

Development of Molecular Diagnostic Assays for Fungicide Resistance in the Dollar Spot Pathogen



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Objectives:

1. Refine RT–PCR assays for detecting DMI resistance in *S. homoeocarpa*, using fungal culturing, as well as detection from dollar spot infected leaf blades.
2. Employ HRM technology for detecting genetic mutations associated with TM–resistance in *S. homoeocarpa*, also from fungal cultures and infected leaf material.
3. Optimize these assays for use as a diagnostic service to golf courses so that they can be provided quickly and inexpensively.

Dollar spot (*Sclerotinia homoeocarpa*) is the most economically demanding of turf disease to treat and is the most geographically widespread, as well. Multiple fungicide treatments are required each year in attempt to control this disease, which aside from economic expense, also has important environmental consequences. The dollar spot pathogen has demonstrated the ability to overcome the DMI fungicides, among those of other classes, across the eastern US. Accurate and quick, yet affordable, diagnostic assay for DMI and TM resistance in dollar spot will not only save golf courses countless dollars, but most importantly, will help to mitigate environmental consequences from overuse and misapplication of fungicides that prove ineffective at sites with resistance.

Currently, detecting fungicide resistance in plant pathogenic fungi relies on culturing and measuring growth on fungicide amended agar petri plates. Our lab provides this service as well for . However, there is at least a 6–10 day turnaround time for such assays, and they are time and labor intensive. Our current molecular diagnostic assay could give accurate results in less than half the time, and requires far fewer resources. In recent years, the field of pharmacogenomics has shown the potential for personalized medicine in treating human disease. Agriculture also has the potential to benefit from such technology, and the development and application of genetic–based diagnostic assays for fungicide resistance could not only be revolutionary for turf diagnostics, but would also be of value to other agricultural systems where fungicide failure is an emerging problem.

Progress

- We have developed assays for detecting resistance to thiophanate–methyl and iprodione using High–Resolution DNA melting analysis (HRM) (Figures 1 and 2).
- An HRM diagnostic test was developed for detecting resistance from infected leaf blades of creeping bentgrass (Figure 3).
- Quantitative Real–Time PCR assays were developed for two genes that will allow for detection of the potential for DMI practical field resistance. One gene has been published on in the following scientific journal article:
Hulvey, J., Popko, J., Sang, H., and G. Jung. 2012. Overexpression of ShCYP51B and ShatrD in *Sclerotinia homoeocarpa* field isolates exhibiting practical field resistance to a demethylation inhibitor fungicide. *Applied and Environmental Microbiology*. 78: 6674 – 6682.
- Another gene was indentified for RT–PCR assays, ShMR4, and has also been developed in a method similar to the gene from Hulvey et al. 2012 (Figure 4).
- Results from the aforementioned research has been presented at the 2013 American Phytopathological Society Meetings in Austin Texas with the following citation:
Hulvey, J., A. Bousquet, H. Sang, and G. Jung. 2013. "Development of molecular diagnostic assays for fungicide resistance in an important turfgrass pathogen, *Sclerotinia homoeocarpa*." *Phytopathology*. 103: 63.



