

Effects of Wild and Commercial Mycorrhizae on *Sorghastrum nutans* and *Sorghum bicolor* Warm-Season Grasses

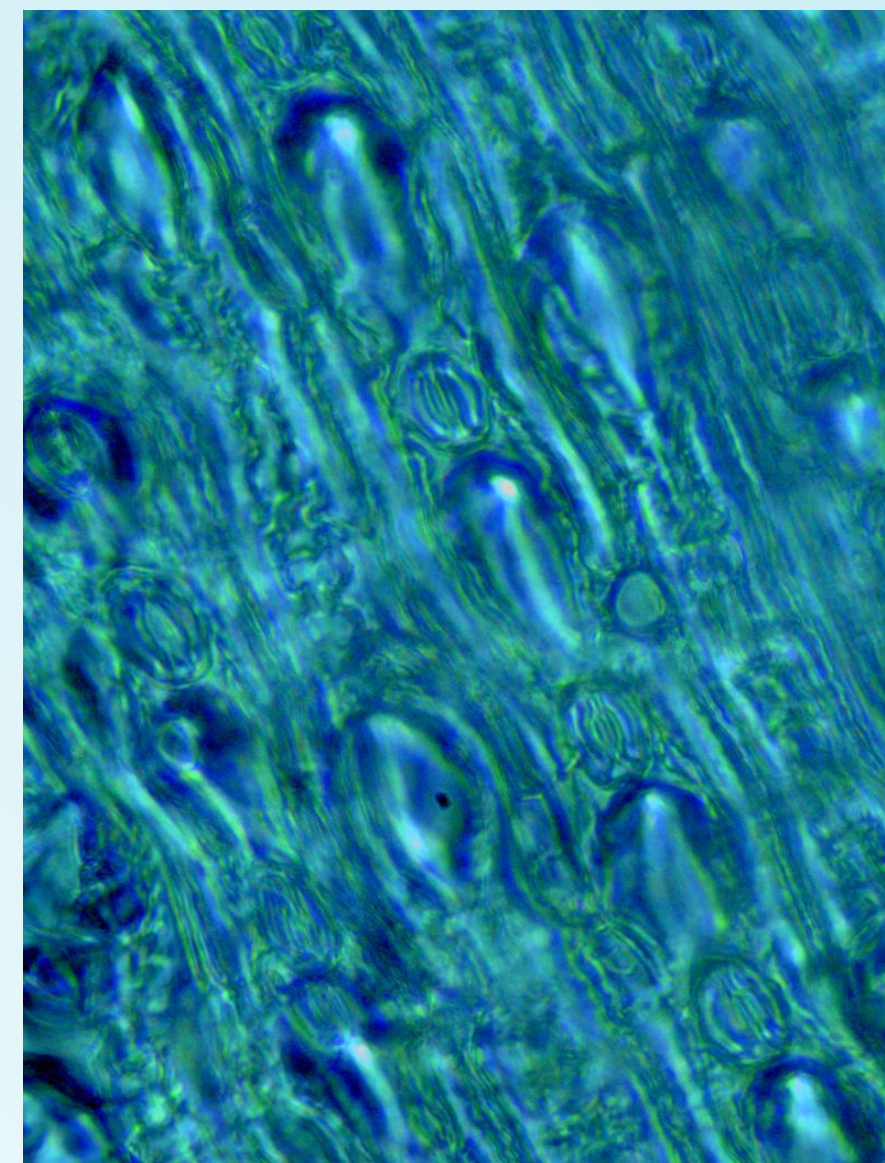
Michael Conover, Dominique Davis, Shelby Tillman

Department of Plant Biology, Ecology, and Evolution

Oklahoma State University

Introduction

- Mycorrhizal fungi maintain a mutualistic beneficial relationship with their host plant. Mycorrhizae support their host plant by increasing mineral nutrition, water absorption, and growth and disease resistance, the host provides the mycorrhiza with energy from photosynthesis (Sheng *et al.* 2008)
- Plants exposed to mycorrhizae tend to be taller, have greater leaf length and area, a larger root diameter, greater biomass, and increased stomata opening and conductance (Augé *et al.* 2015, Yang *et al.* 2017).
- Mycorrhizae-dependent prairie grasses need mycorrhizae to compete in the wild. Less-dependent plants are more likely to be able to compete with other plants in the absence of mycorrhizae (Hartnett *et al.* 1993)
- In addition, seedlings exposed to mycorrhizae tend to have more successful growth (Ortas 2010)
- Warm-season grasses with coarse root systems are extremely dependent on mycorrhiza for growth (Hetrick *et al.* 1988)
- The objective of this experiment was to study how two warm-season grasses, *Sorghastrum nutans* and *Sorghum bicolor*, were effected by wild and commercial mycorrhizae.
- We expected that grasses inoculated with commercial mycorrhiza will yield an overall higher biomass, leaf length, stem diameter, and stomata counts compared to wild mycorrhiza alone and non-inoculated plants



