Project Update 12/31/13

Project Title: "Implementing Quick and Inexpensive Molecular Diagnostic Assays for Fungicide Resistance in the Dollar Spot Pathogen, *Sclerotinia homoeocarpa*"

- 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 identified for RT-PCR assays, and has also been developed as a marker for DMI resistance in a method similar to the gene from Hulvey et al. 2012 (Figure 4).
- This research project was introduced to a group of golf course superintendents and members of the turf industry at the UMASS Turf Research Field Day.
- Results from the research have been presented at the 2013 American Phytopathological Society Meetings in Austin Texas and the Northeastern Division Meeting of APS with the following citations:

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.

Sang, H., J. Hulvey, J. T. Popko, and G. Jung. 2012. "A potential multidrug ABC transporter gene from field isolates of Sclerotinia homoeocarpa involved in propiconazole resistance." Phytopathology. 102: 105.

Hulvey, J., H. Sang, and G. Jung. 2013. "High-resolution DNA melting analysis as a tool to screen for genetic polymorphism in fungicide resistance genes for two important turfgrass fungal pathogens, Sclerotinia homoeocarpa and Colletotrichum cereale" (Poster). Northeastern American Phytopathological Society Meeting, Southbury, CT.

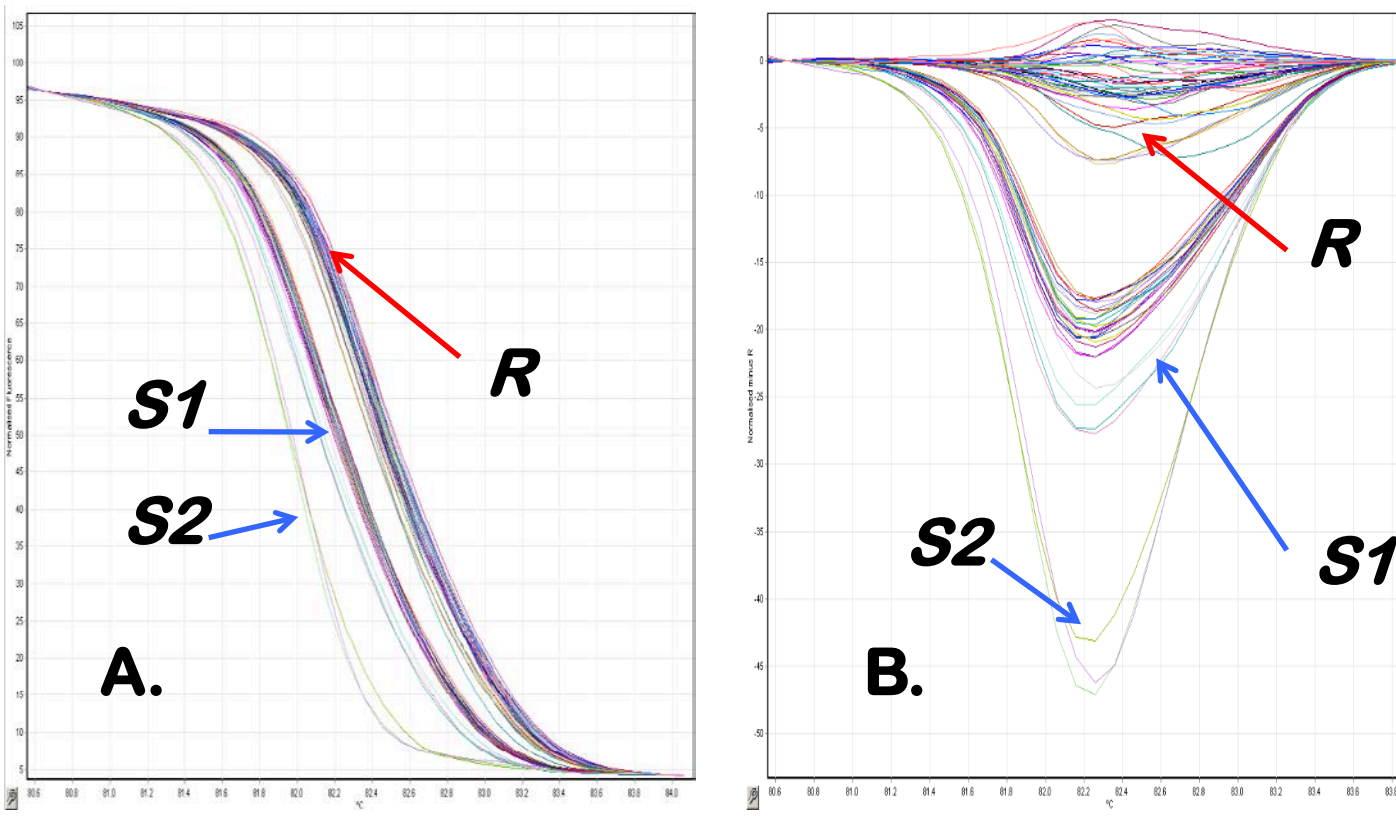

Figure 1. A. Normalized melt curves of TM resistant (R) and sensitive (S1 and S2) genotypes from 24 isolates of *S. homoeocarpa* from five sites in New England in MA and CT. **B.** Difference curves of data from panel A.

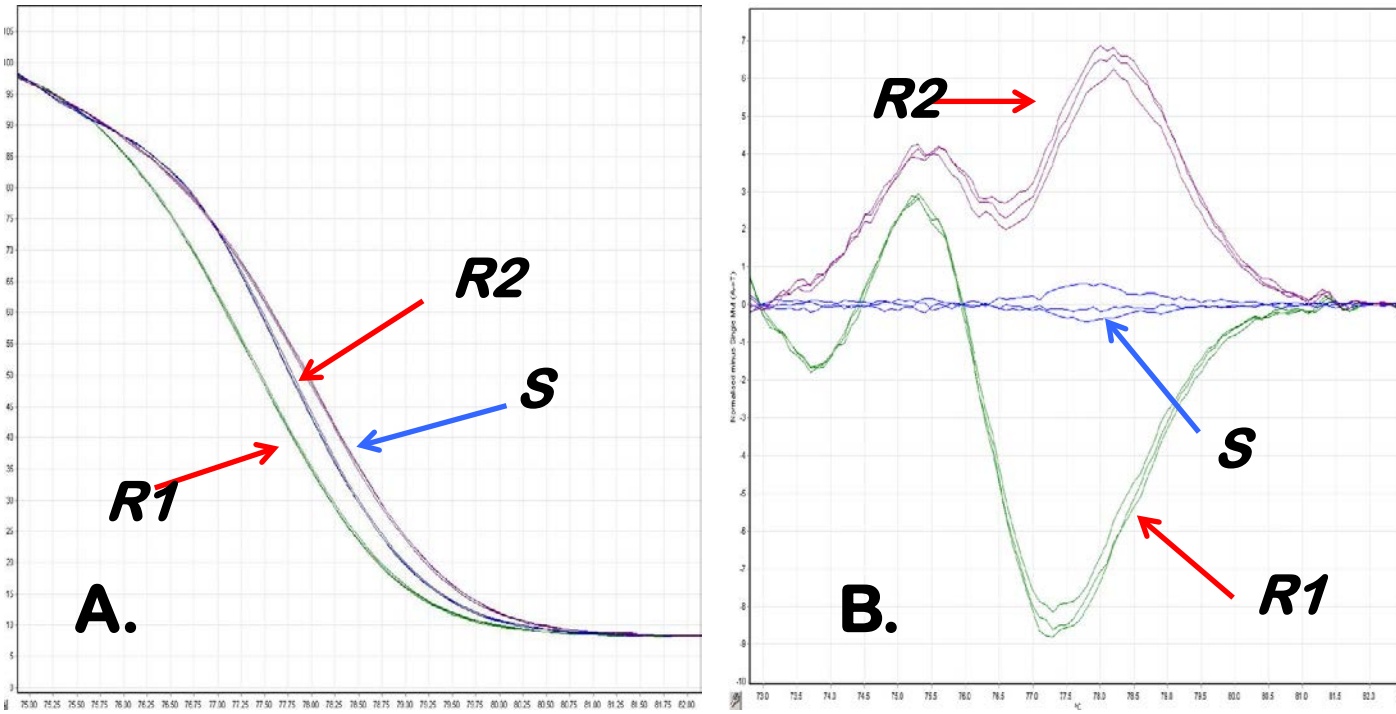

Figure 2. A. Normalized melt curves of dicarboximide resistant (R1 and R2) and sensitive (S) genotypes from isolates of S. homoeocarpa from OH, CT, and MA. **B.** Difference curves of data from panel A.

