How Fungicides Affect Turfgrass Phylloplane Microbes

DEPARTMENT OF PLANT SCIENCE & LANDSCAPE ARCHITECTURE

Joseph Doherty[1], Megan Botti-Marino[3], David Ritchie[2], James Kerns[2], Joseph Roberts[1]

[1]University of Maryland, College Park, [2]North Carolina State University, [3]Michigan State University

NC STATE UNIVERSITY

Introduction

- The abundance and ubiquity of turfgrass pathogens leads golf course managers to frequently apply fungicides to combat the pathogens.
- It is unknown if fungicides applied to combat disease may impact the microorganisms present in and on the leaf.
  - Applications of fungicides in an apple orchard led to a decrease in species richness (Walter et al., 2007).
  - Streptomycin applications did not have an effect on microbial communities in an apple orchard phyllosphere (Yashiro, 2012).
  - Microbial diversity was increased as a result of the application of enostroburin in a wheat phyllosphere (Gu et al., 2010).
  - In the phyllosphere of creeping bentgrass applications of propiconazole and azoxystrobin lowered yeast populations (Buck, 2002).

Objectives

- **Elucidate changes in the culturable microorganisms of an "A-1" creeping bentgrass phyllosphere due to fungicide applications.**

Materials and Methods

Treatment Application

- Six 0.9 by 1.3 meter plots were establish on an "A-1" creeping bentgrass putting green at the Lake Wheeler Turfgrass Research Facility in Raleigh, NC.
- Fungicide treatments were:
  - fluazinam (FLZ)
    - Secure 636.717 g a.i./ha 14 d
  - chlorothalonil (CHL)
    - Daconil Ultrex 8,056.08 g a.i./ha 14 d
  - fosetyl-Al (FAL)
    - Chipco Signature 9,763.5678 g a.i. /ha 14 d
  - fluxapyroxad (FPY)
    - Xzemplar 177.441 g a.i./ha 14 d
  - pyraclostrobin (PYR)
    - (2014) Insignia 549.4133 g a.i./ha 14 d

Fig 1. Sample of turf tissue prior to pulverization

Sampling

- A baseline sample was taken prior to initiating treatments
- Samples were retrieved 5 d post-treatment
  - Samples consisted of five individual turf plants from 4 random locations in each treatment plot
  - Samples were kept on ice to prevent degradation of material in the field.

Sample Processing

- Weight of samples was taken for normalization of colony forming units (CFUs).
- All samples were pulverized in 1 mL of sterile DI water with 425-600 µm glass beads and a plastic pestle.
- Pulverized material was diluted to 10^{-3} and 10^{-5} concentrations.
- Dilutions were plated onto 4 different growth media.
  - Actinomycete Isolation Agar (AI)
  - Acidified Potato Dextrose Agar (APDA)
  - Nutrient Agar + 1% sucrose (NASC)
  - King's B (Fluorescent pseudomonad isolation agar)
- Counts of CFUs were taken 2 and 7 d post plating on media

Quantification of Data

- Post-normalization all data were subjected to ANOVA using GLIMMIX procedure in SAS 9.4 for Windows.
- Means separation determined at the 0.05 level.

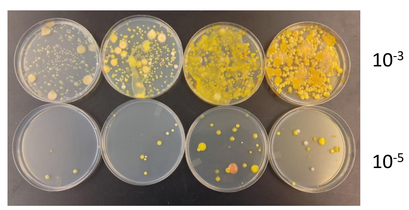

Fig 2. Seven day colony populations on the different media, from left to right, AI, APDA, NASC, and King's B.

Results

Actinomycetes

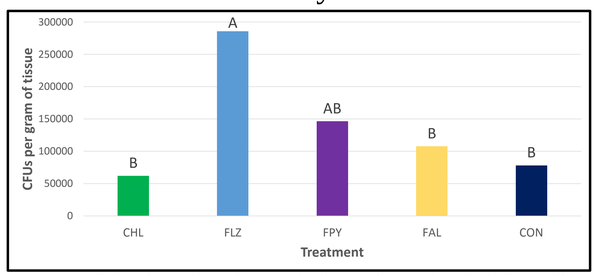

Fig 3. Fungicide effects on actinomycete populations in year 1. Letter designations indicate significant differences at the 0.05 level. Means separation determined from analysis of square root transformed data.

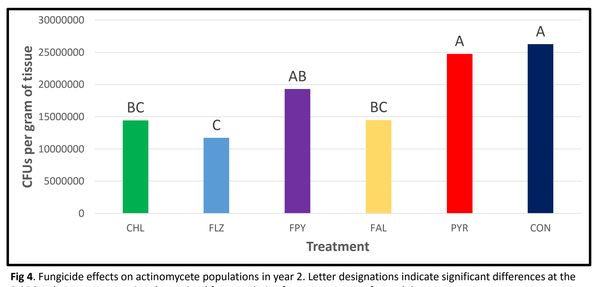

Fig 4. Fungicide effects on actinomycete populations in year 2. Letter designations indicate significant differences at the 0.05 level. Means separation determined from analysis of square root transformed data.

Fungi

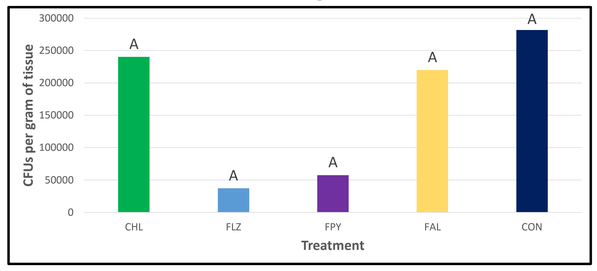

Fig 5. Fungicide effects on fungi populations in year 1. Letter designations indicate significant differences at the 0.05 level. Means separation determined from analysis of logarithmic transformed data.

