Effect of Inoculation on Nodule Occupation and Rhizobia Diversity of Cover Crop Legumes

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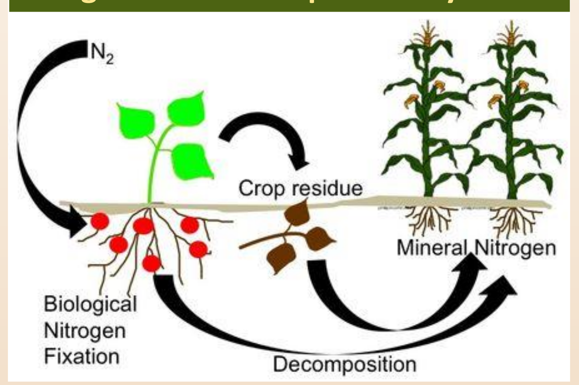
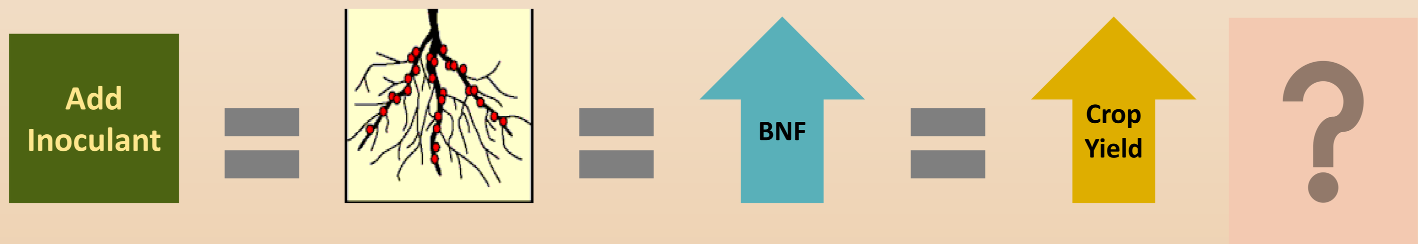
Introduction

Legume Cover Crop Based System



- Biologically fixed nitrogen (BNF) fertility in the form of legume cover crops is of particular importance on organic farms where applications of chemical fertilizers are prohibited.
- Inoculation is the practice of adding compatible rhizobia to a legume host.
- Despite the ecological and economic benefits of legume cover crops, little is known about the effectiveness of inoculant strains to occupy the nodules of their intended cover crop host when introduced on organic farms in North Carolina.



Adding commercial inoculant to seed

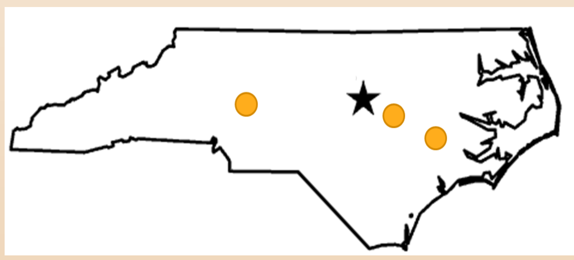
Objectives

1. To further our understanding of how inoculation directly affects the growth of legume cover crops of local interest.
2. To determine if inoculant strains occupy nodules of inoculated cover crops through the use of genetic screening techniques.

Experimental Design

Three Organic Farm Sites

Salisbury Clayton Kinston



Four Cover Crops

Austrian Winter Pea *Pisium sativa*

Hairy Vetch *Vicia villosa* R.

Woolypod *Vicia villosa* R.

Crimson Clover *Trifolium incarnatum*

+ Inoculant - Inoculant

- Randomized split plot design
- Four replicated plots per site
- Main plot cover crop
- Subplot [+ Inoc or – Inoc]

Methods

MPN

Pre-plant soil samples were diluted in stepwise series for Most Probable Number (MPN) enumeration of resident soil rhizobia populations.

Nodules

In early spring, 3 plants per treatment were harvested for nodule count and weight per plant.

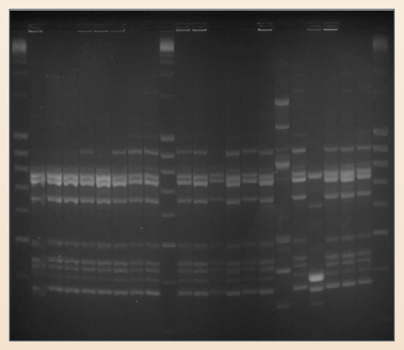
Biomass Harvest

In late spring, ½ m quadrat above ground biomass was harvested per treatment for dry weight. Plants were ground for % nitrogen analysis.

Rhizobia Isolation

Two nodules per plant were surface sterilized and cultured onto selective media (YMA) to isolate single rhizobia colonies.

