Soybean Host Control of Nodulation by Strains of *Bradyrhizobium*

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Background Information

- Soybean roots nodulate and form a nitrogen fixing symbiosis with "rhizobia" that are classified as *Bradyrhizobium japonicum* and *B. elkanii.* Bacterial induced chlorosis by *B. elkanii*
- Variability in the effectiveness of N2 fixation among *B. japonicum* strains and the competitiveness of *B. japonicum* in U.S. soils
- *B. japonicum* of serogroup USDA 123 occupy a large proportion of nodules of soybeans grown in northern Midwestern soils.
- N2 fixation and seed yield of serogroup USDA 123 strains versus other *B. japonicum*
- Research to use the soybean host to reduce nodulation by indigenous serocluster 123 *B. japonicum* strains

Background Information

Soybean roots nodulate and form a nitrogen fixing symbiosis with bacteria that are classified as *Bradyrhizobium japonicum* and *B. elkanii*

- **Jordan (1982): Renaming of the slow-growing Legume nodulating bacteria based upon growth rate in yeast extract-mannitol medium**

  The soybean nodulating bacteria previously referred to as "*Rhizobium japonicum*" were renamed "*Bradyrhizobium japonicum*"

- **Kuykendall *et al*. (1992): Based upon DNA homology, RFLP analysis and other traits divided the soybean *Bradyrhizobia* into two groups**

  Bradyrhizobium elkanii was the name given to a portion of the soybean-nodulating Bradyrhizobia stains. These are the stains that had previously been shown to produce *Rhizobium*-induced chlorosis on susceptible soybean genotypes.

Background Information

Bacterial-induced chlorosis by strains of *B. elkanii*

- **Erdman et al. (1957): *Rhizobium*-induced chlorosis produced by strains of *B. elkanii* seen in Southern U.S. soybean fields especially in the cultivar Lee.**

  "Seed yields are not measurably reduced" by Rhizobium-induced chlorosis

- **Johnson and Means (1960): *Rhizobium*-induced chlorosis varied in soybean genotype x *Rhizobium* strain combinations grown in GH and growth chamber trials**

  "In the presence of severe *Rhizobium*-induced chlorosis, the relationship between bacteria and host can hardly be regarded as symbiotic"

- **Fuhrmann (1990): 18% of *Bradyrhizobium* isolates from Delaware soils produced Rhizobitoxin chlorosis symptoms**

  In a greenhouse pot experiment plant N content significantly lower with Rhizobitoxin-producing strains

- **Teaney and Fuhrmann (1992): In the absence of discernable foliar chlorosis, the effect of nodulation by Rhizobitoxin-producing *Bradyrhizobium* strains in minimal**

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Background Information

Variability in the effectiveness of N2 fixation has been reported among strains of *B. japonicum*

- **Abel and Erdman (1964): The seed yield of "Lee" soybean grown on *B. japonicum*-free soil with 23 *B. japonicum* inoculation treatments and an uninoculated control**
  - Seed yields of inoculation treatments ranged from 1564.9 kg/ha to 2968.4 kg/ha (USDA Strain 110). Uninoculated control yielded 1477.5 kg/ha
- **Caldwell and Vest (1970): Yield trails were conducted for three years on *B. japonicum* free soil using 28 *B. japonicum* strains and two commercial inoculum treatments**
  - Significant yield differences were found between strains. No significant strain x cultivar interactions were detected.
- **Ham (1980) and Chamber *et al.* (1983): Yield tests conducted on *B. japonicum*-free soil with inoculation with various *B. japonicum* strains**
  - Inoculent quality strains such as USDA 110 and USDA 138 produced superior seed yields.

