Oxalic Acid Production by *Sclerotinia homoeocarpa*: the Causal Agent of Dollar Spot

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Abstract

Fungi in the genus *Sclerotinia* include some of the most devastating pathogens known. Dollar spot, caused by *S. homoeocarpa*, is the most prevalent and sprayed for disease of golf course turf. The production of oxalic acid and pectolytic cell wall-degrading enzymes by species within this genus is well documented. A series of laboratory-based assays were used to determine whether *S. homoeocarpa* produces oxalic acid. Potato dextrose agar (PDA) adjusted to pH 4 and pH 6, with and without bromophenol blue (Bb), was used to assess the growth of and acid production by *S. homoeocarpa* between 5-35 C. When added to PDA, Bb only slightly hindered the growth of *S. homoeocarpa*. Acid production by *S. homoeocapra* on PDA + Bb occurred between 15-30 C at both pHs, but was first observed on media adjusted to pH 6. Maximum acid production occurred between 20-30 C. Acid production by *S. homoeocarpa* was also observed when grown in potato dextrose broth (PDB) at 25 C. High Performance Liquid Chromatography analysis of spent culture broth collected from *S. homoeocrapa* inoculated PDB revealed the presence of oxalic acid.

Introduction

Dollar spot, caused by *Sclerotinia homoeocarpa*, is the most prevalent and sprayed for disease of golf course turf (Figure 1). The fungus is widespread in North America, Europe, and Australia and affects most turfgrasses. Disease management is an economic, environmental, and public health strain, which requires ample resources. Despite its impacts, relatively little is known about the biology, ecology, and epidemiology of the dollar spot fungus. Other fungi in the genus *Sclerotinia* include some of the most devastating agricultural pathogens known. The production of oxalic acid and pectolytic cell wall-degrading enzymes by these species is well documented and is believed to contribute to virulence and pathogenicity (Cessna et al). The purpose of this study was to investigate oxalic acid production by *S. homoeocarpa*.

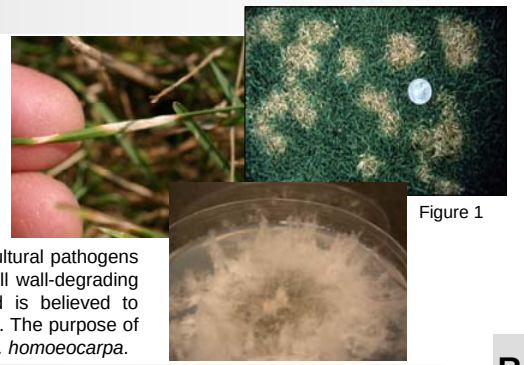

Figure 1

Materials and Methods

A series of laboratory-based assays were used to determine whether *S. homoeocarpa* produces oxalic acid. Potato dextrose agar (PDA) adjusted to pH 4 and pH 6 by 85% lactic acid and 1M potassium hydroxide, respectively, with and without bromophenol blue (Bb), was used to assess the growth of and acid production by *S. homoeocarpa* between 5-35 C. PDA and PDA + Bb at pH 4 and pH 6 were inoculated with *S. homoeocapra* isolate B1 obtained from The Ohio State University, Ohio Turfgrass Foundation Research & Educational Facility and incubated for ten days at five degree increments from 5-35 C. Mycelia diameter growth was recorded every 24 hours to calculate the area under the growth curve (Graph 1). On PDA + Bb, acid production was qualitatively documented, as evident by a color change in the media from purple to yellow (Figure 2). Acid production was ranked on a scale from no production (-) to maximum production (+++).

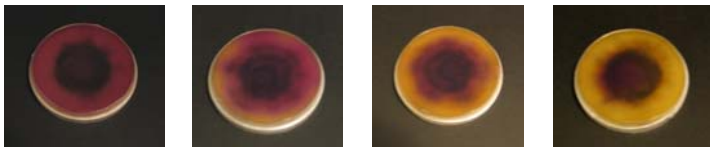

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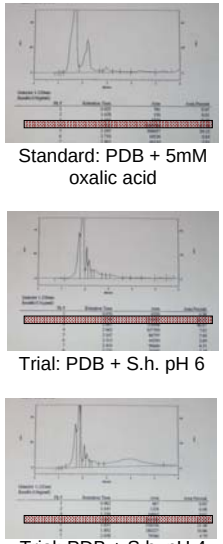
Figure 2

Potato Dextrose Broth (PDB) adjusted to pH 4 and pH 6, following the aforementioned procedures, was inoculated with *S. homoeocarpa* and incubated at 25 C for 17 days. The pH was recorded every three days. After 17 days, the spent broth was tested for the presence of oxalic acid via High Performance Liquid Chromatography (HPLC) analysis (Figure 3). A standard curve of oxalic acid in PDB was prepared.

HPLC Analysis



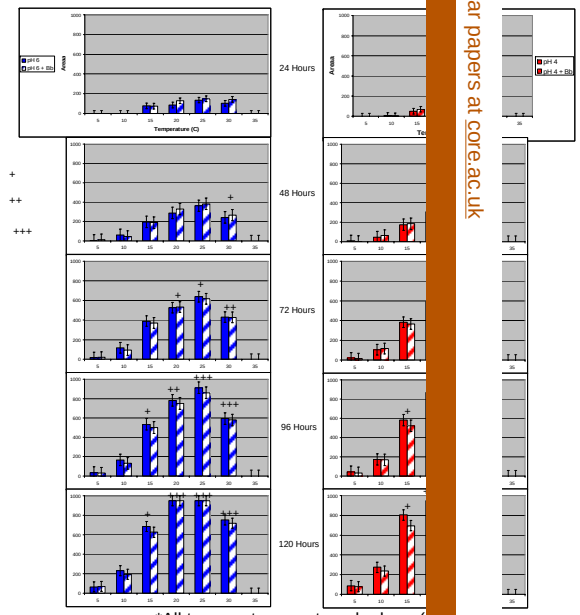
Standard: PDB + 5mM oxalic acid

Trial: PDB + S.h. pH 6

Trial: PDB + S.h. pH 4

Figure 3

***S. homoeocarpa* Growth Curve and Aci** **ction**



*All temperatures not marked are (

Graph 1

Results and Conclusions

- *S. homoeocarpa* produces oxalic acid
- Oxalic acid production occurs between 15-30 C, with 20-30 C proving optimal
- Oxalic acid production occurs first at pH 6
- *S. homoeocarpa* mycelia growth is most rapid between 15-30 C, with 25 C proving
- *S. homoeocarpa* mycelia growth is most rapid at pH 4
- Bb slightly hinders growth of S. homoeocarpa on PDA

Future Studies

Further studies will investigate oxalic acid concentrations under different con such as temperature and pH.

Acknowledgements

The authors would like to thank Dr. Terrence Graham for his help with the HPL is, John Koenig and Ainhoa Medina Martínez for their help reviewing materials, protocols, an through data, and the rest of the members of the Boehm lab for their continued support.

References

Cessna, Stephen A., Valerie E. Sears, Martin B. Dickman, and Phillip S Low. "Oxalic Pathogenicity Factor for *Sclerotinia sclerotiorum*, Suppresses the Oxidative Burst o t Plant." The Plant Cell. November 2000. Vol. 12, pp. 2191-2199.