# Nodulation of Pea Plants by Two *Rhizobium leguminosarum* Biovars

By: Chelsea Cereghino

Advisor: Dr. Mark Williams

# Background

- The crop yield demand in the United States is high
- Farmers apply inorganic nitrogen fertilizer to supplement soil
- About 167 million acres of major crops receive inorganic nitrogen fertilizer[1]
  - Excess is commonly applied
- Growing population will increase crop yield demand

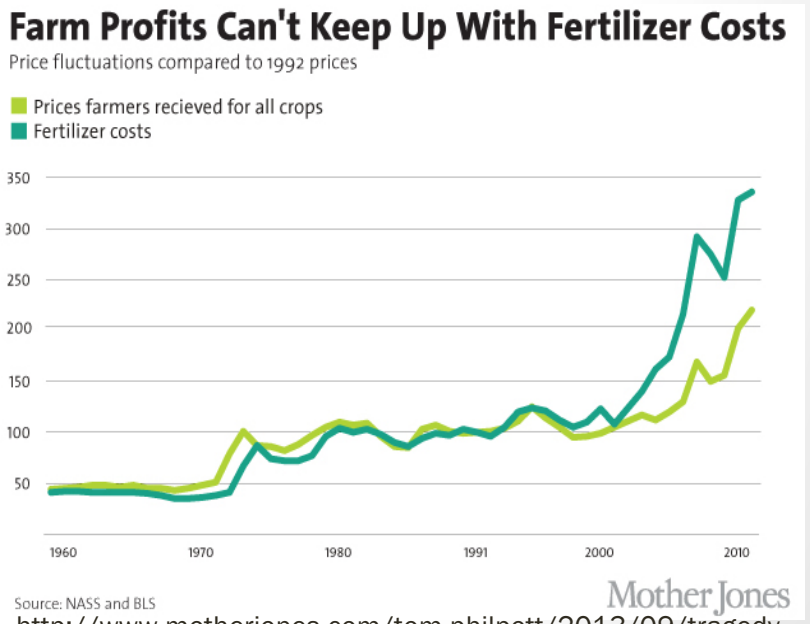
http://www.keyword-suggestions.com/Y3JvcA/

# Negative Impacts of Fertilizer Use

- Environmental concern
- Human health concern
- Costly to farmers

http://www.aquascapeinc.com/blogs/ed-beaulieu/how-to-stop-or-reverse-aging-ponds

https://hengsuliblog.wordpress.com/2015/03/06/monoculture-farming-part-2-pollution-and-health-impacts-2/




http://www.motherjones.com/tom-philpott/2013/09/tragedy-industrial-farming-charts

# The Solution

- Nitrogen fixers
- Rhizobia-legume symbiosis
- Legumes are selective in which rhizobia can nodulate them
- Legumes can give their nitrogen back to the environment

http://bio.vtn2.com/biohome/harvey/lect/lectures.html?flnm=mspg5&ttl=Introduction&ccode=rm&mda=prnt

# Purpose

- Amplify the quantity of nitrogen fixed by the rhizobia-legume relationship
  - Determine a species of rhizobia that is more efficient at fixing nitrogen
  - Future use of more efficient rhizobia for genetic engineering
- Use pea plants (*Pisum sativum*, *L*.) and bacteria that typically nodulate peas (*Rhizobium leguminosarum* biovar *vicaea*)[2,3]
- Use white clover plants (*Trifolium repens*, *L*.) and bacteria that typically nodulate white clover (*Rhizobium leguminosarum* biovar *trifolii*)[4,5]

# Hypotheses

- Two *Rhizobium leguminosarum* biovars, *trifolii* and *vicaea*, will promote growth in plant mass differently when in a symbiotic relationship with clover and pea, respectively.

- The relative growth promotion of biovar *vicaea* for pea will be greater than that of biovar *trifolii* for clover.

# Experimental Design

| Plant Type | Treatment | | | |
|---|---|---|---|---|
| | Without Nitrogen | | | With Nitrogen |
| Pea | 10 plants inoculated with *R. leguminosarum* biovar *vicaea* | 10 plants inoculated with *R. leguminosarum* biovar *trifolii* | 10 plants without bacteria | 10 plants without bacteria |
| White Clover | 10 plants inoculated with *R. leguminosarum* biovar *vicaea* | 10 plants inoculated with *R. leguminosarum* biovar *trifolii* | 10 plants without bacteria | 10 plants without bacteria |

# Methods

1. 40 clover seeds and 40 pea seeds were sterilized and germinated on water agar for 2 weeks
2. The seedlings were placed in small jars containing Thornton agar
   - 1 clover plant and 1 pea plant in each jar; 40 jars total
   - 10 jars were supplemented with nitrogen
3. Jars were placed under light (16 hr photoperiod) in a controlled environment for 6 days
4. 10 jars without nitrogen were inoculated with log phases cultures of vicaea, and 10 jars without nitrogen were inoculated with log phase cultures of trifolii