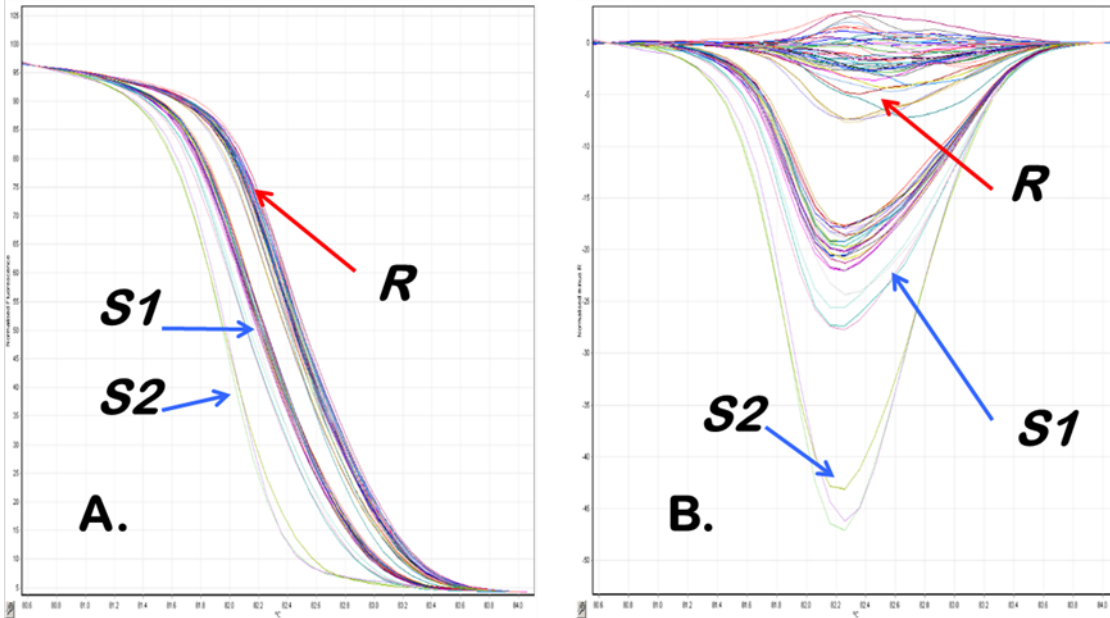


Figure 1. A. Normalized melt curves of TM resistant (R) and sensitive (S1 and S2) genotypes from 24 isolates of *S. homoeocarpa* from five sites in New England in MA and CT. B. Difference curves of data from panel A.

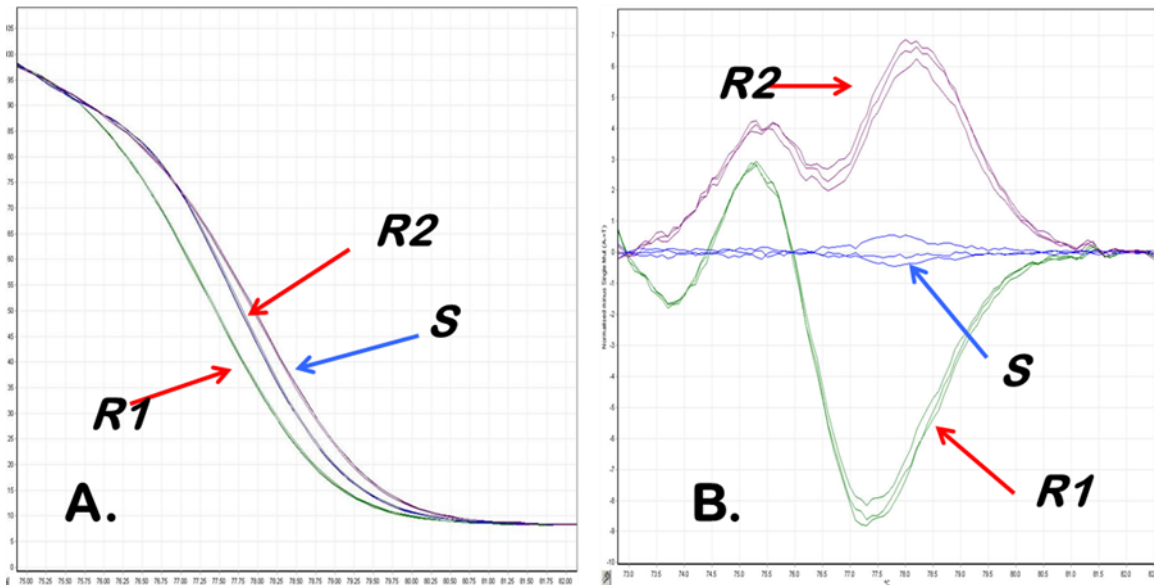


Figure 2. A. Normalized melt curves of dicarboximide resistant (R1 and R2) and sensitive (S) genotypes from isolates of *S. homoeocarpa* from OH, CT, and MA. B. Difference curves of data from panel A.

