

In Vitro Phenotypes of *Pythium* Isolates are Correlated with Disease Symptoms in the Greenhouse

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Introduction

Oomycetes are eukaryotic microbes that are pathogenic to plants and animals. *Pythiaceae* is a family of oomycetes that can infect a variety of hosts causing *Pythium* Root Rot. Phenotypic differences between isolates of the same species may indicate aggressiveness. These isolates were used in *in vitro* plate experiments, growth rates, and in oogonia counts. *In planta* these isolates were used to infect maize from line B73.

Hypotheses:

- There is phenotypic diversity across different isolates of oomycetes within the same species when grown on different media.
- Some phenotypes are correlated with increased disease severity or aggressiveness in maize.

Material and Methods

9 isolates were available: 2 isolates from *Pythium irregulare*, 2 isolates from *P. aphanidermatum*, 3 isolates from *P. ultimum* var. *ultimum*, and 2 isolates from *Phytophthora sojae* (Non-pathogen on maize).

In vitro assay:

Media: lima bean V8, potato carrot, potato dextrose, cornmeal agar.
Growth rate: Isolates grown on PDA or LBDV8 for *P. sojae*, measured daily with callipers, slope & total growth analyzed
Oogonia count: Potato carrot agar plates, measured with microscope and hand counter.

In planta assay:

- 9 isolates + 1 control: 10 flasks of inoculated cornmeal and sand mixture
- Conetainers, pots, coffee filters, aluminium foil, B73 seeds
- Grown 11 days on BM2 soil, measured root lengths, masses, disease severity



Figure 1: One replication of *in vitro* plate experiment

Analysis:

ANOVA in R Studio measured phenotype differences. TukeyHSD determined significance between isolates and species. cor.test assessed phenotype correlations. Data were normal.

Significance codes:

p < 0.001 = '***'; p < 0.01 = '**'; p < 0.05 = '*'; p < 0.1 = '.'

Figure 4 (right): 120 seeds of B73 were inoculated and planted into conetainers in the greenhouse. (A) Infected seedling compared control. (B) Percent root necrosis, controls were less diseased than inoculated. (C) Root lengths as a percent of mock inoculated controls. (D) Root mass as a percent of mock inoculated controls

