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Fungicide Sensitivity of *Sclerotinia sclerotiorum* Isolates from Five States with Different Fungicide Treatments

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Summary

Introduction

Sclerotinia sclerotiorum is a fungal plant pathogen responsible for $252M in yield losses every year. Resistance to the most effective fungicides can emerge and spread in pathogen populations; however, there are few studies on this matter.

Methods

S. sclerotiorum isolates are inoculated onto PDA plates amended with a discriminatory concentration of Boscalid, tetraconazole, picoxystrobin, and thiophanate methyl fungicides. Mycelial growth is measured after 30 hours and EC50(D) is calculated.

Results

- No differences in $EC_{50(D)}$ found between groups screened against Boscalid
- 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
- No growth was observed on plates amended with thiophanate methyl

Conclusions

Differences in $EC_{50(D)}$ in different states hints at *S. sclerotiorum* developing resistance to commonly used fungicides. However, the hypothesis is only partially supported by the data since the baseline isolates $EC_{50(D)}$ is not the lowest for all fungicides.

Background

Sclerotinia sclerotiorum is the causal agent of a disease called white mold that can infect more than 450 plant species including soybeans, dry beans, green beans, canola, and sunflower. It is estimated to cause $252M in losses every year to disease (U.S. Canola Association, 2014).

No dry bean varieties are completely resistant to *S. sclerotiorum,* making fungicides critical in disease management

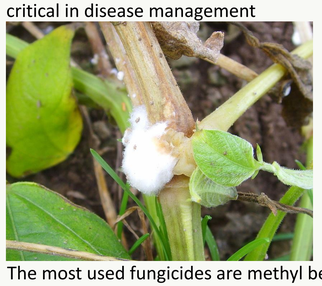

Figure 1. Common symptoms of white mold, the disease caused by *S. sclerotiorum*

The most used fungicides are methyl benzimidazole carbamates (MBC), demethylation inhibitors (DMI), succinate dehydrogenase inhibitors (SDHI), and quinone outside Inhibitors (QoI)

Fungicide field resistance to an MBC in Brazil for dry bean (Lehner et al. 2015), and to a dicarboximide in China for oilseed (Ma et al. 2009) have been reported Limited number of studies have been done in North Central U.S. (Mueller et al. 2002)

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 dry bean fields of five states to thiophanate methyl, tetraconazole, Boscalid, and picoxystrobin 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).

Isolates were selected in an attempt to represent as many fields from the five states selected from the selection of isolates in the Everhart lab, while still having ≥ 5 isolates per field.

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

Reactivating Sclerotia

Sclerotia are hyphal aggregate containing melanin considered as the primary fungal long-term survival structures.

- Sclerotia are treated with a 50% bleach solution and inoculated in water agar plates to induce mycelial growth

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

Inoculation of PDA Plates

- Plugs of the mycelial growth are transferred into control and fungicide amended PDA plates
- Thiophanate methyl, tetraconazole, Boscalid, and picoxystrobin fungicides were used in this study at the discriminatory concentration of 10 ppm, 2 ppm, 0.2 ppm, and 0.01 ppm respectively
- Radial growth in two perpendicular directions was measured after 30 hours.
- In the case of thiophanate methyl, a qualitative comparison between sensitive or resistant was done instead

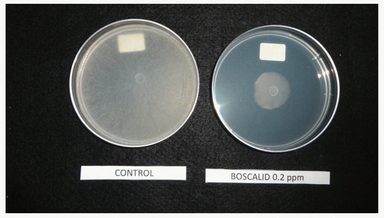

Figure 4. *S. sclerotiorum* growth after 30 hours under control and Boscalid treatment

$EC_{50(D)}$ Determination

The EC_{50} is the concentration of which a fungal pathogen grow at 50% from a dose–response curve. $EC_{50(D)}$, a predictor of the EC_{50}, will be calculated by measuring percent growth on plates amended with a given discriminatory concentration previously determined.