Rep-PCR

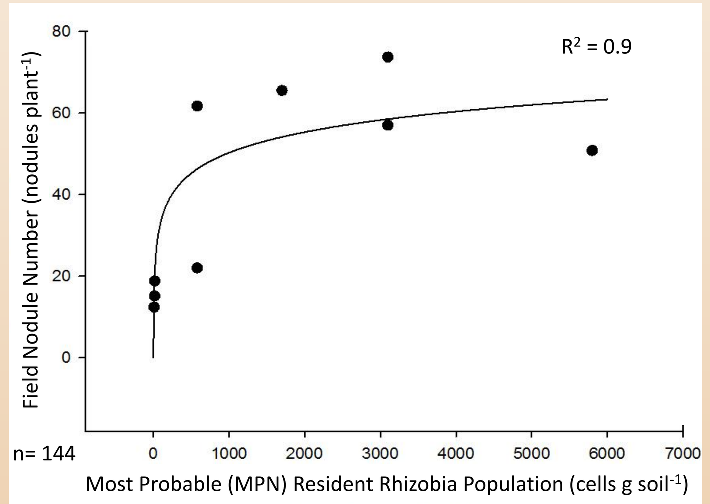


487 nodule strains were amplified with rep-PCR BOX primer. Individual DNA fingerprints were analyzed for genetic similarities using Bionumerics Gel Compar II Software.

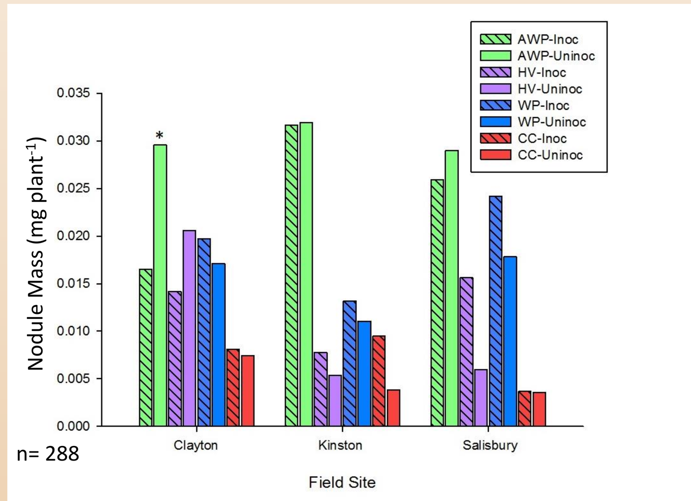
Results Objective 1: Is Inoculant Effective?

- Overall, inoculation did not increase nodule number or nodule mass per plant, nor did it increase cover crop biomass, biomass nitrogen, or %N of any tested cover crop species.
- Population size of compatible resident rhizobia was the main driver of nodule number per cover crop plant.

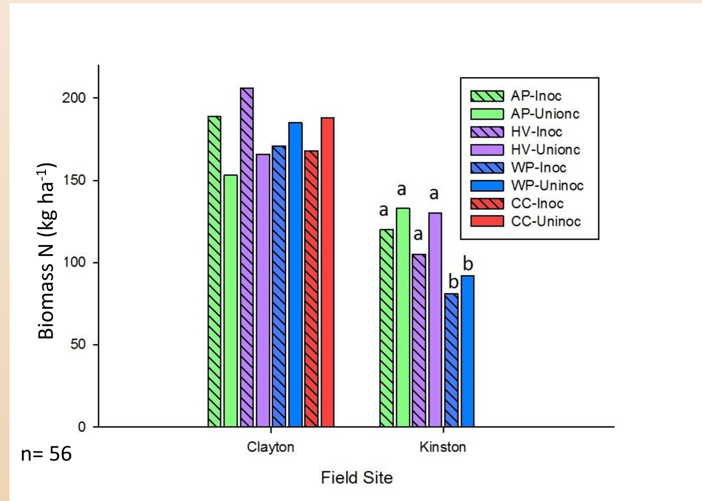
Effect of Resident Rhizobia Population Size on Nodule Number