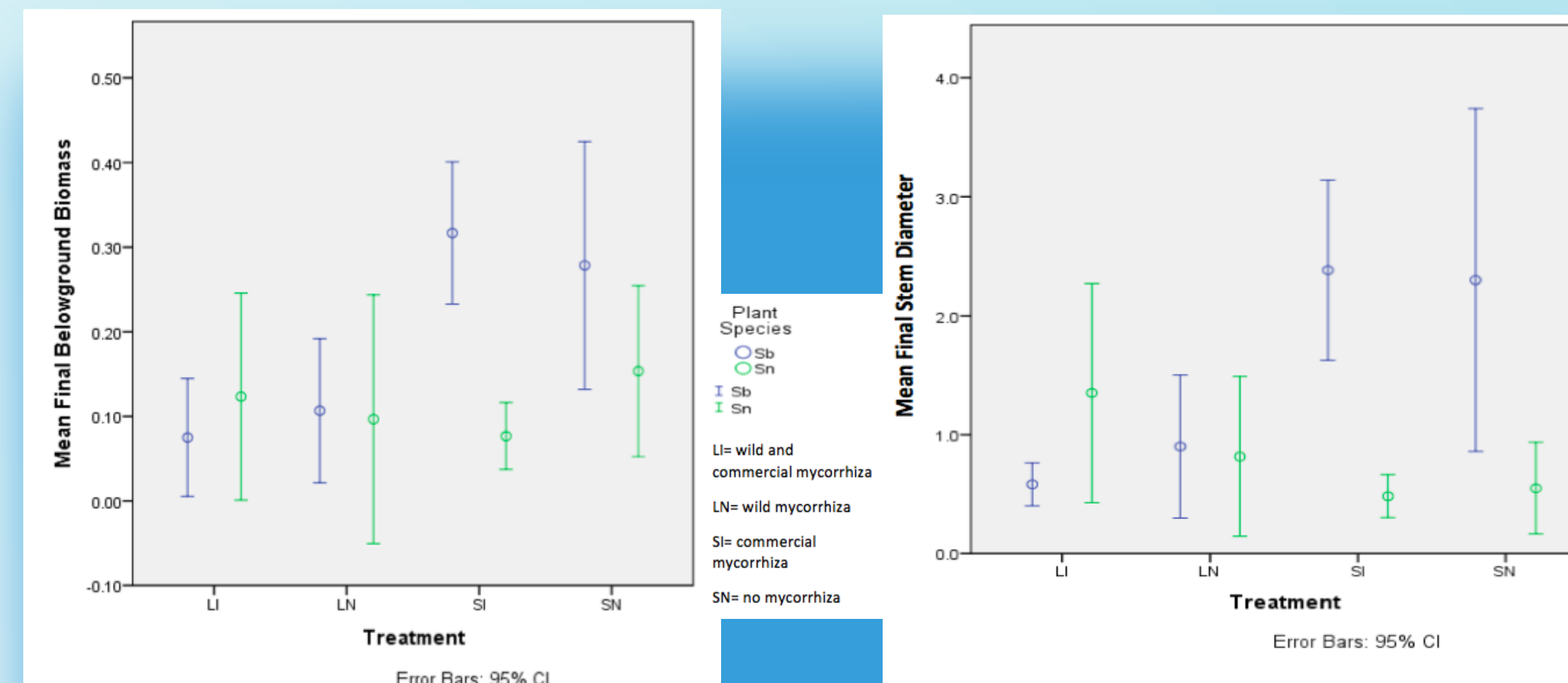
Picture 1 Microscope view of leaf print, used to count stomata