# Methods (Continued)

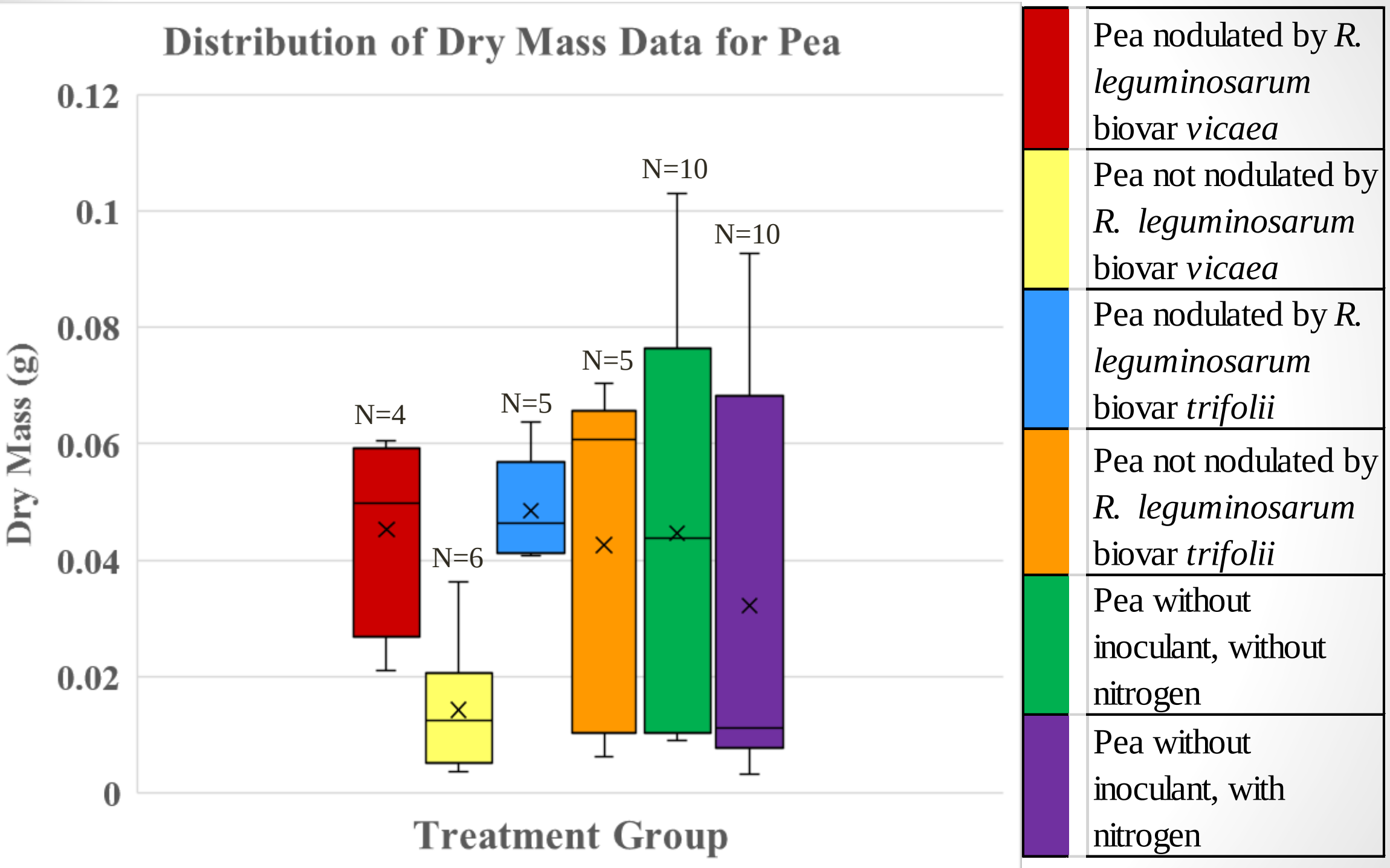
5. All plants were grown for 21 days
6. Nodule count number on root was recorded
7. Plants were dried in an oven for 24 hours at 80°C and then for 13 hours at 65°C
8. Mean dry mass was measured for each plant

# Results

| Plant Type | Treatment | | | |
|---|---|---|---|---|
| | Without Nitrogen | | | With Nitrogen |
| Pea | 10 plants inoculated with *R. leguminosarum* biovar *vicaea;* **NODULATION** | 10 plants inoculated with *R. leguminosarum* biovar *trifolii;* **NODULATION** | 10 plants without bacteria | 10 plants without bacteria |
| White Clover | 10 plants inoculated with *R. leguminosarum* biovar *vicaea;* **NO NODULATION** | 10 plants inoculated with *R. leguminosarum* biovar *trifolii;* **NO NODULATION** | 10 plants without bacteria | 10 plants without bacteria |