Results

Thiophanate Methyl Fungicide: MBC

- No growth was observed in all isolates treated against thiophanate methyl
- All isolates are categorized as sensitive to thiophanate methyl
- These are preliminary results since 30 isolates were not screened against thiophanate methyl due to time constraints

Tetraconazole Fungicide: DMI

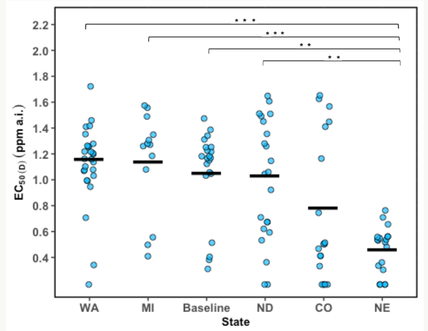

Figure 5. Estimated $EC_{50(D)}$ from isolates against Tetraconazole fungicide

- Washington, Michigan, Baseline (group that has supposedly never been exposed to fungicides), and North Dakota isolates have statistically higher $EC_{50(D)}$ than Nebraska isolates when screened against tetraconazole, meaning that the Nebraska isolates are less resistant to tetraconazole than the isolates from the other 4 states mentioned.

Boscalid Fungicide: SDHI

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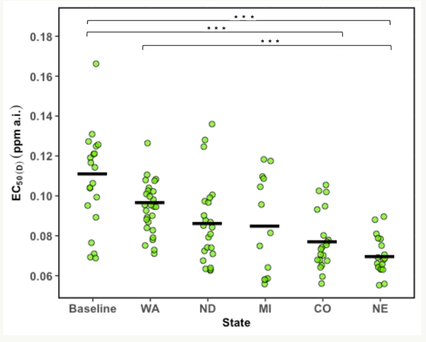

Figure 6. Estimated $EC_{50(D)}$ from isolates against Boscalid fungicide

- Baseline isolates have statistically higher $EC_{50(D)}$ than Nebraska, and Colorado isolates when screened against boscalid
- Washington isolates have statistically higher $EC_{50(D)}$ than Nebraska isolates when screened against boscalid

Picoxystrobin Fungicide: QoI

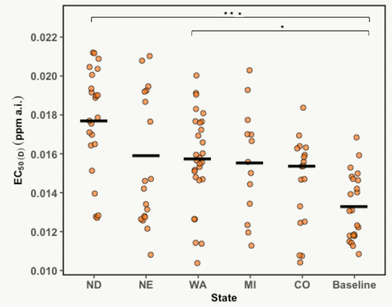

Figure 7. Estimated $EC_{50(D)}$ from isolates against Picoxystrobin fungicide

- North Dakota and Washington isolates have statistically higher $EC_{50(D)}$ than baseline isolates against picoxystrobin

Conclusions & Future Directions

- Differences in $EC_{50(D)}$ in different states hints at *S. sclerotiorum* developing resistance to commonly used fungicides.
- According to the hypothesis, the $EC_{50(D)}$ from baseline isolates should be the lowest for all the fungicides since baseline isolates have supposedly not been exposed to fungicides and therefore have not been able to develop resistance through natural selection. This is what we see happen for picoxystrobin but not in thiophanate methyl, boscalid, or tetraconazole. Therefore, the hypothesis is only partially supported by the data.
- Associations per fields instead of states could be done to analyze variability of resistance to fungicides within particular states. This might yield results that support the original hypothesis.
- In the future, we will be genotyping the least sensitive (likely resistant) isolates to check for single nucleotide polymorphisms (SNPs) that could be related to heritable fungicide resistance.

Acknowledgements

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References

1. U.S. Canola Association. 2014. National Sclerotinia Initiative. Retrieved from: uscanola.com/research/national-sclerotinia-initiative/
2. 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
3. Ma, H.-X., Chen, Y., Wang, J.-X., Yu, W.-Y., Tang, Z.-H., Chen, C.-J., and Zhou, M.-G. 2009. Activity of carbendazim, dimethachlon, iprodione, procymidone and boscalid against Sclerotinia stem rot in Jiangsu province of China. Phytoparasitica 37:421–429
4. D. S. Mueller, A. E. Dorrance, R. C. Derksen, E. Ozkan, J. E. Kurle, C. R. Grau, J. M. Gaska, G. L. Hartman, C. A. Bradley, and W. L. Pedersen. 2002. Efficacy of fungicides on *Sclerotinia sclerotiorum* and their potential for control of Sclerotinia stem rot on soybean. Plant Disease. 86:1, 26-31