Maximizing Legume Efficiency in South Texas

Stephanie Kasper[1], Bradley Christoffersen[2], Pushpa Soti[2], Alexis Racelis[1]

[1]School of Earth, Environmental and Marine Sciences, [2]Department of Biology, University of Texas Rio Grande Valley

INTRODUCTION

Many farms use leguminous cover crops as a nutrient management strategy to reduce the need for nitrogen fertilizer. This strategy depends on a symbiotic relationship between the legume and nitrogen-fixing *Rhizobia* bacteria (Taiz et.al. 2015). Under certain conditions, this symbiosis fails to form despite legume inoculation with *Rhizobia*. Nodulation failure occurred in a 15 acre winter cover crop trial at Hilltop Gardens in Lyford, TX , where three inoculated legume species (crimson clover, hairy vetch, and forage pea) were grown with little evidence of nodulation and nitrogen fixation.

Many factors can inhibit nodulation in legumes including phosphorus and micronutrient deficiencies, extreme temperatures, overly acidic or alkaline soil, excessive nitrogen, and water stress (Graham 1981; Sadowsky 2005).

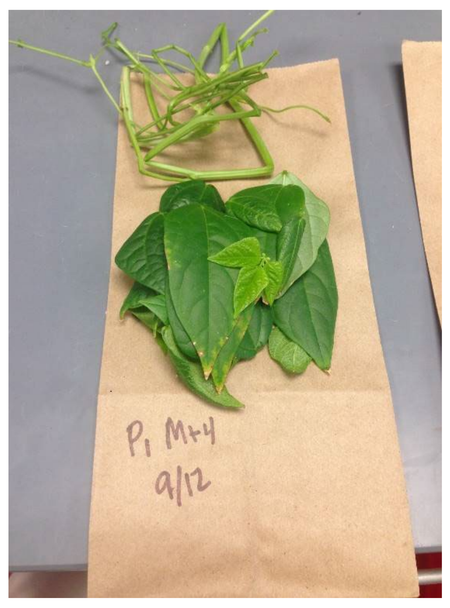
OBJECTIVES

- Determine the likely cause(s) of legume nodulation failure in the cover crop trials in Lyford, Texas. Potential causes include: a) micronutrient deficiency, b) moisture stress, c) phosphorus inaccessibility, and d) nitrogen inhibition.
- Assess the potential of inoculation with arbuscular mycorrhizal fungi to enhance nodulation and N fixation by buffering the legume-*Rhizobium* symbiosis from these stressors.
- Improve South Texas farmers' access to regionally-specific information on nitrogen fixation by cover crops, so they can make informed decisions about cover cropping practices.

METHODS

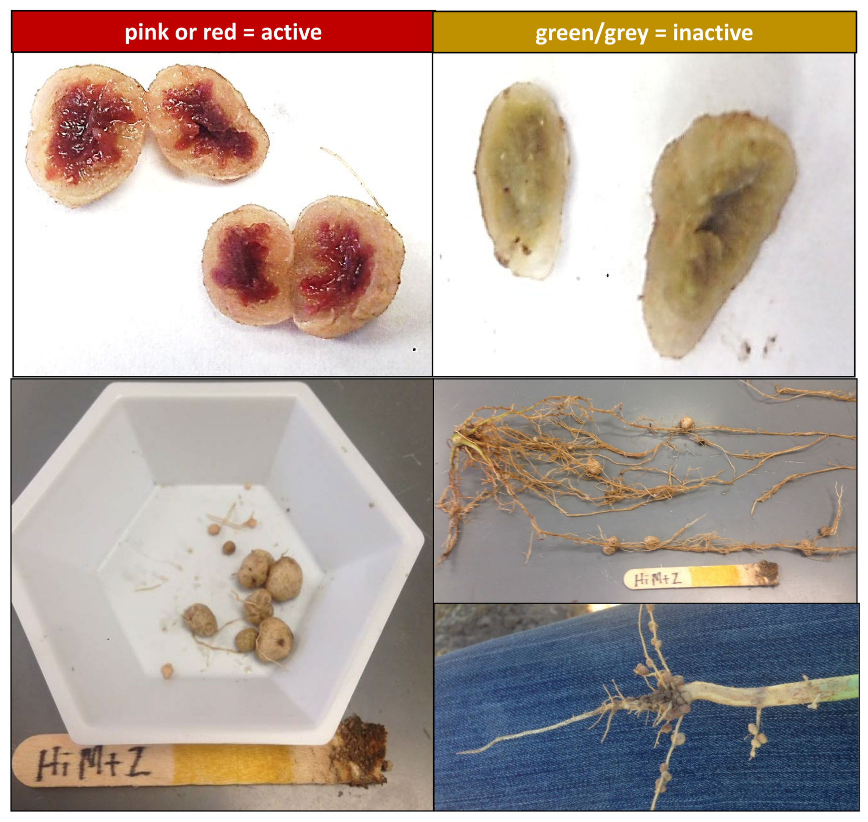
Greenhouse nodulation experiment, Summer 2018