Cover Crop Nodule Mass



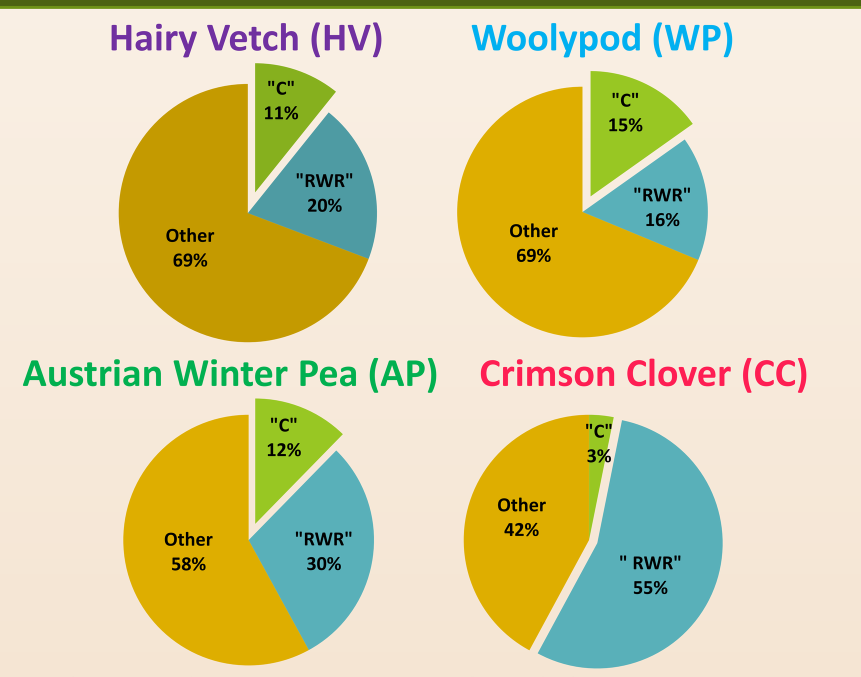
Effect of Rhizobia Inoculation on Cover Crop Biomass Nitrogen



Results Objective 2: Who Is In The Nodule?

- Genetic analysis revealed 13 distinct DNA fingerprint patterns.
- Only 4% of nodule occupants had DNA patterns genetically similar to that of the commercial inoculant strains.
- No nodule strains selected for *nodC* gene sequencing were genetically identical to the commercial inoculant strains.
- No inoculant strains were found in nodules.

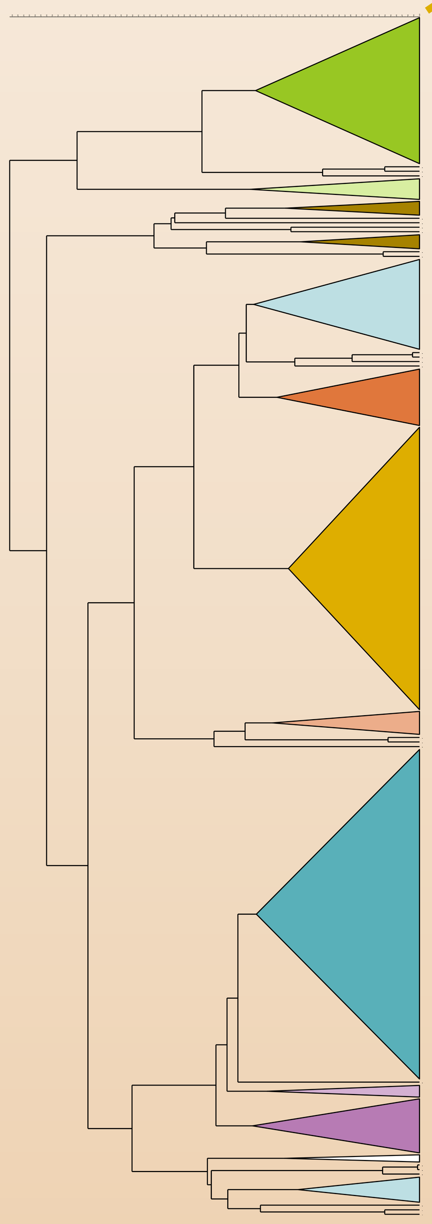
Nodule Occupant Genetic Identity



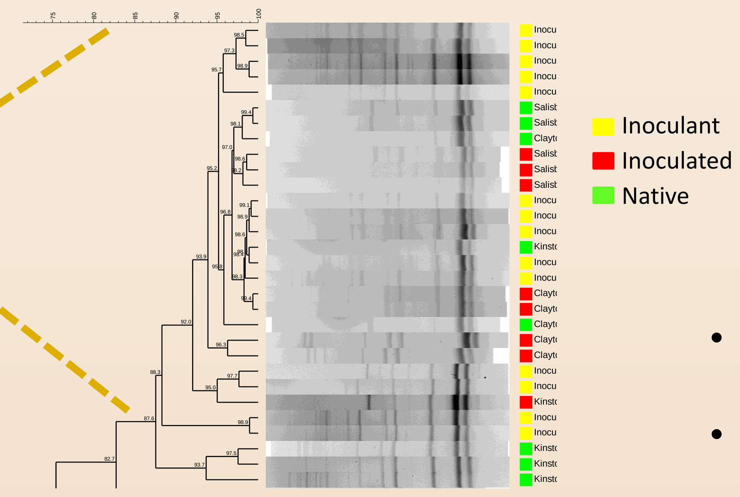
- Vetches and Peas: 11-15% of nodule occupants were genetically similar to strains of their intended inoculant.
- Clovers: 55% of nodule occupants were genetically similar to strains of their intended inoculant.