Methods

- In our study, we tested the effects of wild and commercial Mycorrhizae on *Sorghastrum nutans* and *Sorghum bicolor* Warm Grass Species.
- We Gathered and sterilized soil from OSU research Range station
- Potted 12 of each species in individual pots with sterilized soil and 12 of each in non-sterilized soil (Picture 2).
- Inoculated 6 plants from each species in sterilized soil and 6 plants from each species in non-sterilized soil with commercial mycorrhizae. This created our 4 treatments: Sterilized soil inoculated with wild mycorrhiza, Sterilized soil, Soil with wild mycorrhiza, and Soil with wild mycorrhiza inoculated with commercial mycorrhiza
- Measured leaf length, stem diameter, stomata count, and final above and belowground biomass. We measured our variables of stem diameter, and leaf length weekly, the stomata count one time, and the final above and below ground biomass at the end of the experiment.
- Measured length of 3 leaves from each plant with ruler in cm and averaged the sum three times over the duration of the experiment.
- Measured stem diameter with caliper each week.
- To count Stomata, we painted an inch area of clear nail polish on the backside of each leaf blade for each plant. We placed tape over the nail polish and then removed the tape and placed onto a microscope slide (Picture 1).
- We counted Stomata in 3 different frames of view at x400 magnification, which is an area of 0.45mm or 450 microns.
- At the end of the experiment, Dried plants and recorded above and below ground biomass
- We used a Two-way ANOVA in the program SPSS to test for effects of treatments on traits.