- Iron and Clay cowpeas (*Vigna unguiculata*) grown in pots
- Factorial experimental design with 8 replicates per treatment
  - Micronutrients X Mycorrhizae (2x2)
  - Moisture x Mycorrhizae (3x2)
  - Phosphorus x Mycorrhizae (3x2)
  - Nitrogen x Mycorrhizae (3x2)
- Directly inoculated radicle of pre-germinated seeds at transplant
  - 1 mL rhizobium (1 g inoculant/250 mL water)
  - 1 mL mycorrhizae (1 g inoculant/500 mL water) or equal amount of water for M-
- Randomly assigned 208 cowpeas to a treatment (listed on right)
  - All treatments except Hi and F were watered (150 mL) with nutrient solution or tap water whenever they reached 5% soil moisture
  - Hi treatments received 200 mL with 15% moisture as their lower threshold; F treatments received 500 mL after three days below 3% moisture
- Measurements taken at 75 days: Maximum photosynthesis, leaf area, nodulation data (number, weight, location, and percent active), plant biomass (root, stem, leaf), and nitrogen fixation with relative ureide method (Unkovich et.al. 2008)
- Statistical analysis - SigmaPlot
  - 2-way ANOVAs for Micronutrients, Moisture, and Phosphorus
    - Followed by Holm-Sidak method for multiple comparisons
  - Kruskal-Wallis 1-way analysis of variance on ranks for Nitrogen
    - Dunn's method for multiple comparisons



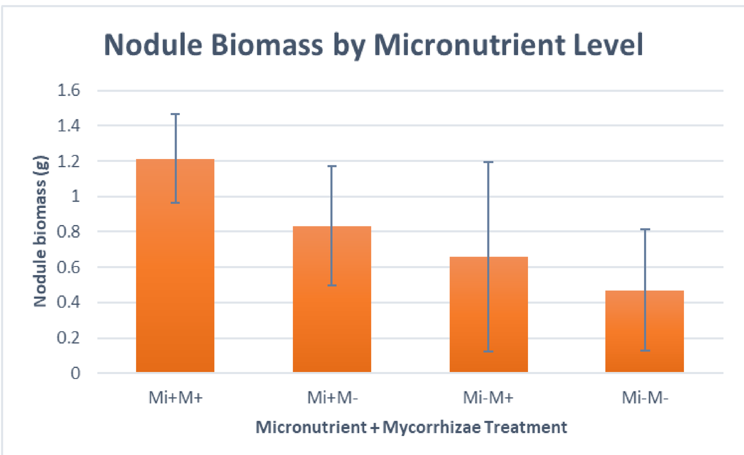
Treatments for Cowpea Greenhouse Experiment

Micronutrient	Moisture	Phosphorus	Nitrogen	Mycorrhizae
• Micronutrients added - Mn, Cu, Zn, Mo, B, Co (Mi+) • Field micro (Mi-)	• High (Hi) 15-25% • Mid range (Mid) 5-15% • Flood/drought cycle (F) 2.5-25%	• Field P (P0) • Low P (P1) 0.1 mM H_2PO^{4-} • High P (P2) 2 mM H_2PO^{4-}	•Field N (N0) •½ Field level (N1/2) •High N (N1) 10mM $Ca(NO_3)_2$	• Inoculated with mycorrhizae (M+) • Control (M-)

