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Characterizing Hormesis and the *in vitro* Effects of Sublethal Fungicide Exposure in *Sclerotinia homoeocarpa*

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INTRODUCTION

Dollar spot is the most economically important disease of turfgrass in the United States. The disease, caused by *Sclerotinia homoeocarpa* F.T. Bennett, has a wide host range, notably impacting the playability and aesthetics of creeping bentgrass used on golf courses. The infection process begins with direct penetration of hyphae through the leaf cuticle or natural openings. Once infection occurs, production of metabolites, such as oxalic acid (OA), aid in pathogenesis by degrading host tissue.

Cultural practices may limit severity of dollar spot, but fungicide use is often necessary to achieve satisfactory control on high amenity turfgrass. Repeated applications are often required due to the wide window of conducive environmental conditions for disease activity and low damage threshold. Although resistance to sterol demethylase inhibiting (DMI) fungicides has been observed in *S. homoeocarpa*, they remain important tools for dollar spot control. These acropetal penetrants act on fungal cells by inhibiting a demethylation step in the biosynthesis of fungal sterols, which are needed for fungal growth.

Hormesis describes a dose response characterized by low dose stimulation and high dose inhibition. Hormetic responses have been recorded in plant pathogens treated with sublethal doses of fungicides, which may lead to increased disease severity in field conditions. This problem could be compounded by fungicide resistant populations. Evidence for the expression of hormesis in *S. homoeocarpa* growth has been observed *in vitro* but not reported, or quantified. Oxalic acid production may also increase due to sublethal fungicide exposure, and contribute to increased disease severity.

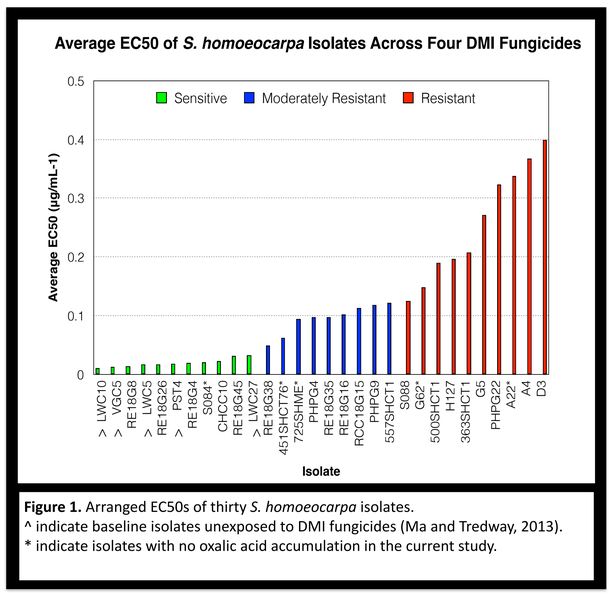
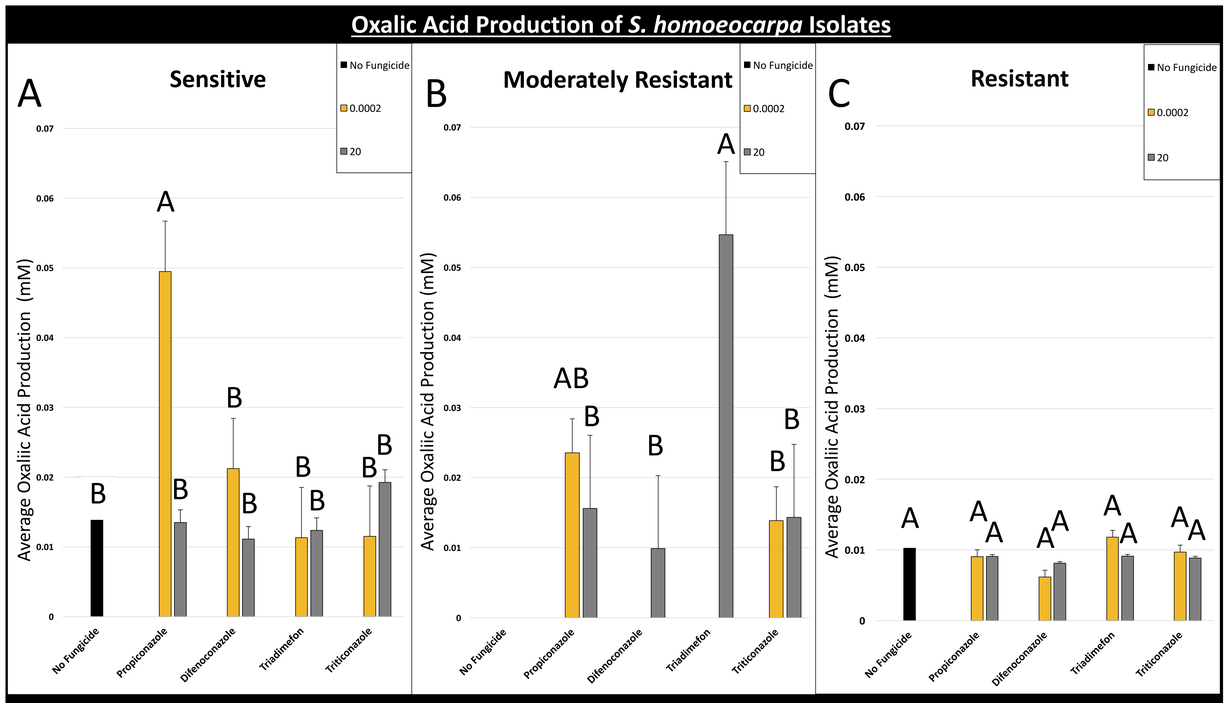