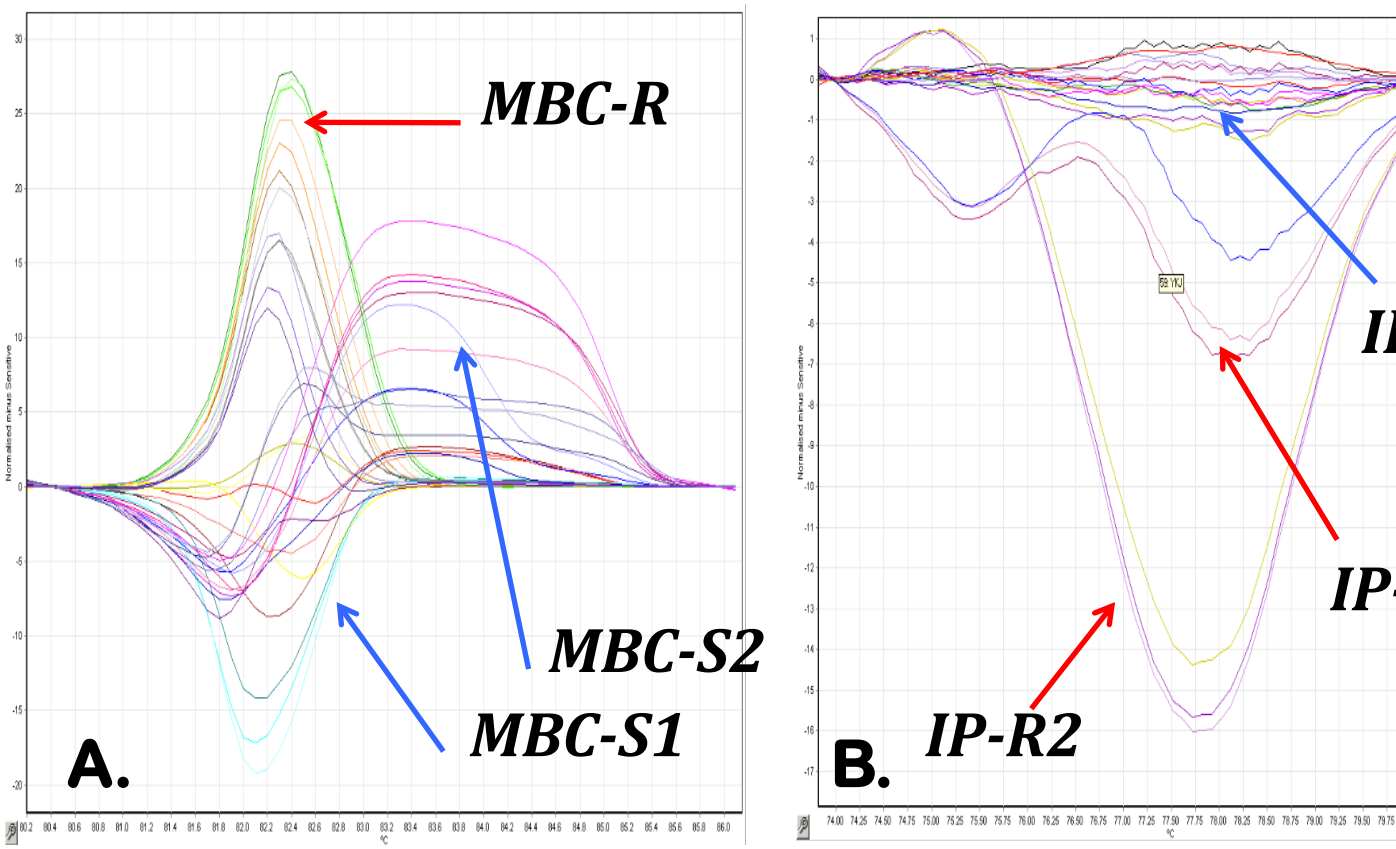

Figure 3. A. Normalized melt curves of *S. homoeocarpa* reference isolates inoculated on creeping bentrgrass (TM resistant denoted by MBC-R and sensitive denoted by MBC-S1 and MBC-S2. **B.** Normalized melt curves of *S. homoeocarpa* reference isolates inoculated on creeping bentrgrass (iprodione resistant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