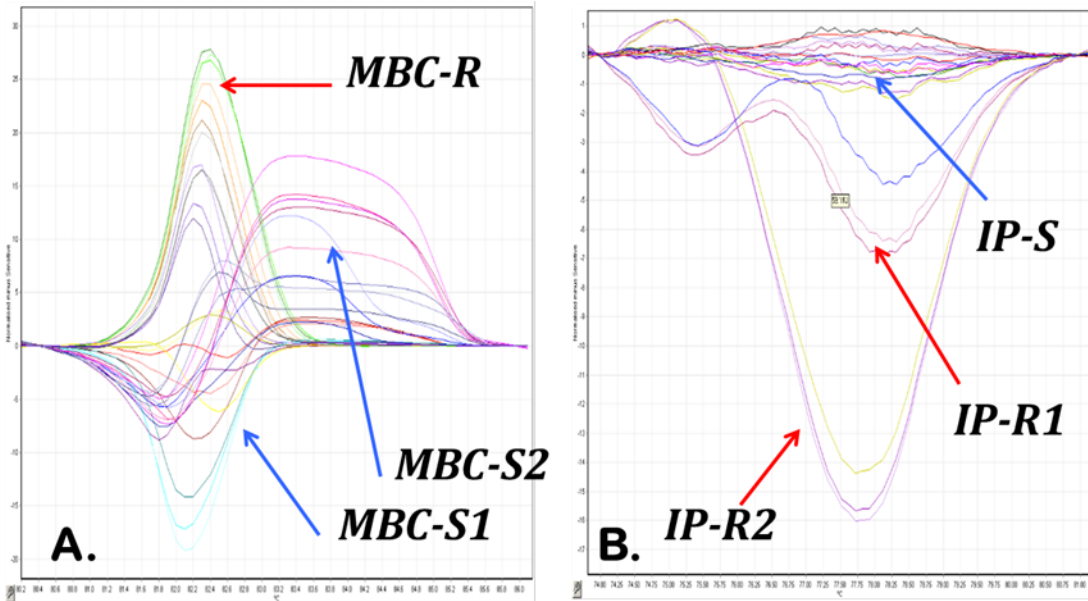


Figure 3. A. Normalized melt curves of *S. homoeocarpa* reference isolates inoculated on creeping bentgrass (TM resistant denoted by MBC-R and sensitive denoted by MBC-S1 and MBC-S2. B. Normalized melt curves of *S. homoeocarpa* reference isolates inoculated on creeping bentgrass (iprodione resistant denoted by IP-R1 and IP-R2) and sensitive denoted by IP-S.

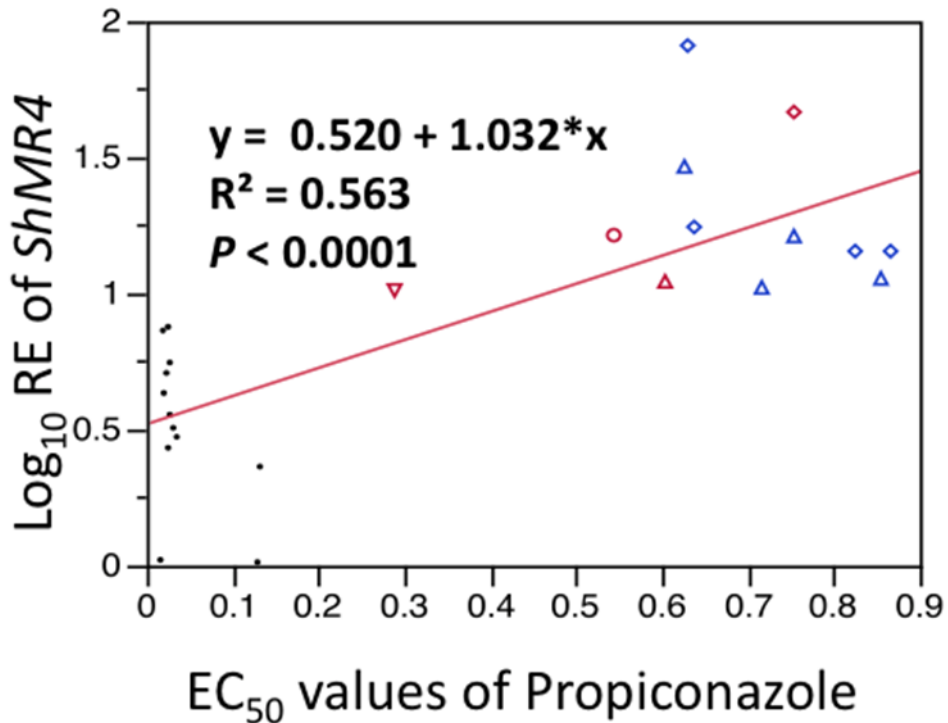


Figure 4. Linear regression of relative expression (R.E.) of a DMI fungicide resistance gene *ShMR4* and transformed propiconazole EC_{50} values. Symbols are data points for propiconazole resistant isolates from two different sites and black dots represent sensitive isolates.