Figure 1. Arranged EC50s of thirty *S. homoeocarpa* isolates.
^ indicate baseline isolates unexposed to DMI fungicides (Ma and Tredway, 2013).
* indicate isolates with no oxalic acid accumulation in the current study.

OBJECTIVES

Evaluate the potential hormetic effects of sublethal DMI fungicide exposure on oxalic acid production in *S. homoeocarpa* F.T. Bennett.



* Error bars indicate standard error of the mean.

Figure 2. Oxalic Acid Production of *S. homoeocarpa* isolates.
Influence of low and high DMI fungicide doses on oxalic acid production of sensitive (A), moderately resistant (B), and resistant (C) *S. homoeocarpa* isolates. Moderately resistant isolates did not produce OA when left untreated or when treated with low doses of difenoconazole or triadimefon. Data is the mean of three replications across all isolates. Columns followed by the same letter are not significantly different according to Fisher's Protected LSD (α = 0.05).

MATERIALS AND METHODS

- Thirty *S. homoeocarpa* F.T. Bennett isolates with a confirmed range of known sensitivities to DMI fungicides, were obtained from previous studies (**Fig. 1**) (Burpee, 1997, Ma and Tredway, 2013). Isolates were grown on modified Melin-Norkrans media for three days.
- Conical centrifuge tubes were filled with sterile 10 mL HPLC grade water and amended with technical grade difenoconazole, propiconazole, triadimefon, or triticonazole at concentrations of 0, 0.0002, or 20 µg ml^{-1}.
- An agar plug (10 mm diameter) was taken from the edge of the 3-day old colonies grown on modified Melin-Norkrans media and placed into each conical centrifuge tube. All tubes were placed on an orbital shaker set at 165 RPM. After 10 days, the agar plug was filtered out of the solution (**Fig. 3C**). Detection and quantity of OA in the solution was assessed at a wavelength of 209 nm with a Dionex Ultimate 3000 UHPLC machine (**Fig 3B**). A standard curve was created from a range of synthetic OA solutions in samples for quantification (**Fig 3A**). Treatments were replicated three times.
- Least squared means for OA accumulation were subjected to analysis of variance using the PROC GLIMMIX (SAS 9.4). LSMeans were separated with Fisher's Protected LSD (α = 0.05).

