Rhizobium leguminosarum strain combination effects on nodulation and biological nitrogen fixation in cover crop *Vicia villosa*

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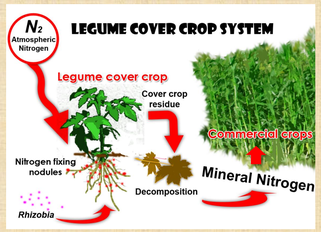
Background



- Cover crops are versatile groundcover that maintain soil stability, suppress weeds, and recycle nutrients. They are especially beneficial to organic producers who avoid synthetic fertilizers.
- Soil bacteria called rhizobia nodulate legume roots and fix atmospheric nitrogen into plant tissue.
- Rhizobia are used as inoculants to enhance biological nitrogen fixation in legume cover crops, but nodule occupancy by the applied inoculant is often challenged by competing native rhizobia.
- Using selected strains, the relationship between biological nitrogen fixation [BNF], nodulation, and nodule occupy was elucidated.

Objectives

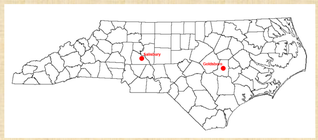
1. Determine the effect of *Rhizobium leguminosarum biovar viciae* strain combinations on biological nitrogen fixation and nodulation of *Vicia villosa*
2. Assess competitiveness for nodule occupancy amongst selected strains

Design and Methods

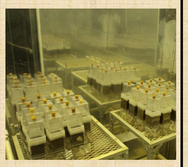
Treatment	Strains Used
1	None (Minus-N)
2	C10
3	NCSU332
4	NCSU 435
5	NCSU478
6	C10/332
7	C10/435
8	C10/478
9	332/435
10	332/478
11	435/478
12	C10/332/435
13	C10/332/478
14	C10/435/478
15	332/435/478
16	C10/332/435/478
17	None (NH_4NO_3)

- Three NC native *Rhizobium leguminosarum* strains and one commercial inoculant strain were used in 15 inoculant combinations.
- In previous studies, strain NCSU332 produced abundant nodules and stains NCSU435 and NCSU478 produced high levels of total plant N.
- Randomized block design with four repetitions per treatment including positive (N-fertilized) and negative (uninoculated).
- A total of 270 nodules were selected from 60 plants

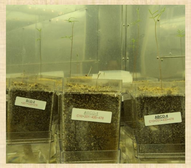
Number of Strains in inoculant	1	2	3	4
Nodules Analyzed from plant	3	6	8	10



Selected rhizobia strains were cultured on YM agar plates before incubating in TY nutrient solution.



Sand and vermiculite planter pots and vetch seeds were sterilized and transferred to a controlled growth chamber.

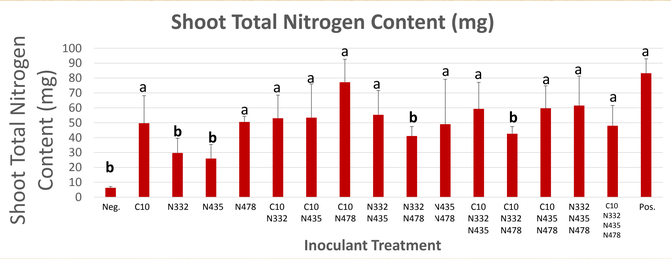


Vetch were inoculated with equal colony forming units of rhizobia strains (2×10^7 total) and grown for 46 days.

Nodules were removed from roots and dried in a desiccator. Shoots were dried and analyzed for N. BNF was determined using N-difference method.

PCR on nodule extracted DNA using *nodC* and BOX-A1R primers was used to identify strains found in nodules. DNA fingerprints were compared using Gel Comprar II software.

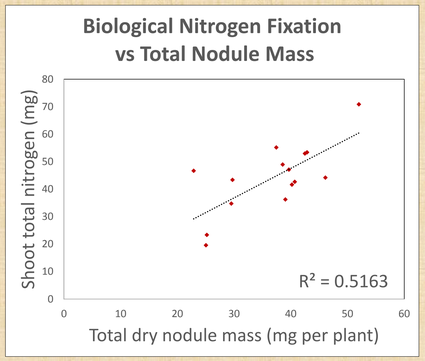
BNF and nodulation



11 of the 15 inoculant treatments contained plants with total shoot N at levels comparable to the fertilized N treatment.