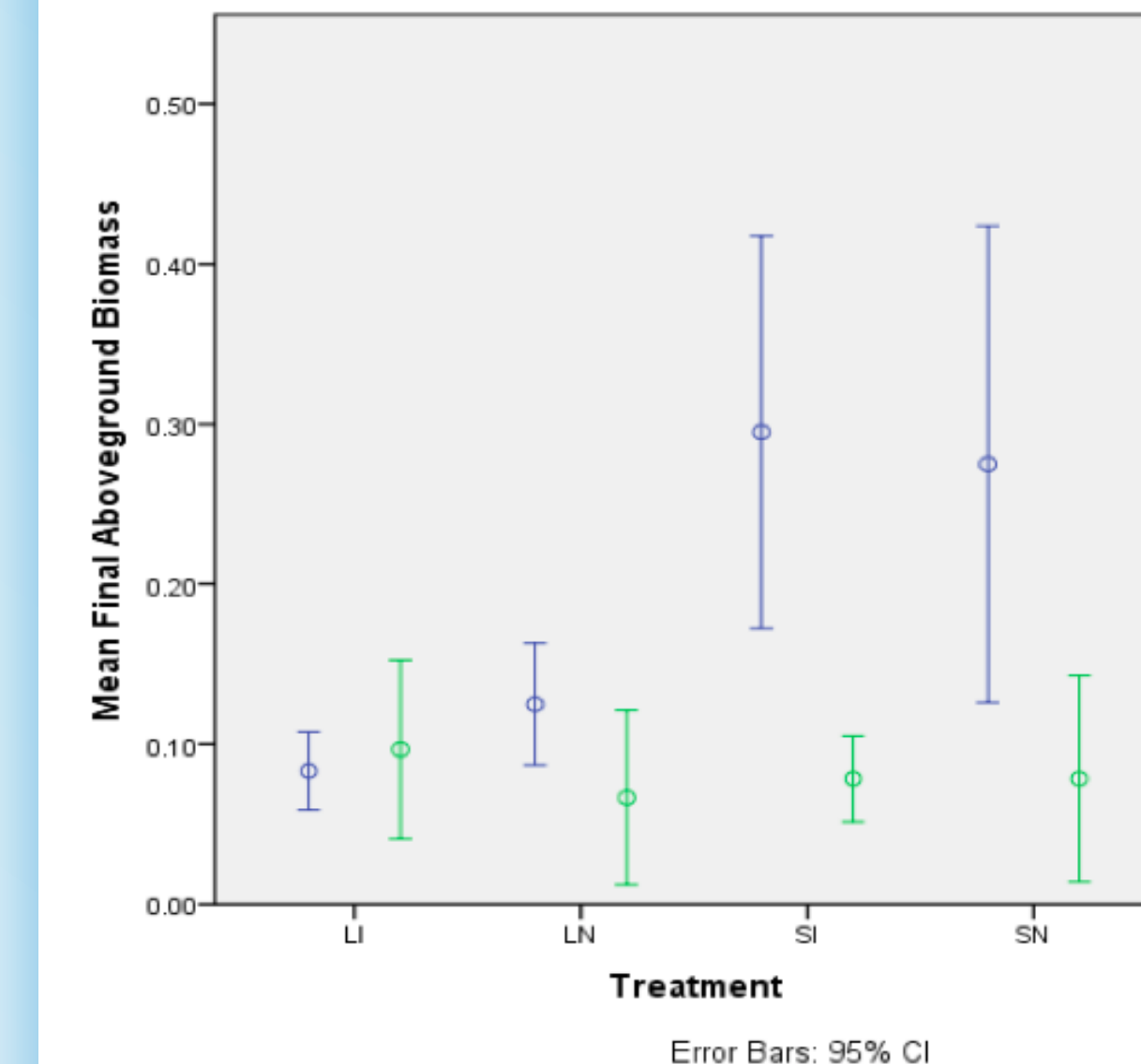
Results

- All analysis was done through a two-way ANOVA.
- The overall effects of treatment on belowground biomass were significant (Significance value $\leq .001$). Both Inoculum and plant species treatments gave no significant values while the soil treatment (sterilized vs. unsterilized) was significant with a value of .001. (Table 1)
- The overall effects of treatment on stem diameter were significant with the significant value < 0.001 . The plant species alone and when paired with soil treatment both produced significant values (.001, .000). (Table 1)
- There were no significant effects of any treatment on stomata count (Table 1)
- The overall effects of treatment on belowground biomass were significant (Significant value < 0.001). Soil treatment, alone and in an interaction with plant species, yielded a significant value < 0.001 (Table 1)
- The results from Graph 1 show a significant difference between live and sterilized soil in relation to belowground biomass
- In graph 2 both species reacted the opposite to the soil treatment in terms of stem diameter.
- In graph 3 sterilized soil yielded an overall higher aboveground biomass resulting in a significant difference.
- There were no significant variations in graph 4 (stomata count).

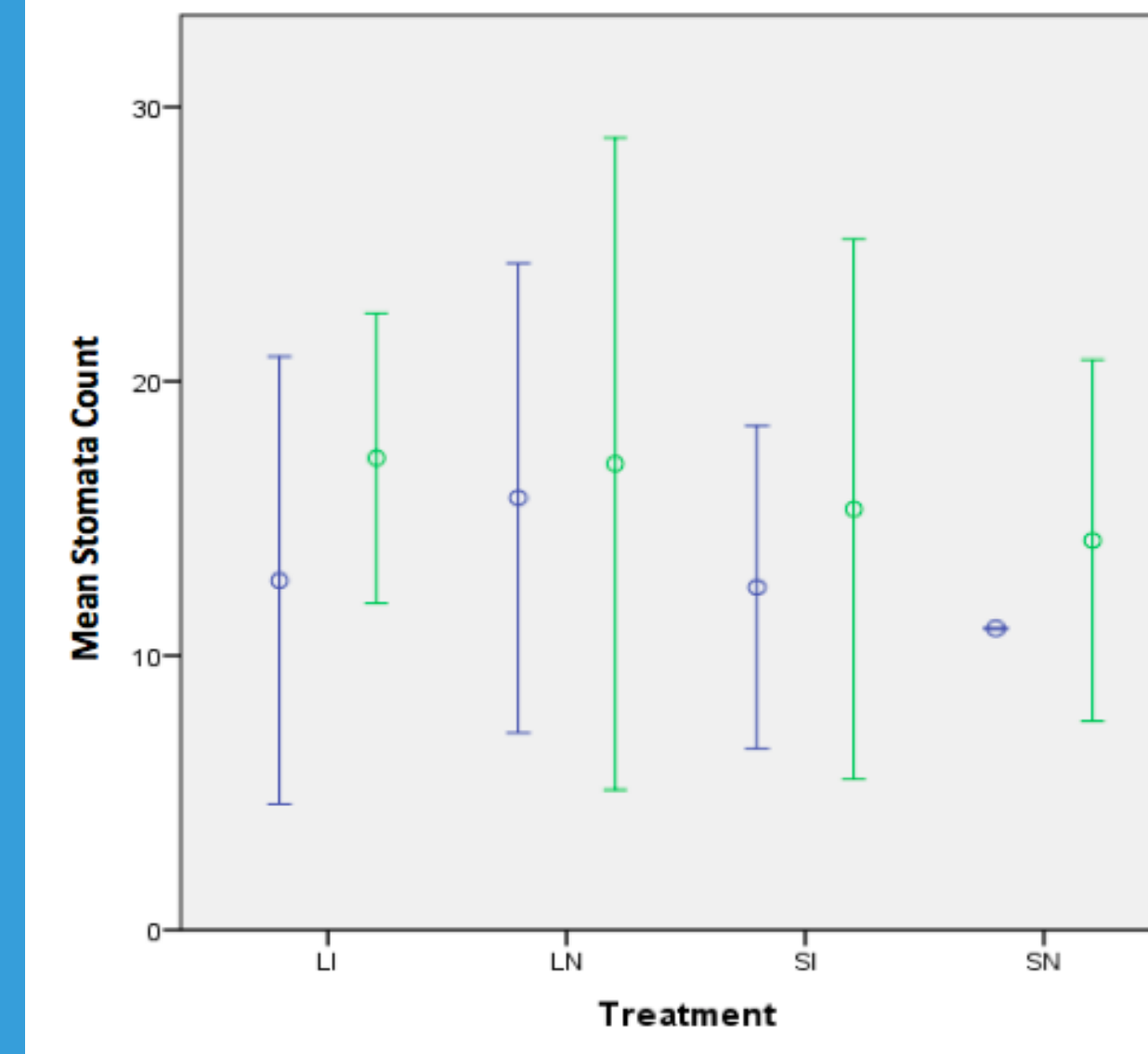


Graph 1 Boxplot showing the effect of four treatments on belowground biomass in two species