In vitro oogonia counts

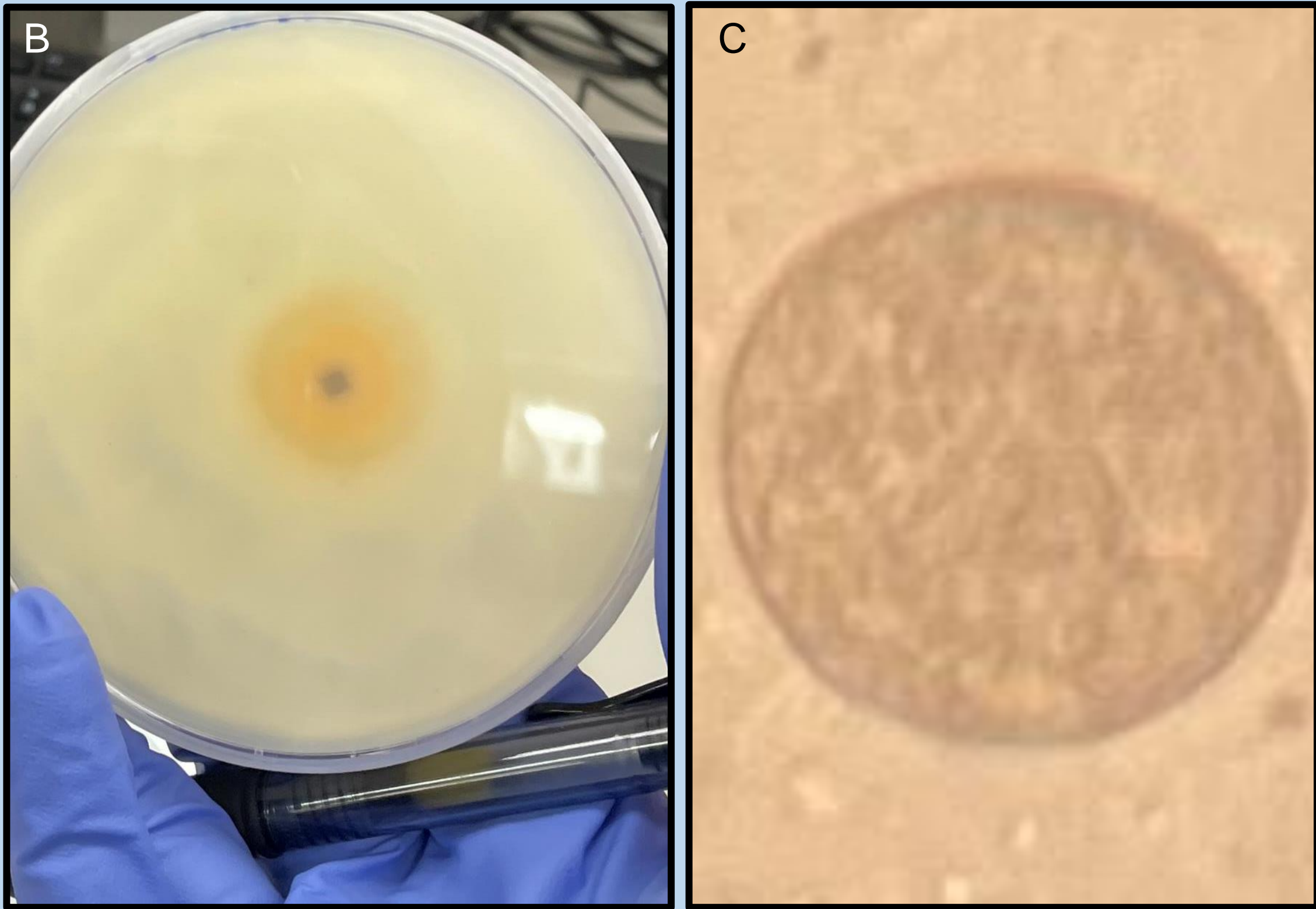
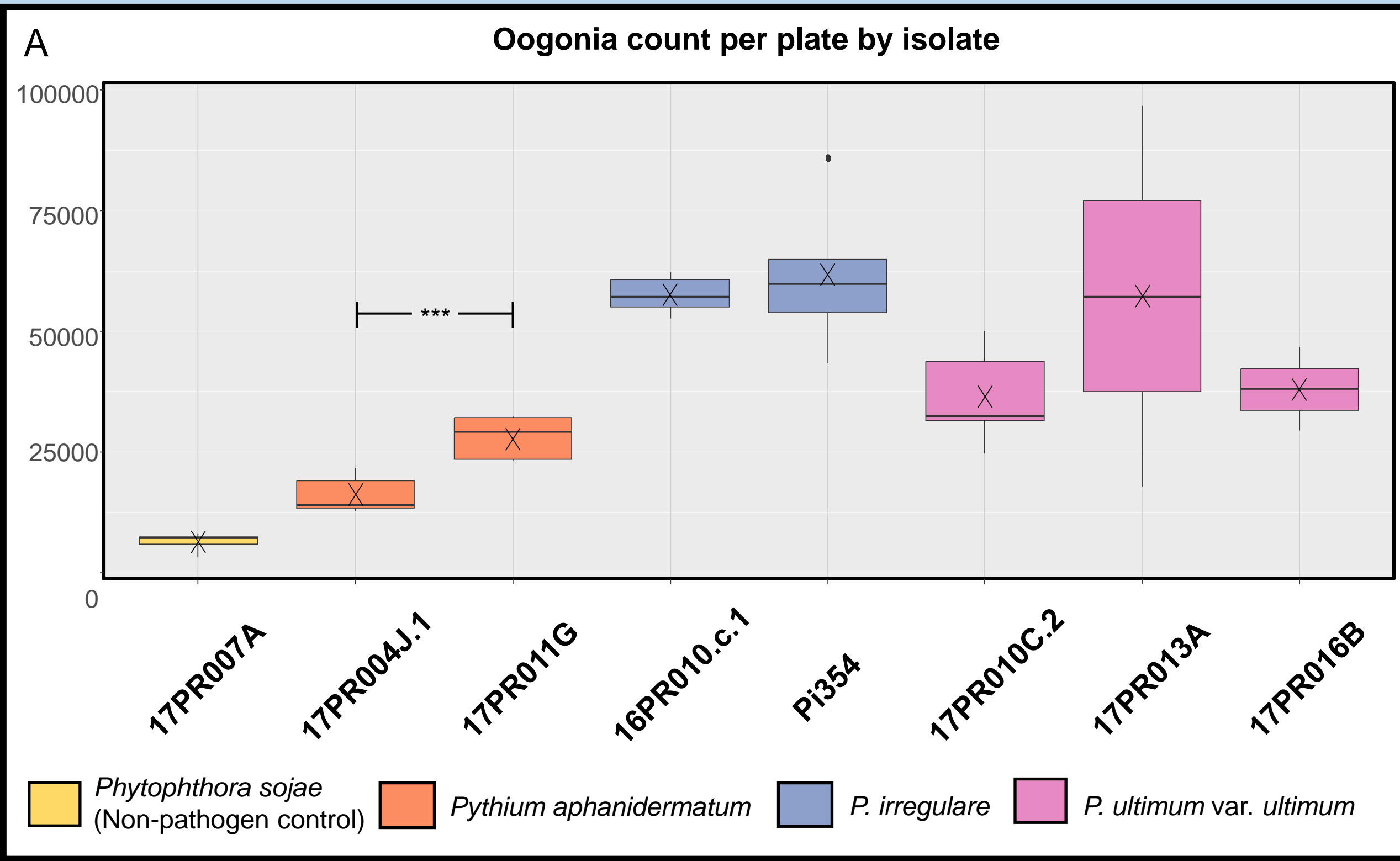