# Results (Continued)

- 4 of 10 pea plants inoculated with vicaea were nodulated
- 5 of 10 pea plants inoculated with trifolii were nodulated


Distribution of Dry Mass Data for Pea
0.12
0.1
0.08
0.06
0.04
0.02
0
Dry Mass (g)
N=4
N=6
N=5
N=5
N=10
N=10
Treatment Group
Pea nodulated by R. leguminosarum biovar vicaea
Pea not nodulated by R. leguminosarum biovar vicaea
Pea nodulated by R. leguminosarum biovar trifolii
Pea not nodulated by R. leguminosarum biovar trifolii
Pea without inoculant, without nitrogen
Pea without inoculant, with nitrogen

# Discussion

- Hypothesis could not be supported based on lack of clover nodulation
- Vicaea and trifolii fix nitrogen differently
- Evolution of biovars for greater host-range
- Pea may have more “options” for nitrogen fixation
- Stressful growth conditions could have induced a more antagonistic relationship of the biovars with pea
- Eliminate possible confounding variables for a more beneficial relationship
- Genetically engineer more efficient biovar for a wider host-range

# References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3. Miller, S. H.; Elliot, R. M.; Sullivan, J. T.; Ronson, C. W., Host-specific Regulation of Symbiotic Nitrogen Fixation in *Rhizobium leguminosarum* bv. *trifolii*. *Microbiology.* **2007**, *153*, 3184-3195.
4. Roest, H. P.; Mulders, I. H. M.; Spaink, H. P.; Wijffelman, C. A.; Lugtenberg, B. J. J., A *Rhizobium leguminosarum* Biovar *trifolii* Locus Not Localized on the Sym Plasmid Hinders Effective Nodulation on Plants of the Pea Cross-Inoculation Group. *Molecular Plant-Microbe Interactions.* **1997**, *10*(7), 938-941.
5. Karunakaran, R.; Ramachandran, V. K.; Seman, J. C.; East, A. K.; Mouhsine, B.; Mauchline, T. H.; Prell, J.; Skeffington, A.; Poole, P.S., Transcriptomic Analysis of *Rhizobium leguminosarum* Biovar vicaea in Symbiosis with Host Plants Pisum sativum and Vicia cracca. *Journal of Bacteriology*. **2009**, *191*(12), 4002-4014.

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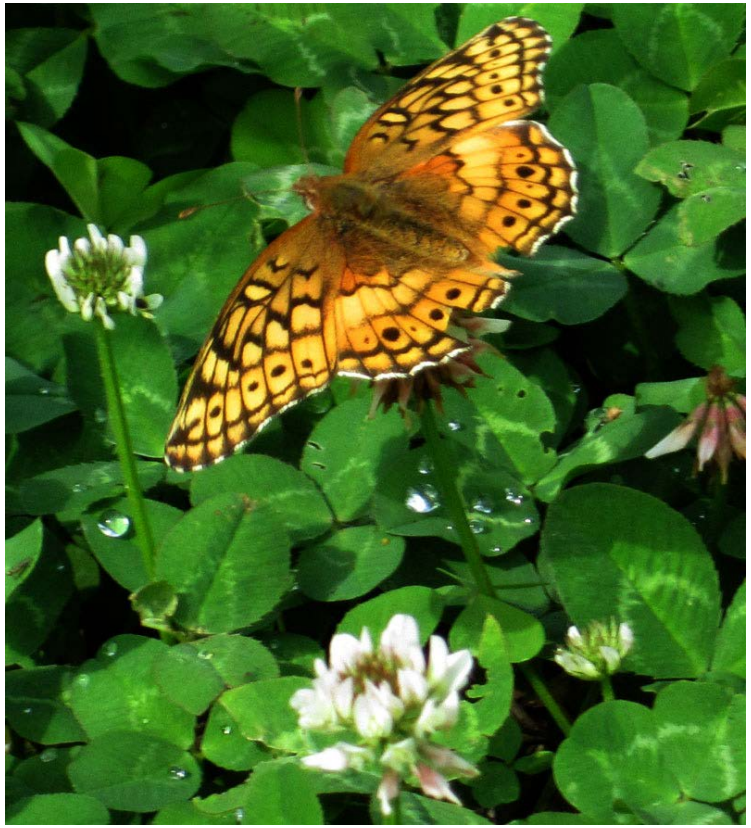
# Questions?



https://butterflygardening.wordpress.com/2012/04/30/the-clover-butterfly-garden/