An Evaluation of Host Adaptation and Detection Methods for *Sclerotinia homoeocarpa*, the pathogen causing Dollar Spot of Turfgrass

Brian A. Aynardi, M.M. Jiménez-Gasco, and W. Uddin
The Pennsylvania State University
Department of Plant Pathology and Environmental Microbiology
University Park, PA 16802

Overall Goals

The purpose of this research is to:

1. Develop a molecular detection method for *Sclerotinia homoeocarpa* using conventional and quantitative PCR
2. Evaluate the genetic diversity of isolates of *S. homoeocarpa* in North America
3. Evaluate the ability of isolates from C3 and C4 turfgrasses to cause disease on the alternative host type

Introduction

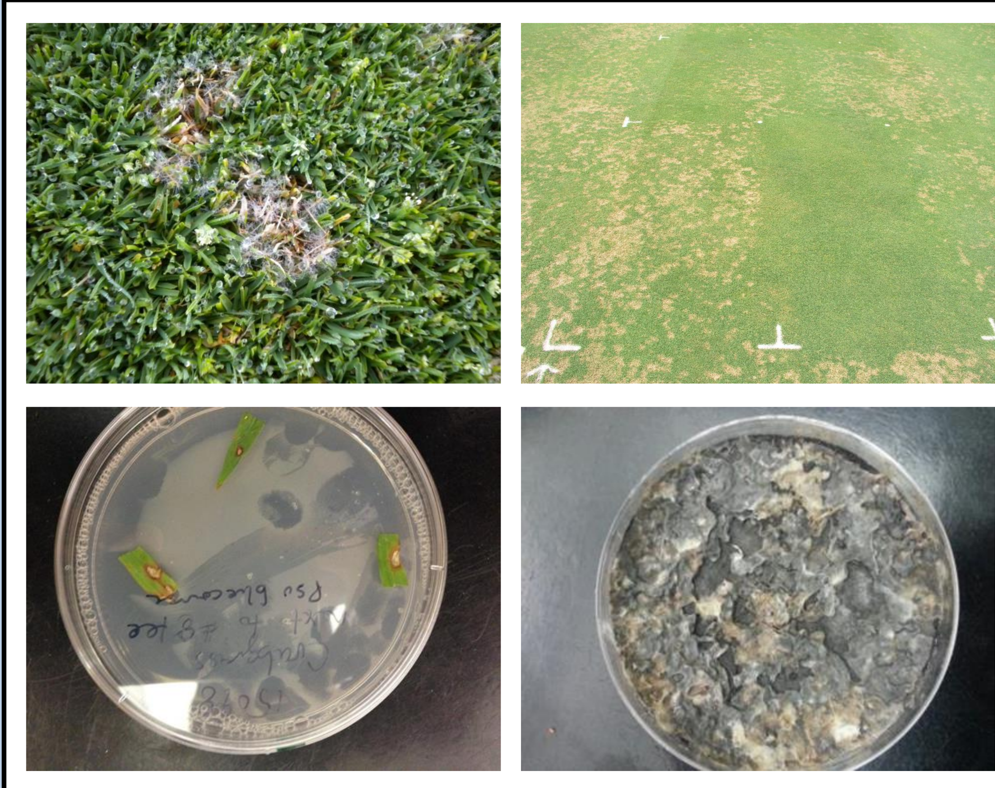
Dollar spot disease of turfgrass is caused by the ascomycete *Sclerotinia homoeocarpa*, F.T. Bennett. The control of dollar spot costs more money than any other disease in high amenity turf (3). The best, and most widely used method of control for dollar spot is though the use of fungicides (1). The disease occurs on nearly every species of turfgrass used in the golf course industry, and is prevalent on cool (C3) and warm-season (C4) hosts (1,3). Isolates collected from C3 hosts are termed C3 isolates, and isolates collected form C4 hosts are termed C4 isolates (2). Cool-season, or C3 grasses (i.e. *Agrostis spp.*), differ from warm-season, or C4 grasses *(*i.e. *Cynodon* spp.) in that C4 grasses are able to perform photosynthesis in the mesophyll and bundle sheath cells, while C3 grasses only photosynthesize in the mesophyll cells.

