Spring 4-4-2020

Fungicide Sensitivity of Sclerotinia sclerotiorum Isolates Selected from Five Different States That Use Different Fungicide Treatments

Cristian Wulkop Gil
University of Nebraska - Lincoln, cristianwulkop@huskers.unl.edu

Fungicide Sensitivity of *Sclerotinia sclerotiorum* Isolates Selected from Five Different States That Use Different Fungicide Treatments

Cristian Wulkop, Sydney Everhart Department of Plant Pathology Contact: cristianwulkop@huskers.unl.edu

UCARE

Summary

Introduction
- *Sclerotinia sclerotiorum* is a plant pathogen responsible for $252M in losses every year. Resistance to the most effective fungicides has emerged and spread in pathogen populations.

Methods
- *S. sclerotiorum* isolates are inoculated in fungicide amended plates. Growth is measured after 30 hours. $EC_{50(D)}$ is calculated.

Results
- No differences in EC50(D) found between groups screened against Bscalid.
- Baseline and Washington isolates have a significantly higher $EC_{50(D)}$ than Nebraska isolates screened against tetraconazole.
- Washington, Michigan, and Baseline isolates have a significantly higher $EC_{50(D)}$ than Nebraska isolates screened against picoxystrobin.

Conclusions
- Differences in $EC_{50(D)}$ in different states hints at *S. sclerotiorum* developing resistance to commonly used fungicides.

Background

- *Sclerotinia sclerotiorum* is a plant pathogenic fungus that can cause a disease called white mold that can infect more than 450 plant species including soybeans, dry beans, green beans, canola, and sunflower
- Estimated to cause $252M in losses every year to disease

U.S. Canola Association 2014

Figure 1. Common symptoms of white mold, the disease caused by *S. sclerotiorum*

- Fungicides are widely used in developed agricultural systems to control disease and safeguard crop yield and quality
- Resistance to the most effective fungicides has emerged and spread in pathogen populations

Lucas 2015

- There have been multiple reports of *S. sclerotiorum* isolates from dry bean, soybean, and canola fields being resistant to certain fungicides.

Lehner et al. 2015

Hypothesis

Since different fields in different states use different fungicide treatments on plants and different numbers of application depending on environmental conditions, isolates with the lowest fungicide sensitivity will be those that come from fields with more intensive fungicide applications.

Goal: Determine the fungicide sensitivity of *S. sclerotiorum* isolates from five states to assess risk of resistance

Methods

Selection of Isolates

- This study examines 95 isolates from dry bean fields from five states of the United States: North Dakota (32), Colorado (28), Nebraska (11), Washington (20), Michigan (4).

Figure 2. Map showing the geographic location of the five states the isolates of this study were collected from. It also show the number of isolates from each state.

- Isolates were selected in an attempt to represent as many fields from the five states selected from the selection of isolates in the Evertart lab, while still having a significant amount of isolates present from each field.

Reactivating Sclerotia

- Sclerotia are hard dark resting bodies of fungi that can remain dormant for long periods
- Sclerotia are treated with bleach and inoculated in water agar plates to induce mycelial growth

Figure 3. Picture of *S. sclerotiorum* sclerotias.

Inoculation of PDA Plates

- Plugs of the mycelial growth are transferred into control and fungicide amended PDA plates
- Boscalid, Tetraconazole, Picoxystrobin fungicides were used in this study at the discriminatory concentration of 0.2 ppm, 2 ppm, and 0.01 ppm, respectively.
- Radial growth in two perpendicular directions was measured after 30 hours

Figure 4. *S. sclerotiorum* isolate's growth after 30 hours under control treatment

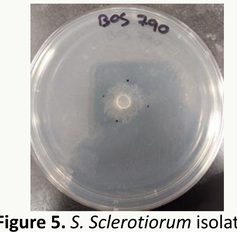

Figure 5. *S. Sclerotiorum* isolate's growth after 30 hours under Boscalid fungicide treatment

$EC_{50(D)}$ Determination

- The EC_{50} is the concentration of a fungicide that gives the half maximal response on a fungal pathogen.
- For this study, we will be calculating the $EC_{50(D)}$ by measuring percent growth on plates amended with a discriminatory concentration previously determined by members of the Everhart Lab.

