Alleviating Environmental Stresses in Native Arkansas Plants by Arbuscular Mycorrhizal Fungi Inoculation

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Introduction

- Plant–microbial interactions may play a crucial role in mitigating the extreme stress plants experience.
- Mutualistic microbes have been shown to confer a diversity of benefits on plants:
  - Tolerance to drought (Song et al., 2015), heavy metals (Zhang et al., 2010), or thermal stress (Khan et al., 2012).
  - Enhanced growth and nutrient/water acquisition (Read, 1999).
- Climate projections indicate that temperate forests will be exposed to increased frequency of drought in the near future (Sheffield and Wood, 2008).
- Several Arkansas plant species have been dramatically reduced from their historical levels due to global warming effects, including drought, introduction of diseases, or pests (e.g., invasive species).
- Mycorrhizal fungi help overcome the nutrient deficiency by extending their external hyphae to areas of soil beyond the depletion zone and increasing the absorptive surface of the root (DeLuca et al., 2002).

Objectives

1. To determine if AMF enhance fitness to native plants.
2. To test experimentally if variation in phenotypic plasticity due to AMF inoculation can be reflected under controlled environment in a greenhouse.
3. To determine if AMF can be used in native species habitat restoration.

Experimental tests and hypotheses

Experiment 1: *Assessment of mycorrhizal colonization in wild populations of native plant species*

Hypothesis: Populations of native species in upland environments will have a higher degree of mycorrhizal colonization compared to foothill or bottomland populations due to the difference in soil characteristics.

Experiment 2: *Mycorrhizal dependency of native species under greenhouse conditions*

Hypothesis: Native plant species are highly dependent on AMF for increased biomass accumulation and nutrient uptake under stressful environmental conditions.

Methods

Experiment 1: Field Assessment – March - August

- 11 different locations in Arkansas covering moist, intermediate, and dry habitats.
- Flowering plants within 10X10 m2 plot at each location were sampled.
- Root samples were cut into 1.5 cm fragments, cleared in 15% KOH at 70˚C for 4 hours, rinsed twice with water, bleached with ammoniated H_2O_2, and acidified with 1 N HCl.
- 0.05% Trypan Blue was used to stain mycorrhizal structures.
- Roots were examined under 40x magnification.
- Presence or absence was established for each location.

Results

Experiment 1: Field Assessment

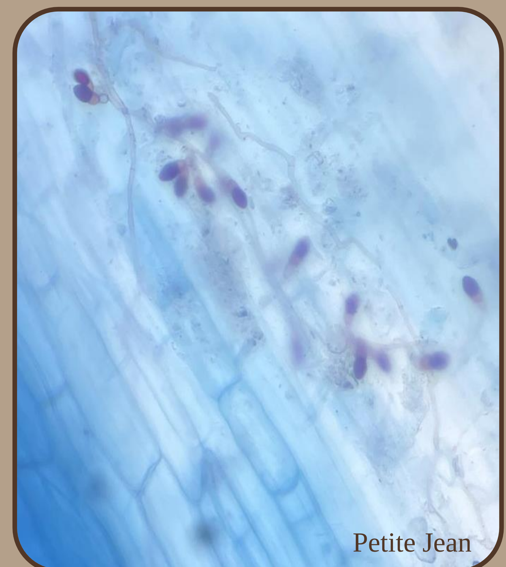
- A total of 11 locations around north/central Arkansas were examined for mycorrhizal presence (Table 1).
- Presence was indicated by hyphae, vesicles, arbuscules or a combination of the three in at least one of the sampled roots.

Location	Coordinates	Presence
Russellville	35.321877 N, 93.139932 W	Y
Cabot	34.91203 N, 92.00509 W	Y
Jasper (H)	36.02025 N, 93.17646 W	N
Jasper (L)	36.0564214 N, 93.2755800 W	Y
Mt. Magazine (H)	35.16481 N, 93.64249 W	Y
Mt. Magazine (L)	35.17810 N, 93.58224 W	Y
Mt. Nebo (H)	35.22940 N, 93.25723 W	Y
Mt. Nebo (M)	35.22369 N, 93.24923 W	Y
Mt. Nebo (L)	35.22429 N, 93.23547 W	Y
Mountain View	35.9641670 N, 92.0987981 W	N
Petite Jean	35.123340 N, 92.921555 W	Y

Table 1. Location and presence of AMF in cleared root samples. Presence: Y – AMF structures, or N – lack of AMF structures.


Mountain View


Mount Magazine (lower)


Petite Jean


Jasper (lower)


Mount Nebo (upper)

Methods

Experiment 2: Greenhouse – June - October

- *Tradescantia ohioensis* and *Solidago arguta* were chosen based compatibility with commercial AMF inoculant, *Rhizophagus intraradices* (MYKOS Xtreme).
- Topsoil collected from field to simulate normal conditions.
  - Sterilized in an industrial autoclave to remove natural microorganisms.
- Factorial design that consisted of two treatments:
  - Species X Drought X Mycorrhizae
  - Mycorrhizae: Mycorrhizal and non-mycorrhizal
  - Water: Drought, intermediate, and saturated
  - 5 replicate pots per treatment combination, 30 of each species
- AMF treatment began immediately after planting:
  - AMF: 2 tablespoons of *R. intraradices* added directly to the roots
  - Non-AMF: Fungicide added to reduce cross contamination
- Water treatment was started two weeks after potting to reduce shock:
  - Drought: 500 ml of water once a week
  - Intermediate: 500 ml every three days
  - Saturated: 500 ml daily
- ***Measurements:*** Height change, leaf number change, leaf area, specific leaf area, and final root length.

