

# Effects of Rhizobial Co-infection on Nodule Occupancy and Plant Fitness

John Mulcahy, R. Ford Denison

Department of Evolution, Ecology, & Behavior, University of Minnesota College of Biological Sciences

## Background

Plants of the legume (Fabaceae) family rely on symbiotic interactions with nitrogen fixing rhizobia to provide them with a sufficient supply of biologically available nitrogen. There is a large amount of diversity among different rhizobial strains in their nitrogen fixing efficiency (how much nitrogen is fixed per unit of plant supplied carbon.)<sup>[5]</sup>. Many plants have the ability to place fitness-reducing sanctions on less efficient rhizobia that “cheat” in the mutualistic relationship between the two organisms<sup>[1],[2],[4],[5]</sup>, however these cheating strains still persist in nature. It has been proposed that ineffective strains were able to evolve in environments where nitrogen is readily available, such as agricultural settings, making legumes less dependent on their rhizobial partners<sup>[3]</sup>. However it has also been noted that much of the experimentation that has calculated the nitrogen fixing efficiency of rhizobial strains has been done in single inoculation scenarios, whereas plants are often infected with several different strains in nature<sup>[3]</sup>. Co-infection with multiple strains could result in increased or decreased nitrogen fixing efficiency due to interactions between strains, different rates of nodulation, or differential plant response to co-infection. This led me to investigate how co-infection affects nodule occupancy and plant fitness.