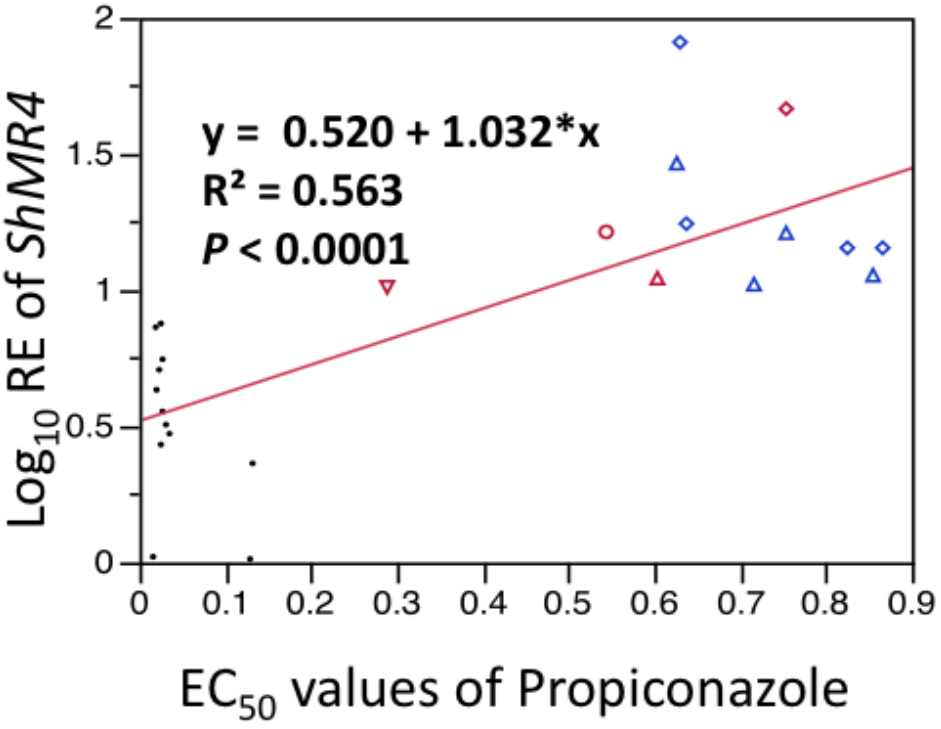

Figure 4. Linear regression of relative expression (R.E.) of a DMI fungicide resistance gene ShMR4 and transformed propiconazole EC_{50} values. Symbols are data points for propiconazole resistant isolates from two different sites and black dots represent sensitive isolates.

Goals for 2014

Our general objectives for this project in the coming year are the following:

1) **Determine whether the rapid diagnostic assays we have developed are suitable for detection of resistance on-site at golf courses.** Our goal with these rapid diagnostic tests is to be able to give results within two to three hours after sample collection. At the very least we believe that the assays will work within 3 days of sample collection if the on-site method proves ineffective.

2) **Provide conventional resistance screening for the participating golf courses in New England in parallel with testing our molecular diagnostic assays.** The traditional screening assays (petri plate growth assay) are a routine service that our lab provides to golf course superintendents and will provide reliable results within two weeks of sample collection. By providing these assays in parallel we can be sure to have reliable comparisons for our molecular assay results.

3) **Present this research at conferences, in the form of research publications, and in meetings with golf course superintendents to share relevant findings.**

As we did in 2013, we plan to present the results of our work at scientific conferences and if possible, extension events, such as the UMASS Turf Research Field Day in 2014.