Use of RNAi technology to control the phytopathogen Downy Mildew.

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Abstract

- RNA interference (RNAi) technology has the potential to provide species-specific control of plant pathogens, thereby eliminating detrimental effects on the environment caused by broad spectrum fungicides.
- Here, RNAi is shown to reduce germination of the pathogen – *Hyaloperonospora arabidopsidis* (Hpa/ Downy Mildew).

Introduction

- Hpa is a biotrophic oomycete (fungi-like) parasite that needs its host plant to survive[1].
- It causes downy mildew in *Arabidopsis thaliana* (Ath) and other plants[1].
- Ath is not a crop plant[1] but learning how to prevent Hpa infections in Ath will help other economically important plants.
- Conventional fungicides produce off target effects, resistance and environmental accumulation.
- RNAi is an effective and eco-friendly alternative that can target and destroy mRNAs of essential genes to control a pathogen or pest[2].
- RNAi does this by using double strand RNA (dsRNA) that is complementary to the target mRNA[2].
- dsRNA that is applied to plants is bio-degradable[3].
- By selecting a gene sequence that is highly specific to our pathogen we can reduce negative effects on other organisms[2].

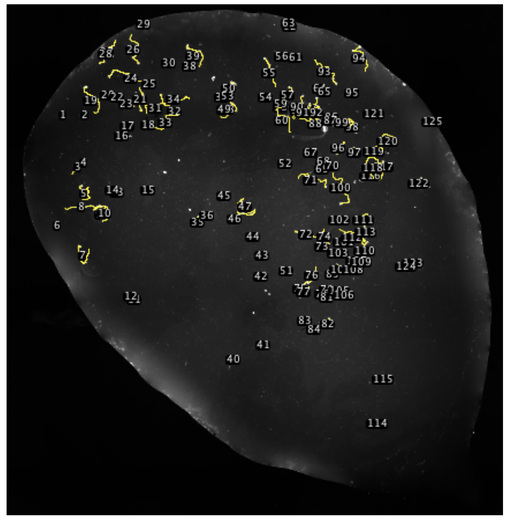
References

1) McDowell, J. M. (2014). Hyaloperonospora arabidopsidis: A Model Pathogen of Arabidopsis. In *Genomics of Plant-Associated Fungi and Oomycetes: Dicot Pathogens* (pp. 209–234). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-44056-8_10
2) Ul Haq, I., & Ijaz, S. (2020). *Plant Disease Management Strategies for Sustainable Agriculture through Traditional and Modern Approaches* (1st ed. 2020.). Springer International Publishing. https://doi.org/10.1007/978-3-030-35955-3
3) Dubelman, S., Fischer, J., Zapata, F., Huizinga, K., Jiang, C., Uffman, J., Levine, S., & Carson, D. (2014). Environmental fate of double-stranded RNA in agricultural soils. *PloS One*, *9*(3), e93155–e93155. https://doi.org/10.1371/journal.pone.0093155

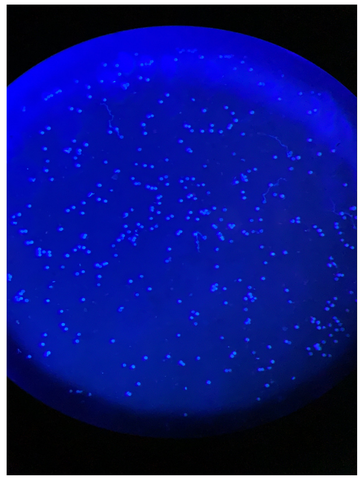
Methods

1. To collect germination-ready Hpa spores, Ath lawns were infected with quiescent Hpa spores and placed in a 17° C growth chamber.
2. 7 days after infection, leaves infected with spores were collected.
3. The spores were separated from soil and leaf debris by centrifugation.
4. Spore concentration was adjusted and applied to cellophane squares on MS media.
5. 2 different doses for each gene target was added (100 ng/µl treatment done by Christopher Manchur).
6. 2 days later, oomycetes were stained and imaged.
7. Germination tube lengths of each spore was calculated, as a measure of growth.

Ath lawns, 7 days after infection with Hpa spores.



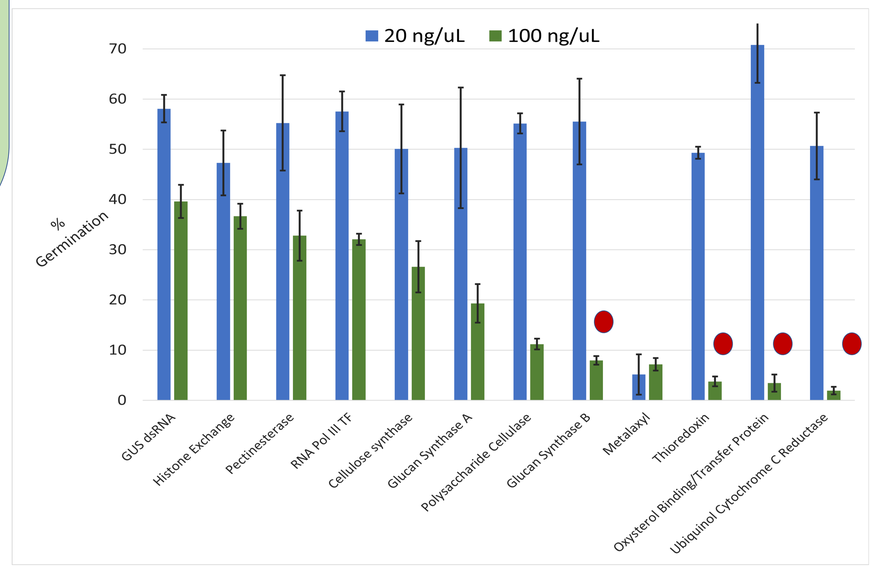
Germination tube calculations using Fiji



Staining with Calcofluor and imaging using ImageXpress

Results

- Two doses of 11 different dsRNAs (X-axis) and the chemical fungicide Metalaxyl (positive control), were tested.
- The percent germination of fungal spores (Y-axis) shows that 8 of the dsRNAs had an impact on Hpa germination with the higher dose of dsRNA.



Discussion

- The Metalaxyl control treatment showed low germination for both doses tested.
- While the low dose (20 ng/µl) of dsRNA had no effect, 8 of the dsRNAs, using the higher dose (100 ng/µl), showed reductions in germination of Hpa. 4 of the dsRNA showed similar levels of germination inhibitions as the Metalaxyl control.
- The choices of target gene sequences can play a key role in the effectiveness of RNAi to control Hpa.
- RNAi has clear potential in Hpa control.

This work was done in the Whyard Lab at the University of Manitoba under the guidance of Dr. Steve Whyard and Christopher Manchur.