Graph 2 Box plot displaying the effect of four treatments on stem diameter in two species



Graph 3 Box plot recording the effect of four treatments on aboveground biomass in two species



Graph 4 Box plot recording the effect of four treatments on stomata count in multiple species

Response Variable	F-value	Overall Significance	Inoculant treatment	Soil treatment	Soil/Inoculum Interaction	Soil/Inoculum/Species
Leaf length	1.451	0.213	0.366	0.896	0.426	0.308
Stem Diameter	6.968	0	0.78	0.017	0.81	0.234
Below Ground Biomass	5.145	0	0.711	0.001	0.775	0.143
Above Ground Biomass	9.103	0	0.924	0	0.718	0.299
Stomata	0.472	0.846	0.985	0.286	0.546	0.691

Table 1 Significance levels of various variables using a two-way ANOVA

Discussion

- Treatments with wild mycorrhizae had less belowground biomass in *S. bicolor* but little effect in *S. nutans*
- S. nutans* seedlings exposed to wild mycorrhizae have greater stem diameter, where *S. bicolor* have smaller stem diameter
- Mycorrhizae do not significantly effect stomata count or leaf length in warm-seasoned grasses
- Treatments with wild mycorrhizae had smaller aboveground biomass in *S. bicolor*, whereas *S. nutans* aboveground biomass was not greatly affected by mycorrhizae
- Our hypotheses were mostly not supported; commercial mycorrhizae did not help these plants grow significantly or affect stomata count. In addition, the grasses reacted quite different even though both are warm-seasoned.
- The increase growth then tends to show in sterilized soil could be due to mycorrhiza bringing nutrients to the plant without it requiring growth to reach it.
- Our research is not supported by previous research in the field that showed growth and stomata increase due to mycorrhizae (Yang *et al.* 2017, Hartnett *et al.* 1993, Ortas 2010, Hetrick *et al.* 1988, and Augé *et al.* 2015)
- This difference could show that there are some issues in our experiment
- We may have run into some biased data for several reason including: watering amounts which could affect the growth of the plants, hard to view slides that may have effected stomata count, different people planting the plants could have resulted in some variation
- In future studies, researchers could look at many other species of plants, try many different kinds of commercial mycorrhizae, set up experiments where plants compete against each other and are only exposed to one mycorrhizae, or on the effect of mycorrhizae on stomata opening



Picture 2 Plants randomly assorted and treated with 4 treatments

Literature Cited

- AUGE, R. M., TOLER, H. D. & SAXTON, A. M. Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. *Mycorrhiza*. 2015. 25 (1): 13-24.
- HARTNETT, D. C., B. A. D. HETRICK, G. W. T. WILSON, AND D. J. GIBSON. 1993. Mycorrhizal influence on intra- and interspecific neighbor interactions among co-occurring prairie grasses. *Journal of Ecology*. 81(4):787-795.
- HETRICK, B. A. D., D. G. KITT, AND G. W. T. WILSON. 1988. Mycorrhizal dependency and growth habit of warm-season and cool-season tallgrass prairie plants. *Can. J. Bot.* 66:1376-1380.
- ORTAS, I. 2010. Effect of mycorrhiza application on plant growth and nutrient uptake in cucumber production under field conditions. *Spanish Journal of Agricultural Research*. 8(S1):S116-S122.
- SHENG, M., M. TANG, H. CHEN, B. YANG, F. ZHANG, AND Y. HUANG. 2008. Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. *Mycorrhiza*. 18(6):287-296.
- YANG, Y., X. OU, G. YANG, Y. XIA, M. CHEN, L. GUO, AND D. LIU. 2017. Arbuscular Mycorrhizal Fungi Regulate the Growth and Phyto-Active Compound of *Salvia miltiorrhiza* Seedlings. *Applied Sciences*. 7(1):68.

Acknowledgements

We would like to acknowledge Dr. Janette Steets and TA Frankie Coburn for their guidance in this research.

hhmi
Howard Hughes
Medical Institute

