Can *In Vitro* Phenotypes of *Fusarium graminearum* Indicate *In Planta* Aggressiveness?

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PRECS Phenotypic Plasticity Research Experience for Community College Students

Introduction

What is Fusarium graminearum and what does it do?

Fusarium graminearum (F. graminearum), causes Fusarium Head Blight (FHB) in wheat and barley. FHB results in crop destruction and toxins that are harmful for human and animal consumption [1].

There are several populations of *F. graminearum* present in North America and rates of in planta aggressiveness and toxin levels vary by Population [2][3].

Visually, it is difficult to determine the aggression or toxin levels of an infection [3]. However, it may be possible to determine this through in vitro growth.

[Fig 1] Example of healthy wheat head, left, next to wheat inoculated with F. graminearum, right. Photo by Keith Weller, public domain.

By using *in vitro* growth, it may be possible to determine *in planta* aggressiveness of *F. graminearum* populations.

Fusarium graminearum Isolates

163 isolates, collected from across the United States, were plated on full strength potato dextrose agar plates. From those, 25 of the healthiest looking *F. graminearum* plates, plus PH1-FT, the positive control, were chosen to measure growth rates and coloration.

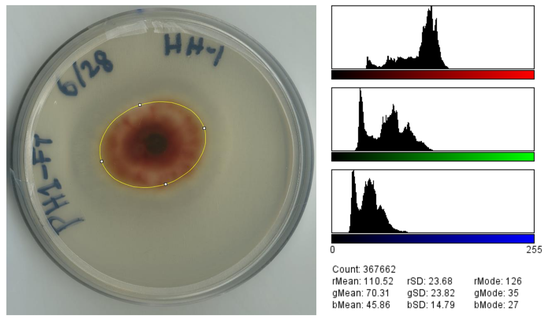
From these 25 healthiest looking isolates, 12 were chosen to use in a greenhouse assay. The isolates were grown on twice sterilized sorghum to acquire a granulated inoculum can be applied both within and on top of the soil. The greenhouse assay will study the *in planta* aggressiveness of these isolates.

[Fig 2] Isolates and inoculum.

Methods

Coloration

Bottom views of isolates were scanned at 600 dpi at 9 days after plating. The area of growth was then selected in imageJ and analyzed with a color histogram. This was repeated with 3 sets.

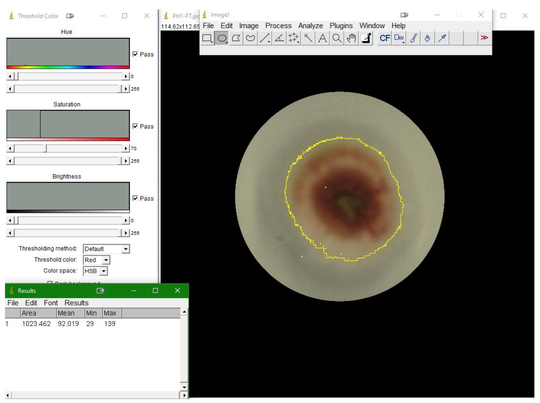


[Fig 3] imageJ histograms show the color range of the highlighted area of isolate PH1-FT, the positive control.

Growth Rate

Using the same isolates for the coloration experiment, radial growth was measured by using the following formula:

Radial growth in mm/day = $(R_2-R_1)/(T_1-T_2)$



[Fig 4] Measuring the area in imageJ, using PH1-FT, the positive control as an example.

Greenhouse Assay

An inoculum was made with 13 isolates of *F. graminearum* and allowed to grow for 2 weeks on twice sterilized sorghum.

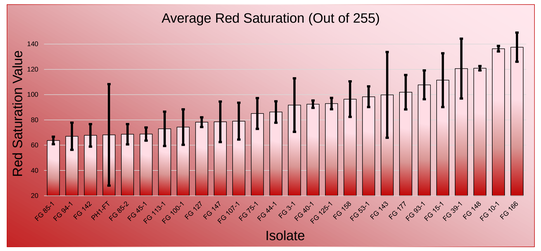
Inoculum will be added to plastic containers with wheat seeds. Some containers will have the inoculum added below the wheat seed, while others will have the inoculum added above the wheat seed.

Wheat will be allowed to grow until noticeable damage by the fungus can be recorded.

Results

Coloration

Results show wide color variation within some isolates, while in others the coloration was reliable.

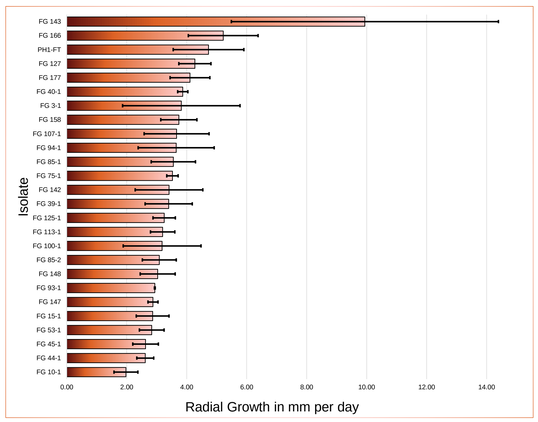


[Fig 5] The highest concentration of color is in the red pigments, with lower saturation values being associated with the darker colored isolates, and higher saturation values being associated with paler colors.

Growth Rate

Growth rates varied widely, with most settling around 3mm of radial growth per day.

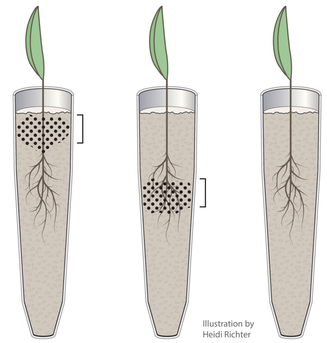
Some isolates had little deviation, while others had extreme deviation.



[Fig 6] Radial growth rate of 26 F. graminearum isolates, from fastest to slowest.

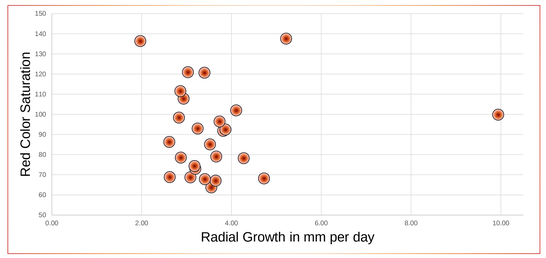
Greenhouse Assay

The greenhouse assay is in progress.



[Fig 7] Diagram of greenhouse assay. By Heidi Richter Used with permission.

Conclusions



[Fig 8] Radial growth versus color saturation, for 26 F. graminearum isolates. No obvious trend. The far outlier on the right side is FG 143, an isolate with a very fast growth rate.

Results suggest that the radial growth rate and average coloration are not obviously correlated. The greenhouse assay is still needed to test *in planta* aggressiveness.

Future Work

There are several ways we could expand this research in the future:

- The greenhouse assay has started
- Testing for toxin levels in wheat
- Top view photos for coloration analysis
- Spore counts

References

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