BOX-PCR Cluster Analysis

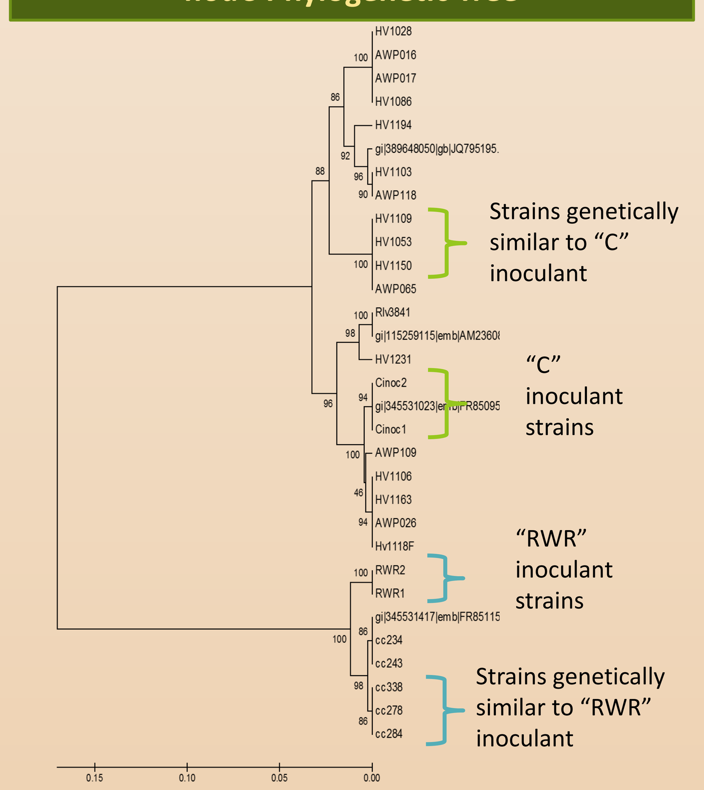
- Colored triangles indicate clusters of strains genetically similar (< 70% fingerprint similarity)



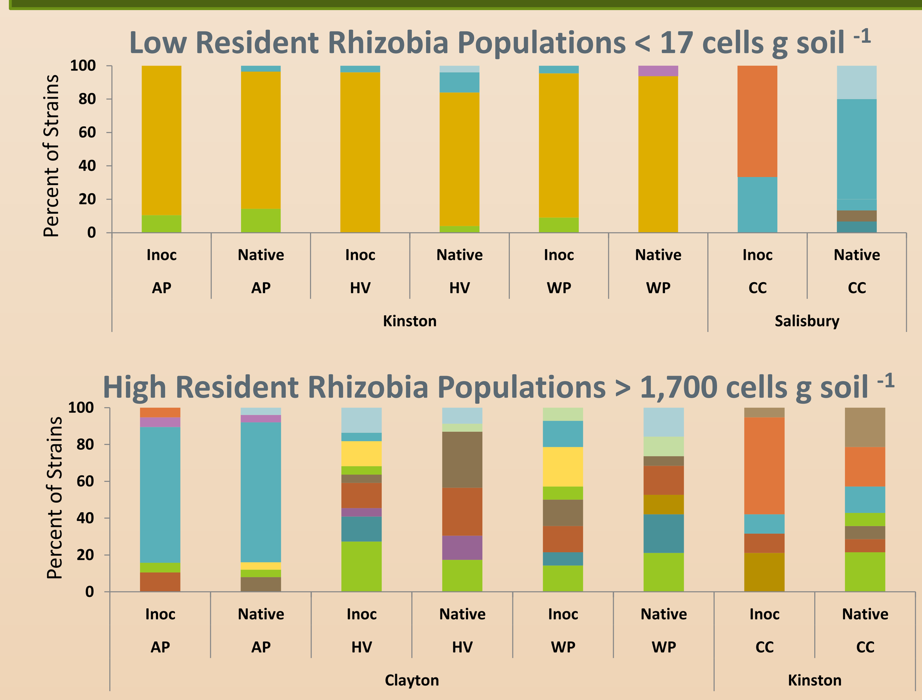
Cluster Including Vetch and Pea Inoculant "C"



***nodC* Phylogenetic Tree**



Diversity of Rhizobia Nodule Occupants from BOX PCR Clusters



- Soils containing large population sizes displayed greater rhizobia diversity.

Conclusions

- Inoculation had no effect on cover crops at these site locations.
- Native rhizobia strains outcompeted inoculants for nodule occupancy.
- Existing rhizobia can supply adequate nitrogen for crop needs – cover crop biomass nitrogen contributions ranged from 90 to 180 kg N ha^{-1}.
- The future success of BNF depends on high quality inoculants and an understanding of how they compete in a variety of soils.

Acknowledgments

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