## Experiment Design

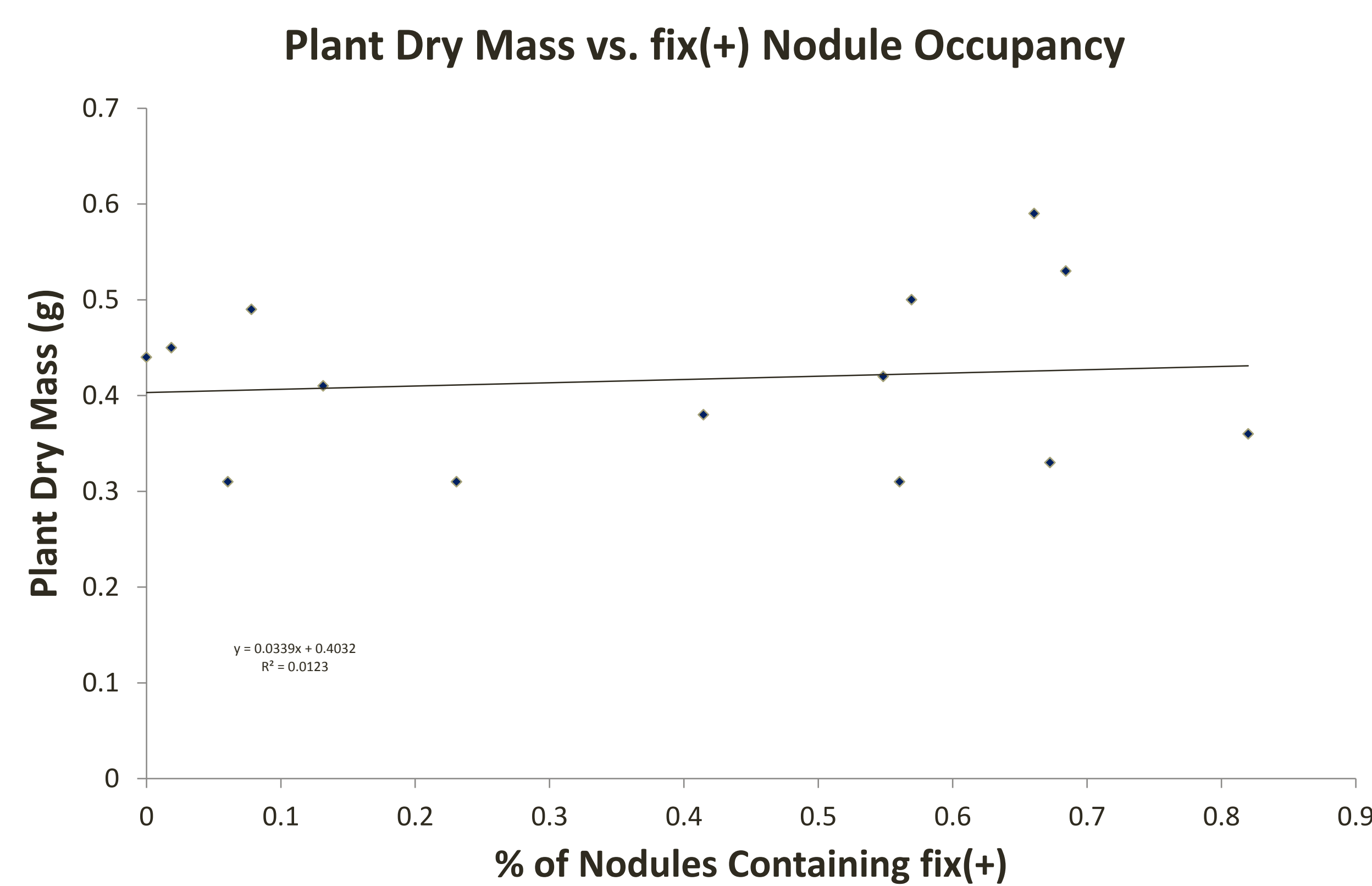
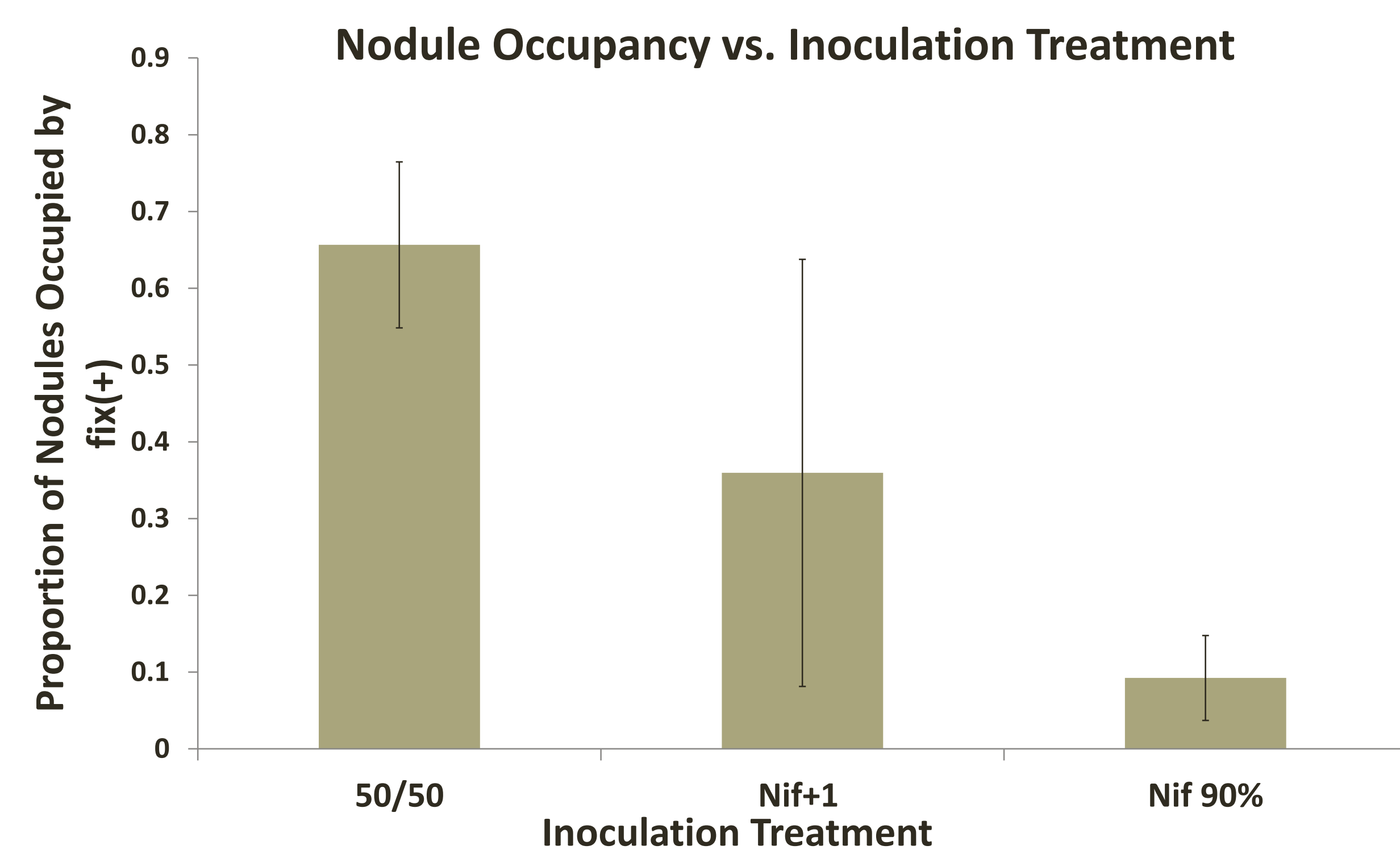
- Soy Bean (*Glycine max*) used as host plant.
- 2 different *B. japonicum* Rhizobia strains used for co-infection.