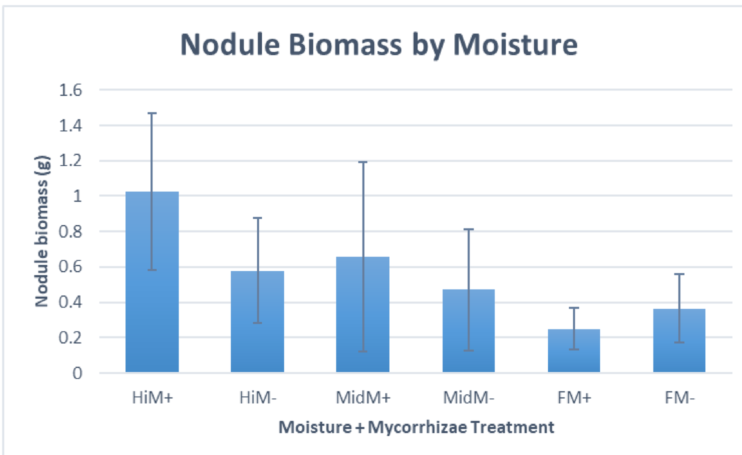


Pictured (top left) are two examples of active cowpea nodules. Active nodules are pink or red, while inactive nodules (top right) are green, grey or brown. The bottom left picture shows cleaned nodules prior to weighing and color check. Bottom right compares cowpea root and nodule structures from the greenhouse experiment (top) and a field experiment (bottom).

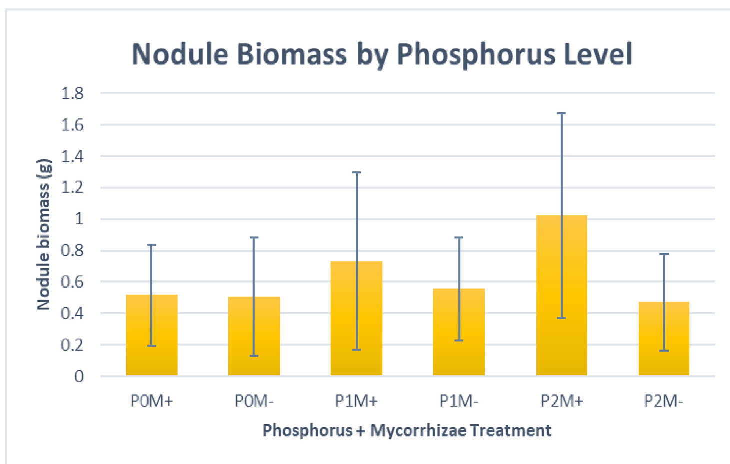
RESULTS



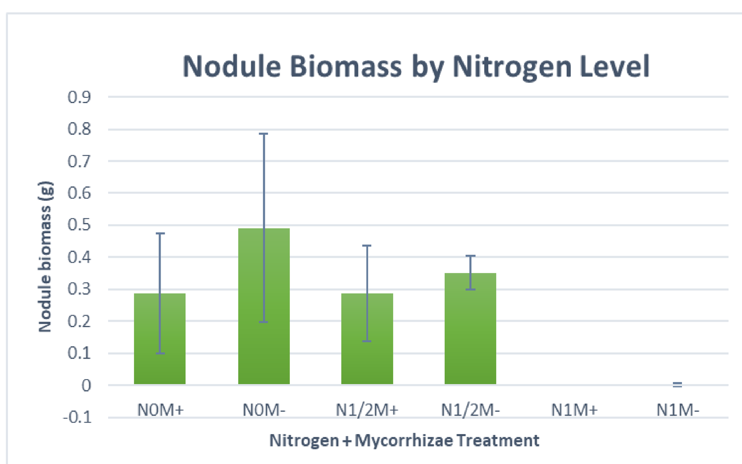
- **Micronutrient addition significantly increased nodule biomass** (p = 0.013)
- Mycorrhizae inoculation increased the average nodule biomass, but not significantly
- All 6 micronutrients (Mn, Cu, Zn, Mo, B, Co) were applied together in solution, therefore the source of the increase cannot be pinpointed in this experiment