RESULTS

- OA was detected in solutions from 25 out of 30 isolates. Sensitive, moderately resistant, and resistant isolates produced OA.
- Fungicide type (P = 0.391) and fungicide concentration (P = 0.308) did not have a significant effect on OA production of individual isolates.
- Sensitive isolates accumulated more OA than resistant isolates (P = <0.0001).
- Oxalic acid accumulation was highest in sensitive isolates treated with a low dose of fungicide compared to all other treatment combinations (P = <0.0001).
- Oxalic acid accumulation was lowest in resistant isolates compared to all other isolates (P = <0.0001) (**Fig 2**).

CONCLUSIONS

Production of OA by *S. homoeocarpa* isolates was sporadic in this study, with 16.9% of solutions having detectable OA in both HPLC injections. Sensitive isolates produced more OA than resistant isolates, which may indicate a fitness cost in DMI resistant isolates. Furthermore, OA production was highest by sensitive isolates treated with a low dose of fungicide, and lowest by resistant isolates treated with a high dose of fungicide. Resistant isolates may require a higher fungicide concentration than the concentration used in this study (0.0002 µg ml $^{-1}$) to induce hormesis, and a sublethal dose increase in metabolism. Future research should investigate other fungicide chemistries, a larger set of sublethal fungicide concentrations, and additional growth factors including disease severity to accurately assess the impact of low fungicide doses of pathogen metabolism.

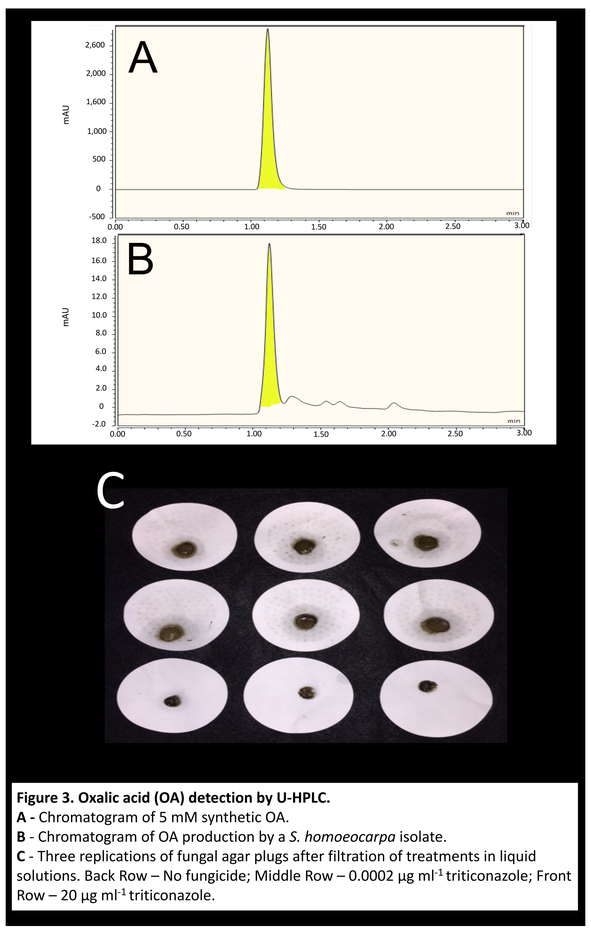


Figure 3. Oxalic acid (OA) detection by U-HPLC.
A - Chromatogram of 5 mM synthetic OA.
B - Chromatogram of OA production by a *S. homoeocarpa* isolate.
C - Three replications of fungal agar plugs after filtration of treatments in liquid solutions. Back Row – No fungicide; Middle Row – 0.0002 µg ml^{-1} triticonazole; Front Row – 20 µg ml^{-1} triticonazole.

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