Figure 2: (A) Oogonia counts by isolate separated by species, *P. aphanidermatum* isolates were significantly different. (B) *P. irregulare* darkened circle around older growth. (C) Oogonia under microscope.

In vitro growth rates

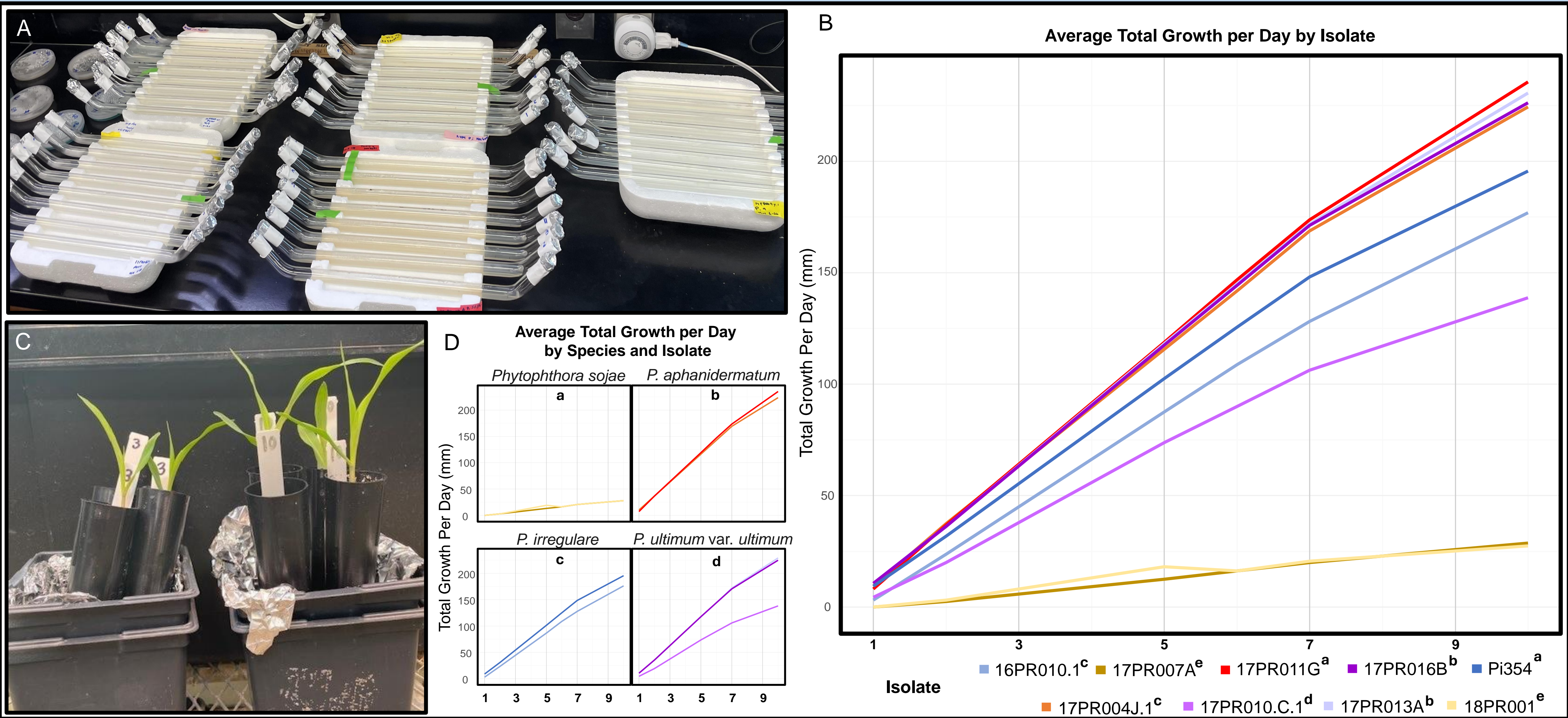
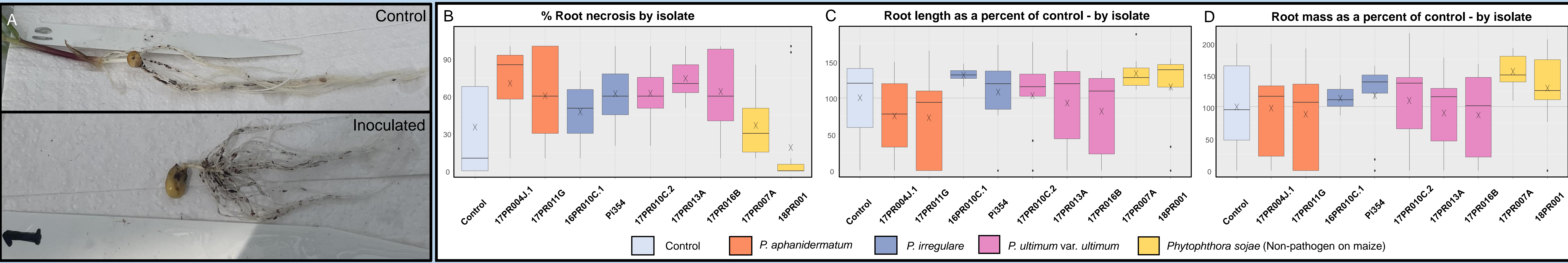