**fix(-) (nif110):** knockout mutant, cannot fix N

**(fix(+)) (dsRed):** Red fluorescence gene, actively fixes N

- Three different inoculation treatments were used to create a range of nodule occupancy
  - Treatment 1:** Plants inoculated with 1:1 strain ratio on same day.
  - Treatment 2:** Plants inoculated with 1:1 strain ratio, fix(-) cells added 1 day early.
  - Treatment 3:** Plants inoculated with a 9:1 fix(-):fix(+) ratio, fix(-) cells added 2 days early.
- All Plants inoculated with a total of  $\approx 5 \times 10^6$  cells.
- 5 Plants in each treatment group.
- Plant dry mass was used as a measure of plant fitness and regressed against % of nodules containing fix(-).

## Results



## Conclusions

- 50/50 and Nif 90% treatments produced fairly consistent nodule occupancy. Nif + 1 treatment did not.
- No significant relationship between percent of nodules containing fix(+) and plant dry mass. Possibly due to variable plant size at inoculation. Further research on how nodule occupancy affects plant fitness is needed.

## Methods

- Plants were grown in hydroponically in growth pouches.
- Nodules were labeled and dated on pouch as nodules formed.
- After Nodulation ceased, nodules were removed, placed in separate wells, crushed, and plated using a 48 well replicator.
- Red fluorescence was used to detect the presence of fix(+).



## References

1. Heath, K. D. & Tiffin P. 2007. Context dependence in the coevolution of plant and rhizobial mutualists. *Proc. R. Soc.* 274, 1905-1915.
2. Kiers, T. E., Hutton, M. G., & Denison F. R. 2007. Human selection and the relaxation of legume defences against ineffective rhizobia. *Proc. R. Soc. B.* 274, 3119-3126.
3. Kiers, T. E., Ratcliff W. C., & Denison, F. R. 2012. Single strain inoculation may create spurious correlations between legume fitness and rhizobial fitness. *New Phytologist* 198, 4-6.
4. Oono, R., & Denison F. R. 2010. Comparing Symbiotic Efficiency between Swollen versus Nonswollen Rhizobial Bacteroids. *Plant Physiology* 154, 1541-1548.
5. Oono, R., Denison F. R., & Kiers T. E. 2009. Controlling the reproductive fate of rhizobia: how universal are legume sanctions? *New Phytologist*, 1-13.
6. Somasegaran, P. & Böhlool, B.B. 1990. Single-Strain versus Multistrain Inoculation: Effect of Soil Mineral N Availability on Rhizobial Strain Effectiveness and Competition for Nodulation on Chick-Pea, Soybean, and Dry Bean. *Applied and Environmental Microbiology* 56, 3298-3303.