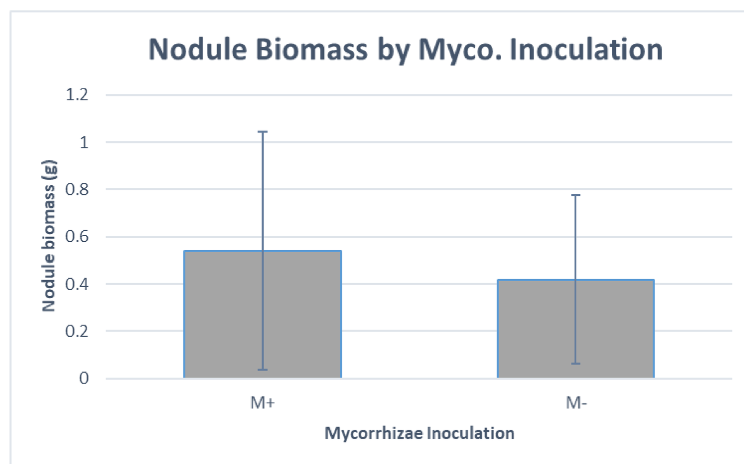
- Moisture level significantly impacted nodule biomass (p = 0.016)
- Cowpeas with the **highest moisture** (HiM+/M-) had **significantly higher nodule biomass** than cowpeas under the flood/drought cycle (FM+/M-) (p = 0.013)
- No significant difference between Hi and Mid treatments or between M+ and M-



- Applying phosphorus did not significantly increase nodule biomass (p = 0.497)
- Mycorrhizal inoculation also failed to increase nodule biomass (p = 0.134)
- Although there were concerns that soil P would be inaccessible in Hilltop's calcareous soils, this suggests that **phosphorus inaccessibility was not responsible for the nodulation failure**



- Nitrogen level significantly impacted nodule biomass (p < 0.001)
- Added nitrogen (N1M+/M-) completely inhibited nodulation
- However, there was no significant difference between field N (N0M+/M-) and ½ field N (N1/2M+/M-)
- Therefore, **excess nitrogen is an unlikely explanation for observed nodulation failures**



- Mycorrhizal inoculation was tested as a potential intervention to improve nodulation However, adding mycorrhizal spores did not significantly increase nodule biomass in any of the four components of this experiment
- The data suggest that **mycorrhizae are not an appropriate strategy** for improving nodulation and nitrogen fixation under these conditions.

DISCUSSION

Effective biological nitrogen fixation depends on the success of the legume-rhizobia symbiosis. The mutualism between these two species is ecologically complex, and there are many pitfalls where trouble can arise. This research looked at a few of the most promising factors in the mystery of the Hilltop nodulation failure hoping to winnow the list down to a few prime suspects. This research strongly suggests that phosphorus inaccessibility and excess nitrogen were not responsible for the nodulation failure in Winter 2017, nor is mycorrhizae an effective intervention to improve nodulation results, at least not with brand of inoculant and inoculation method employed.

In contrast micronutrients and moisture were confirmed as major determinants of nodule biomass in this agroecosystem. Nodules are the first to lose their water supply when a plant enters a period of water stress and nitrogen fixation rates are more sensitive to drought than plant growth (Serraj et al. 1999). However, in a non-irrigated setting like Hilltop, not much can be done to improve legume outcomes. Micronutrient deficiencies, on the other hand, could be addressed through targeted amendments if the responsible micronutrient were isolated.

FUTURE WORK

Biological nitrogen fixation is a powerful tool for sustainable agriculture and deserves our attention to ensure that it is implemented as effectively as possible. Many questions remain about how to best maximize legume efficiency in South Texas. Follow-up work is planned for the following:

- Of the 6 micronutrients applied in this experiment, which are most responsible for observed increases in nodulation? How could micronutrient deficiencies best be rectified, especially in organic fields?
- Liquid inoculant applied directly to plant roots showed much higher nodulation rates than cowpeas in the field at Hilltop where dry inoculant was applied (93% in greenhouse + vs 38% in field). How can field inoculation techniques be improved to maximize inoculant-seed contact? Could sticking solutions like gum Arabic or mesquite gum be an effective addition to the inoculation protocol?

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