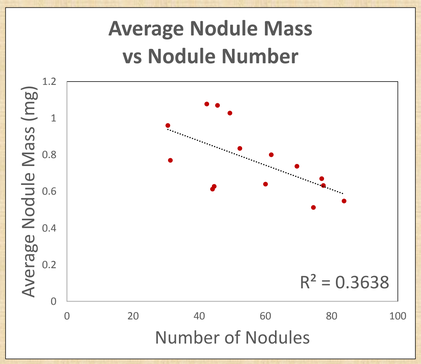
Total shoot nitrogen in treatment NCSU478 from BNF was comparable to total shoot nitrogen in N-fertilized control.

Treatment NCSU332/NCSU478 contained less total shoot N than NCSU478 alone. NCSU332 was found in 67% of selected nodules against NCSU478.



Inoculant treatments with more total shoot nitrogen had higher total nodule mass.

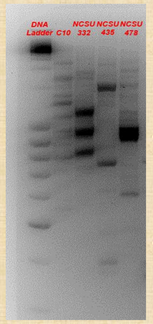
Inoculated root system with large nodules.



As nodule number increased, the average mass of nodules decreased, which indicated a limited pool of plant resources for nodule growth.

Inoculated root system with small nodules.

Nodule Occupancy



- C10, the commercial inoculant, was found in 67% of nodules against NCSU332, the strain previously exhibiting extensive nodulation, and appeared in 44% of nodules against the other strains.
- NCSU332 was found in 61% of nodules against NCSU478, which had generated higher levels of total plant N.
- NCSU478 was competitive for nodulation against C10, found in 56% of nodules, but was overall the lowest competitor for nodule occupancy.

Nodule occupancy of *V. villosa* roots as determined by BOX-A1R DNA fingerprints generated from 1 µL of nodule extract DNA in 24 µL of PCR reaction. Occupancy expressed as the percentage of selected nodules in which the strain was found for the treatment.

Treatment		C10 (%)	NCSU 332 (%)	NCSU 435 (%)	NCSU 478 (%)	Not Determined ** (%)	No Amplification (%)
	Strains						
2	C10	100	0	0	0	0	0
3	NCSU 332	11*	78	0	11*	0	0
4	NCSU 435	0	0	100	0	0	0
5	NCSU478	0	0	0	100	0	0
6	C10/332	67	33	0	0	0	0
7	C10/435	44	0	50	0	0	6
8	C10/478	44	0	0	56	0	0
9	332/435	0	28	67	0	0	6
10	332/478	0	61	0	33	0	6
11	435/478	0	0	83	17	0	0
12	C10/332/435	13	25	34	0	0	29
13	C10/332/478	17	21	4*	13	33	13
14	C10/435/478	8	9*	54	4	0	25
15	332/435/478	0	21	55	9	0	17
16	C10/332/435/478	27	17	23	7	17	10

*** Strains found outside of an intended treatment possibly due to growth chamber conditions.**
**** Nodule DNA that generated a fingerprint different from inoculant strains.**

- NCSU435 had the lowest total BNF per plant but was the most competitive strain for nodule occupancy. In two strain inoculants it was found in 50%, 67%, and 83% of nodules against C10, NCSU332, and NCSU478 respectively. NCSU435 was also the most frequent nodulator in three strain inoculants.
- Strain competitiveness varied more in four strain inoculants compared to inoculants with fewer strains.
- DNA amplification with BOX-A1R and *nodC* PCR primers was unsuccessful in 9.6% of nodules, which were considered to be unoccupied

Conclusions

- BNF of inoculated vetch is comparable to N-fertilized vetch for sufficient cover crop growth and nitrogen fertility management.
- Total nodule mass is the main factor for BNF regardless of the size distribution of nodules.
- BNF capacity is not closely linked to competitiveness
- Rhizobia strains competitive for nodulation are unlikely to remain competitive as rhizobia diversity increases in native soil conditions.

Acknowledgements

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