Figure 3: Race tubes were used to measure growth rate (A) Depicts sample size. (B) Growth rate of all isolates after 10 days, letters in legend adjacent to isolate ID indicate significant differences between isolates. (C) Diseased greenhouse plant inoculated with 17PR013A (left) next to mock-inoculated control (right). (D) Total growth of isolates by species, letters below species name indicate significant differences of growth rate between species.

In Planta greenhouse phenotypes



Correlation and P-Values				
Race Tube Growth Rate	$r = -0.26$ $p = 0.027$	$r = -0.15$ $p = 0.207$	$r = -0.13$ $p = 0.277$	$r = -0.56$ $p = 0.000$
Oogonia Per Plate	$r = 0.32$ $p = 0.005$	$r = 0.16$ $p = 0.175$	$r = 0.17$ $p = 0.134$	
% Root Necrosis	$r = 0.71$ $p = 0.000$	$r = 0.77$ $p = 0.000$		
Root Mass	$r = 0.83$ $p = 0.000$			
Root Length				

Figure 5: Significant correlations were found between root phenotypes; root length with *in vitro* phenotypes.

Conclusions

- Significant difference of growth rates between isolates of *P. ultimum* var. *ultimum*; significant difference of oogonia production between isolates of *P. aphanidermatum*
- Oogonia production was significantly correlated with disease in plants: more oogonia associated with less disease
- Faster *in vitro* growth rate was significantly correlated with disease in plants: slower *in vitro* growth associated with more disease

Future Work

- Additional replications using more isolates and species of *Pythium* & *Globoisporangium*
- Continuing Koch's Postulates with greenhouse plants

References

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Acknowledgments

Financial support was provided by the National Science Foundation under grant #NSF REU 1950819/1950786, as part of the Phenotypic Plasticity Research Experience for Community College Students, through the University of Illinois at Urbana-Champaign Institute for Genomic Biology and Parkland College. <http://precs.igb.illinois.edu/>

I would like to thank the Mideros and Jamann labs for helping me along the way. Especially my mentor, Harrison Hall. Also, I would like to thank my family and friends for always supporting me.