C3 and C4 isolates of *S. homoeocarpa* have been found coexisting in the same locale within the transition zone. These isolates are genetically distinct from one another (1,2,4). Research regarding the potential effects of isolates of *S. homoeocarpa* infecting opposite host types from which they were collected is limited(1,4). As warm-season turfgrass, such as bermudagrass, is increasingly used north of the transition zone, it is imperative to investigate the potential implications on disease severity.

Molecular methods for the detection and quantification of *S. homoeocarpa* have been developed. Although further research is essential, the potential for these tools to be incorporated into a disease prediction model may allow turfgrass managers to reduce the number of annual fungicide applications by limiting unwarranted applications, particularly before disease becomes active.

Selected References

1. Liberti, et al. 2012. Evidence for morphological, vegetative, genetic, and mating-type diversity in *Sclerotinia homoeocarpa*. Phytopathology 102:506-518.
2. Putman, A.I., Tredway, L.P., and Carbone, I. 2015. Characterization and distribution of mating-type genes of the turfgrass pathogen *Sclerotinia homoeocarpa* on a global scale. Fungal Genetics and Biology. 81:25-40
3. Smiley, R. W., Dernoeden, P. H., and Clarke, B. B. 2005. Compendium of turfgrass diseases. 3rd ed. APS Press, St. Paul, MN.
4. Viji. et al. 2004. Genetic Diversity of *Sclerotinia homoeocarpa* isolates from turfgrasses from various regions in North America. Plant Dis. 88:1269-1276.



Materials and Methods

1. Used the ITS region of sequences of *S. homoeocarpa* and closest known relatives in the Rutstroemiaceae for developing species-specific primers to use in conventional PCR and a Taqman® probe for use in quantitative PCR

2. Phylogenetic tree was constructed using maximum likelihood analysis of the ITS region of isolates of *S. homoeocarpa* and Rutstroemiaceae fungi

3. Creeping bentgrass (*Agrostis palustris*) and hybrid bermudagrass (*Cynodon dactylon* x *transvaalensis*) were cross inoculated with C3 and C4 isolates to determine if isolates were capable of causing disease on the opposite host type, and if so, to what extent this interaction had on disease severity

Results

1. Developed species-specific primers which detect only *S. homoeocarpa*, including isolates from the Type 1 and Type 2 groups

2. q-PCR protocol developed; validated *in planta*

3. Phylogenetic analysis indicates there are two distinct groups of *S. homoeocarpa* isolates; Type 1 and Type 2. However, C3 and C4 isolates do not always group with Type 1 or Type 2

4. Cross inoculation studies indicate C3 isolates incite greater disease severity on both C3 and C4 host plants, therefore indicating by using C4 plants father north, there could be increased dollar spot severity on these hosts

Molecular detection

ITS species-specific primers

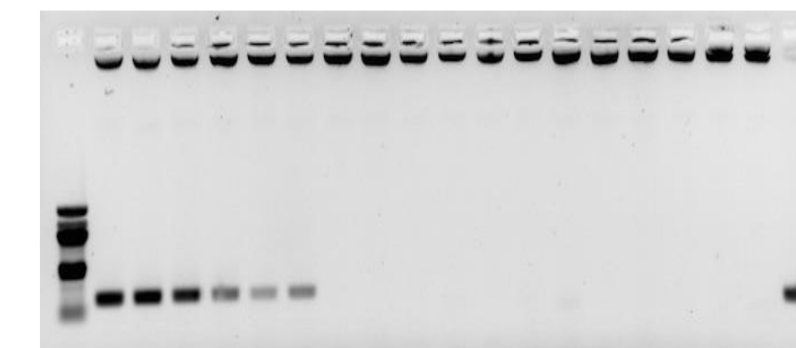


Fig. 1. PCR products on a 1.0% agarose gel using synthesized ShFWD2 and ShREV2 primers with 100 BP Ladder in lane 1; *Sclerotinia homoeocapra* isolates Fl.Sh.A; Fl.Sh,B; 44cad; 11046; 5008AA; *Poculum henningsianum*; *P. sydowianum*; *Lanzia luteovirescens*; *Rutstroemia bolaris*; *Lambertella subrenispora*; *R. firma*; *R. cuniculi*; *R. cuniculi*; *Rutstroemia sp.*; empty lane; negative control; empty lane; *S. homoeocarpa* S-30 (positive control)