Results

Experiment 2: Greenhouse

An ANOVA was done for each of the five response variables to examine the effects of individual and combined treatments.

- **Height change:** *S. arguta* had a much larger height increase than *T. ohiensis*, but both species had an increase height change in the AMF treatment, independent from the water treatment (Fig. 1).
- **Final root length:** Root length was optimal in the intermediate water treatment for both species (Fig. 2).
- **Leaf Area:** The non-AMF treatment increased in leaf area as the water supply increased, while the AMF treatment had the highest leaf area at the intermediate water level (Fig. 3).
- **Leaf number change:** In both species, leaf number increased as water supply increased (Fig. 4). There was an increase in leaf number for *S. arguta* in the AMF treatment, while *T. ohiensis* had more leaves without AMF (Fig. 5).


Mrs. Loewer (*Tradescantia ohioensis*)


Rhizophagus intraradices


Forest goldenrod (*Solidago arguta*)

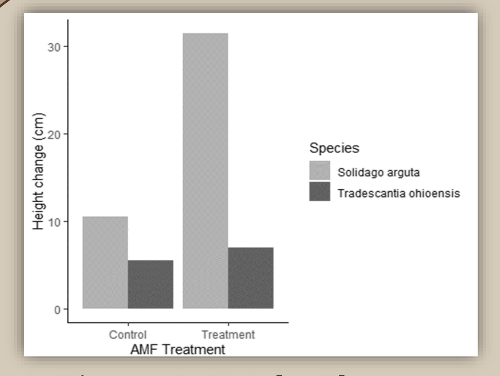


Figure 1. Height Change with AMF and Species.

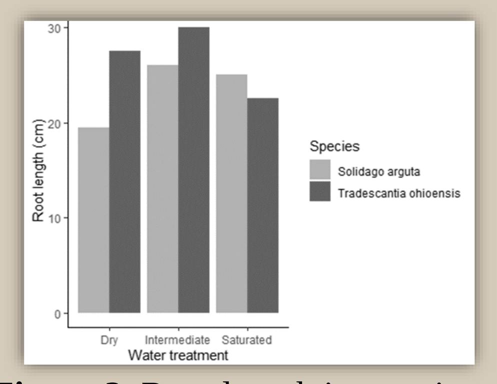


Figure 2. Root length interaction with water treatment by species.

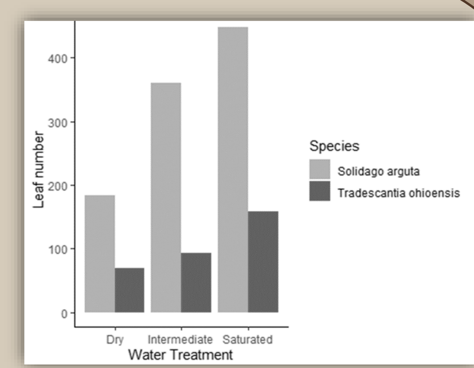


Figure 3. Change in leaf number compared between water treatments and species.

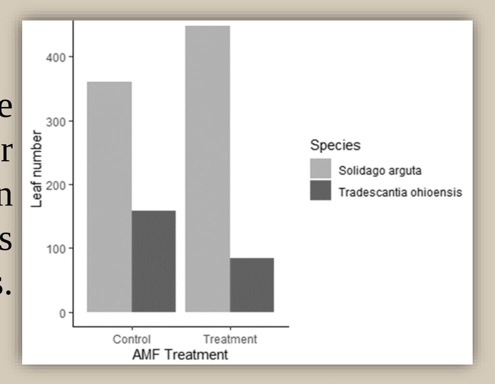


Figure 4. Change in leaf number compared between AMF treatments and species.

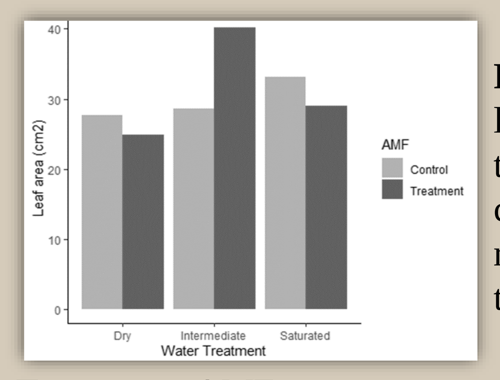


Figure 5. Change in leaf area per AMF treatment for combined species in response to water treatment.

Control - No AMF, Treatment - AMF.

Discussion

- AMF associations varied based on soil characteristics.
- Soils with low clay content provide more suitable conditions for root-AMF interactions (Vieira et al., 2020).
- AMF had a positive relationship with the overall success of the plant, but stressful water treatment did not factor in.
- AMF interactions increase with optimal water supply.
- Setbacks due to pest infestation and heatwaves caused foliage to die before the end of the four-month period.

Conclusions

- AR native species have a high degree of mycorrhizal colonization.
- AMF can aid native plants in nutrient cycling.
- AMF could be a useful tool for habitat restoration projects in suboptimal soil conditions.
- There are limits to the efficacy of the mycorrhizae, as some species require certain soil profiles to be productive.
- In future studies, leaf chemistry analysis (C, N, and P) from the leaves of the experimental plants should be examined.

References

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