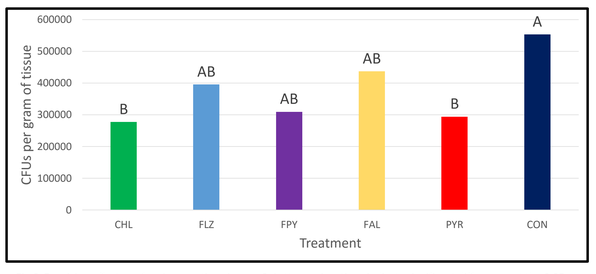

Fig 6. Fungicide effects on fungi populations in year 2. Letter designations indicate significant differences at the 0.05 level. Means separation determined from analysis of square root transformed data.

General Bacteria

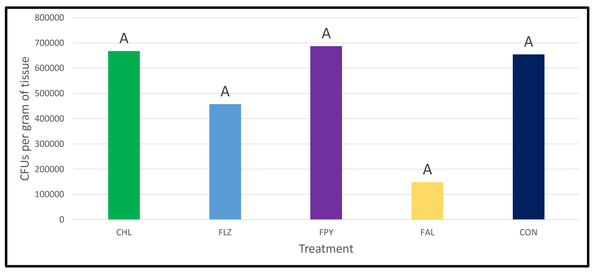

Fig 7. Fungicide effects on bacterial populations in year 1. Letter designations indicate significant differences at the 0.05 level.

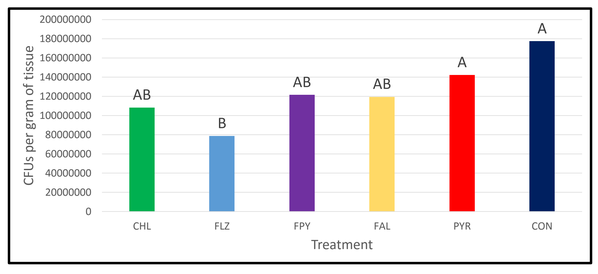

Fig 8. Fungicide effects on bacterial populations in year 2. Letter designations indicate significant differences at the 0.05 level. Means separation determined by analysis of square root transformed data.

Fluorescent Pseudomonads

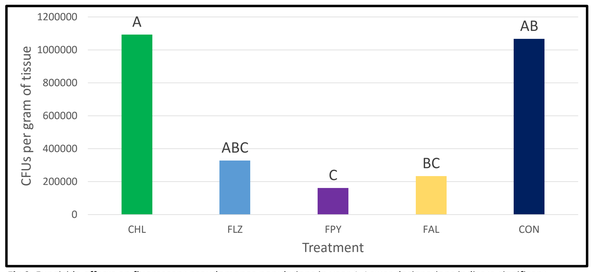

Fig 9. Fungicide effects on fluorescent pseudomonas populations in year 1. Letter designations indicate significant differences at the 0.05 level. Means separation determined by analysis of square root transformed data.

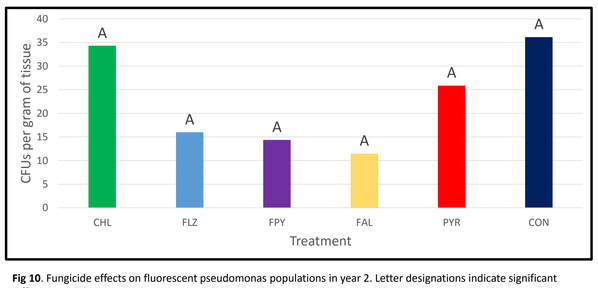

Fig 10. Fungicide effects on fluorescent pseudomonas populations in year 2. Letter designations indicate significant differences at the 0.05 level.

Discussion

- All treatment effects lowered microbial populations except for fluazinam (2013)
- All chemicals exhibit at least one effect across the trial
- Some treatments exhibited large variations in populations while lacking any statistical difference
  - Populations could have already recovered prior to sampling
  - Weather impact on microbial populations is not well understood
  - Fungicides were potentially degraded by microbes
- More methods will be utilized in future experiments to broaden scope
  - Illumina sequencing for capturing population changes of non-culturable organisms

Acknowledgements

Funding provided by the Kelman Scholars Program at North Carolina State University. The authors would like to thank the staff of the Lake Wheeler Turfgrass Research Facility in Raleigh, NC for management and maintenance of the research plots. We would also like to thank Dr. Consuelo Arellano for assistance in statistical analysis of data.

References

Buck, James W, and Leon L Burpee. 2002. The Effects Of Fungicides On The Phylloplane Yeast Populations Of Creeping Bentgrass. Canadian Journal Of Microbiology 48.6: 522-529.

Gu, Likun, Zhihui Bai, Bo Jin, Qing Hu, Huili Wang, Guoqiang Zhuang, and Hongxun Zhang. 2010. Assessing the Impact of Fungicide Enostroburin Application on Bacterial Community in Wheat Phyllosphere. Journal of Environmental Sciences 22.1: 134-141.

Walter, Monika, Frampton, Christopher M., Boyd-Wilson, Kirsty S.H., Harris-Virgin, Patricia, Waipara, Nicholas W. 2007. Agrichemical Impact on Growth and Survival of Non-target Apple Phyllosphere Microorganisms. Canadian Journal of Microbiology 53(1):45-55.

Yashiro E. and McManus P. S. 2012. Effect of Streptomycin Treatment on Bacterial Community Structure in the Apple Phyllosphere. PLoS ONE 7.5: e37131.

UID 92901