Detection

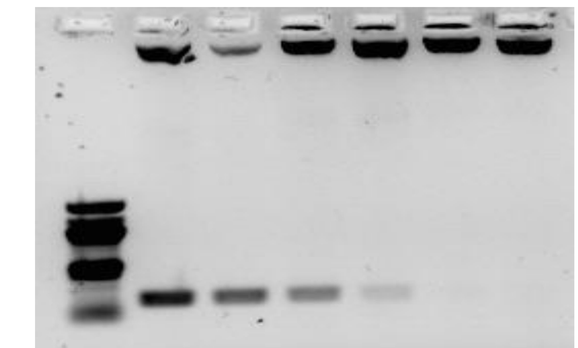
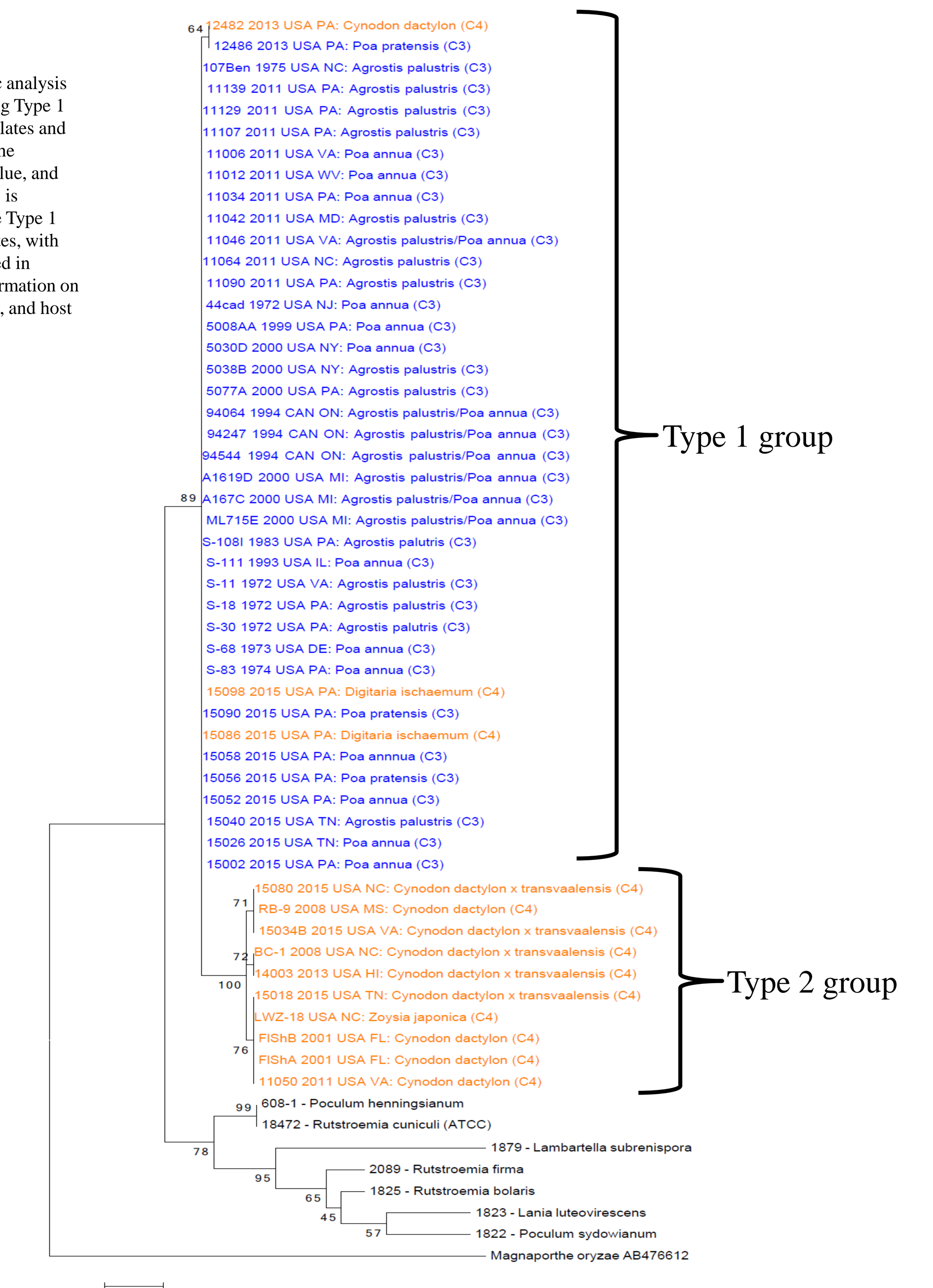


