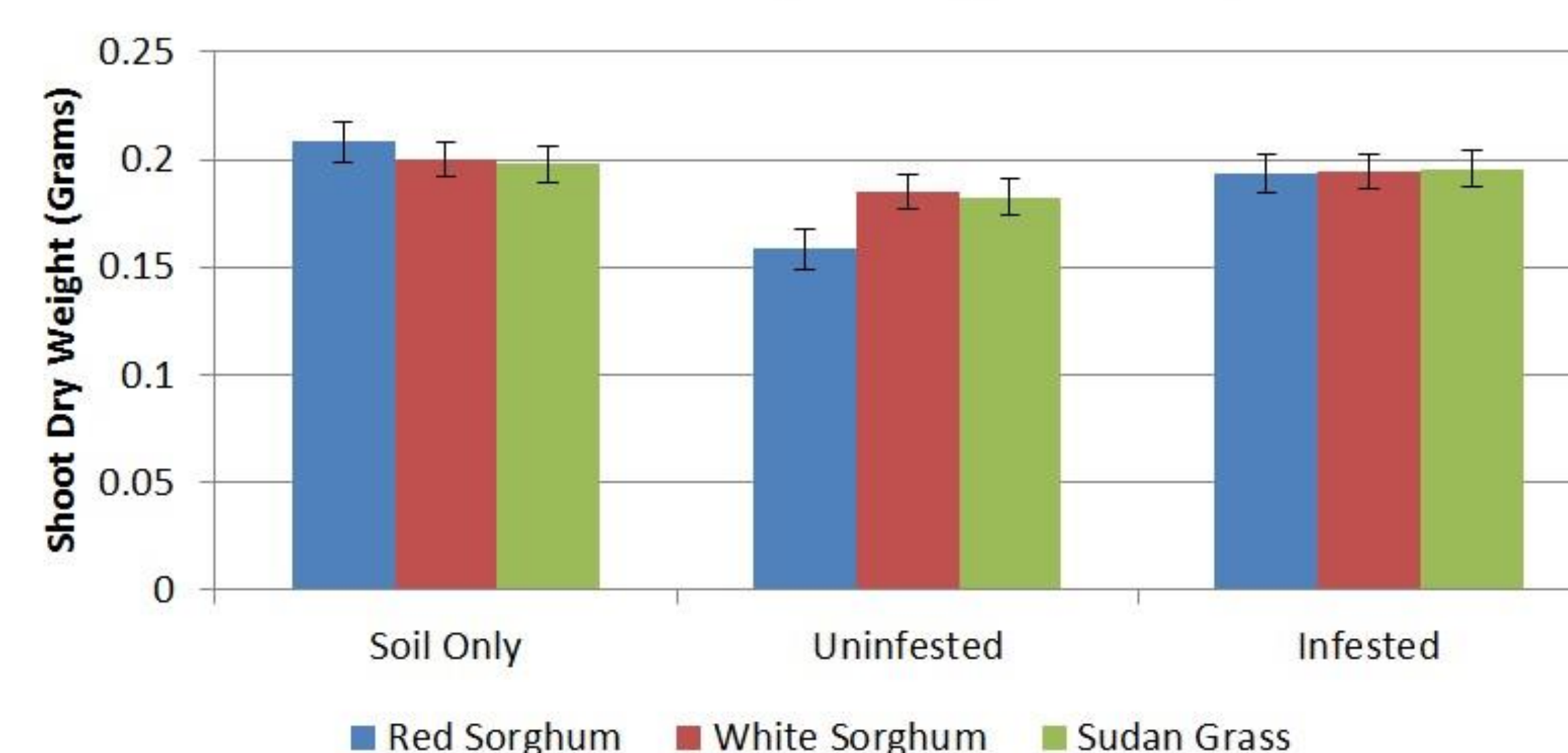
**Chart 1** – Shows the variance in root necrosis between the different substrates and treatments. As the graph shows roots for the uninfested treatment, on average, had more necrotic symptoms than the infested treatment.

## Substrate Root Dry Weight Comparison



**Chart 2** – Shows the change in root biomass between the three substrates and treatments. As the graph shows, root biomass is severely reduced when comparing the uninfested and soil only treatments. The infested treatment yielded roots with more mass than the soil only treatment.

## Substrate Shoot Dry Weight Comparison



**Chart 3** – Shows the variance in shoot biomass between the different substrates and treatments. Uninfested and infested treatments had less mass than the soil only treatment; uninfested had the smallest average masses.

## RESULTS

- **Red Sorghum**
  - The uninfested treatment decreased shoot and root biomass 24 and 34% respectively.
  - Infested treatment decreased shoot biomass 7% yet increased root biomass 4%.
- **White Sorghum**
  - The uninfested treatment decreased shoot and root biomass 7 and 6% respectively.
  - Infested treatment decreased shoot biomass 3% yet increased root biomass 10%.
- **Sudan Grass**
  - The uninfested treatment decreased shoot and root biomass by 8 and 13% respectively.
  - Infested treatment decreased shoot biomass 1% and increased root biomass 2%.

## CONCLUSIONS

- All substrates impacted root growth and symptom development.
- Red sorghum exhibited the most severe symptoms in both infested treatment and uninfested treatments which makes it difficult to determine if the infested treatment symptoms are from *F. solani* or the substrate.
- Sudan grass had the smallest impact on soybean growth and also the smallest impact on root necrosis indicating that of the substrates sudan grass will not influence the data significantly yet also might not be an optimal carrier of the fungus from the lack of symptoms in the inoculated treatment.
- Due to the unpredictable extent to which fungal carriers effect soybean growth it is recommended that a new inoculation technique be developed eliminating the need of a carrier.

## FUTURE RESEARCH QUESTIONS

- (1) Will using different soybean varieties replicate the results observed with McCall?
- (2) If different cultivars of the same substrates were used, would the same results be observed?
- (3) To what extent do substrates contribute to root and shoot necrosis symptoms and development?

## ACKNOWLEDGEMENTS

A special thank you to: Dr. James E. Kurle, Grace Anderson, Kurle Lab: John Lencowski, Erin Walch, Colin Zumwalde, Marissa Scherven, and Dante Leyva.