Results

Boscalid Fungicide: Succinate Dehydrogenase Inhibitor

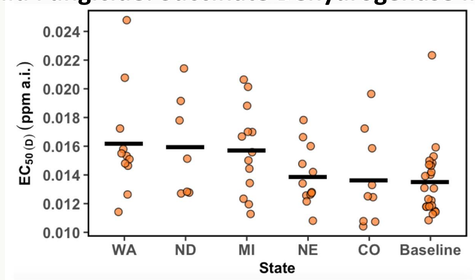

Figure 6. Estimated $EC_{50(D)}$ from isolates against Boscalid fungicide

- Very good control; no differences found between groups

Tetraconazole Fungicide: Demethylation Inhibitor

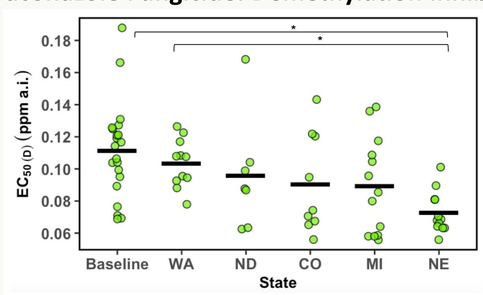

Figure 7. Estimated $EC_{50(D)}$ from isolates against Tetraconazole fungicide

- Fair control; Baseline and Washington isolates have a significantly higher $EC_{50(D)}$ than Nebraska isolates

Picoxystrobin Fungicide: Quinone Outside Inhibitor

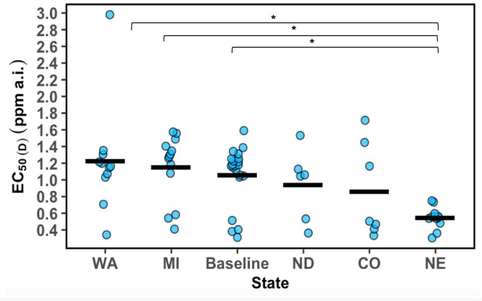

Figure 8. Estimated $EC_{50(D)}$ from isolates against Picoxystrobin fungicide

- Very good control; Washington, Michigan, and Baseline isolates have a significantly higher $EC_{50(D)}$ than Nebraska isolates.

- These are preliminary results from the first experimental replica of the project. The second experimental replica has now been completed along with the screening of a fourth fungicide, thiophanate methyl. However, due to time constraints, the statistical analysis of this data has yet to be performed.

Conclusions & Future Directions

- Differences in $EC_{50(D)}$ in different states hints at *S. sclerotiorum* developing resistance to commonly used fungicides.
- The data shows a significant difference between the $EC_{50(D)}$ of Nebraskan isolates compared to baseline isolates and Washington, Michigan, and baseline isolates against tetraconazole and picoxystrobin, respectively.
- Further studies or surveys must be done to assess the differences between the treatments of dry bean fields in different states. This would allow investigation into whether differences in fungicide sensitivity are caused by natural selection driven by the application of fungicides.
- Once this is done, some recommendations on fungicide applications for farmers can be developed in order to stop the spread of fungicide resistance.
- In the future, we will be genotyping the least sensitive (like resistant) isolates to check for single nucleotide polymorphisms (SNPs) that are related to the lack of sensitivity.

Acknowledgements

- Sydney Everhart
- Rebecca Higgins
- Edgar Nieto-Lopez
- UCARE
- Institute of Agriculture and Natural Resources

References

1. U.S. Canola Association. National Sclerotinia Initiative. Retrieved from http://www.uscanola.com
2. Lucas JA, Hawkins NJ, Fraaije BA (2015). Chapter Two – The Evolution of Fungicide Resistance. Advances in Applied Microbiology 90: 29-92
3. Lehner MS, Paula Júnior TJ, Silva RA, Vieira RF, Carneiro JES, Schnabel G, Mizubuti ESG (2015) Fungicide sensitivity of *Sclerotinia sclerotiorum*: a thorough assessment using discriminatory dose, EC50, high-resolution melting analysis, and description of new point mutation associated with thiophanate-methyl resistance. Plant Dis 99:1537–1543