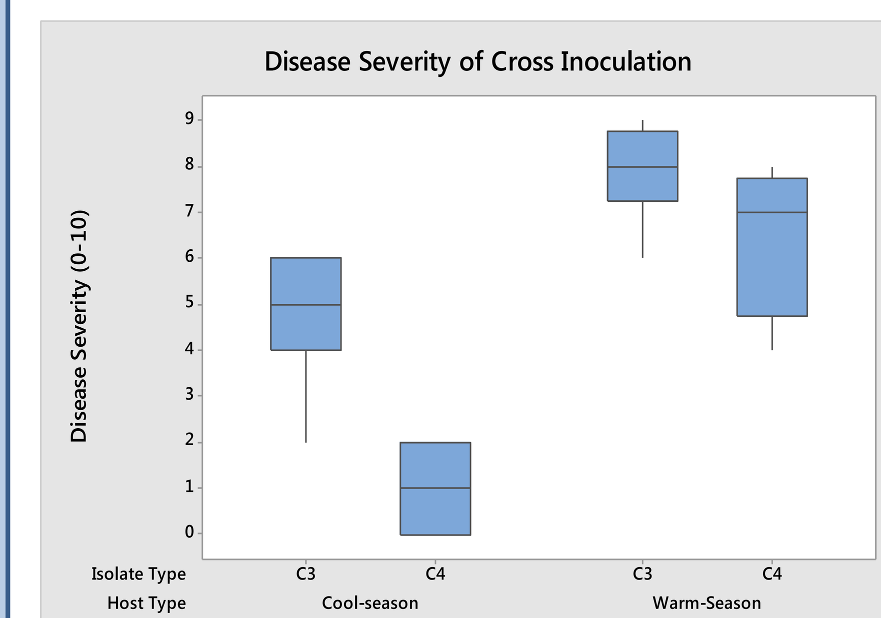
Fig. 2. PCR gradient of DNA concentration dilutions of isolate S-30. Lane 1: 100BP Ladder; 20 ng/µL; 2 ng/µL; 0.2 ng/µL; 0.02 ng/µL; 0.002 ng/µL; negative control.

Phylogenetic analysis of rDNA using the ITS region

Fig. 3. Maximum likelihood phylogenetic analysis with 1000 bootstrap replications indicating Type 1 and Type 2 groups of *S. homoeocarpa* isolates and their inferred relatedness to members of the Rutstroemiaceae family. C3 isolates are blue, and C4 isolates are orange. The Type 2 group is comprised solely of C4 isolates, while the Type 1 group consists of predominately C3 isolates, with the exception of three C4 isolates collected in Pennsylvania. Isolates labels contain information on year collected, country and state of origin, and host species.



Cross inoculation study



General Linear Model: Severity versus Host Type, Isolate Type
Analysis of Variance, $\alpha = 0.05$
Host $p=0.000$
Isolate $p=0.000$
Host*Isolate $p=0.016$