Background Information

The competitiveness of soybean nodulating *Bradyrhizobium* in U.S. soils and nodulation by inoculant strains

- **Johnson, et al. (1965): Inoculated Maryland and Iowa field plots with 25, 100, 200, 400 and 800 times the normal inoculation rate + other additional treatments**

  High levels of inoculation did sometimes increase the level of inoculant strains in nodules but standard inoculation rates did not

- **Weaver and Frederick (1974): Inoculated Iowa field plots with 1.0 ml per cm of row using liquid cultures with up to 3 x 108 cells/ml of *B. japonicum* USDA 138:**

  "If the inoculum rhizobia are to form 50% or more of nodules an inoculum rate of at least 1,000 times the soil population (per g soil) must be used"

- **Kvien et al. (1981): Examined the competitiveness of an applied inoculum strain against the indigenous *Bradyrhizobium* at Minnesota field sites in which serotype strains USDA 123 predominated**

  Highly significant soybean genotype differences in the recovery of inoculant strain USDA 110 as well as large differences in recovery between field sites and years

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Background Information

B. japonicum of serogroup USDA 123 occupy a large proportion of nodules of soybeans grown in northern Midwestern soils

- **Damirgi et al. (1967): Determined the serotype of *B. japonicum* in the nodules of soybeans collected from various soil types "in widely separated areas of Iowa"**
  - Nodules obtained were in one of four serogroups (USDA 123, 135, 31 and 3). Serogroup 123 was dominant averaging 52% across soil types.
- **Ellis et al. (1984): Used *B. japonicum* inoculation rates up to 50 times the normal rate and determined the % of nodules with inoculant strain vs. indigenous serogroup 123**
  - 93.2% of nodules were occupied with the indigenous serogroup 123 strain.
- **Moawad et al. (1984): Measured rhizosphere densities of serogroup USDA 110, 123 and 138 and nodule occupancy by these serogroup strains on 3 soybean genotypes**
  - "Although serogroup 123 gave no evidence of dominance in early host rhizospheres it clearly dominated in nodule composition, occupying 60 to 100% of the nodules."

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Background Information

Nodulation by indigenous *B. japonicum* of serogroup USDA 123 does not provide highly effective N2 fixation or the highest seed yields

- **Caldwell and Vest 1970: 28 *B. japonicum* strains and 2 commercial inocula used in replicated yield trials on *B. japonicum*-free soil for 3 years**

  The yield of cultivars inoculated with USDA 123 ranked 16th among the 30 strains.

- **Ham et al. (1980): Seed yield of three soybean cultivars grown on *B. japonicim*-free soil with 7 *B. japonicum* strains**

  Seed yield with USDA 123 ranked last among the 7 strains.

- **Kvien et al. (1981): Yield trials with 12 genotypes conducted on typical Minnesota soils with and without inoculation. Nodule occupancy determined for all treatments.**

  In the uninoculated treatments 95% of the nodules were occupied with serogroup 123 strains. "The 12 soybean lines responded with yield increases whenever 50% or more of the nodules were formed by the inoculant strains. "

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Background Information

Could the genetics of the soybean host be manipulated to stop nodulation by indigenous serogroup 123 *B. japonicum*?

- **Caldwell (1966): Reported the single dominant gene *Rj*2 that restricts the nodulation of all available strains of the USDA 122 and c1 serogroups.**
- **Vest (1970): Reported the single dominant gene *Rj*3 that restricts the nodulation of strain USDA 33 but no other strains that are serologically related.**
- **Vest and Caldwell (1972): Reported the single dominant gene *Rj*4 that restricts nodulation with strain USDA 61.**

Does a soybean genotype exist that would restrict nodulation by strains of indigenous serogroup USDA 123 while allowing normal nodulation and N2 fixation with other more effective strains?

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Background Information

Identification of soybean genotypes that restrict the nodulation of strain USDA 123 and other serologically related strains

- **Cregan and Keyser (1986): 1287 soybean cultivars and Plant Introductions were screened in the greenhouse for nodulation with strain USDA 123.**
  - Two genotypes (PI371607 and PI377578) were identified that restricted the nodulation of strain USDA 123.
  - In competition studies with USDA 123 vs. inoculant quality strains, greater than 75% of the nodules on the control genotype Williams were occupied with USDA 123 while less than 10% of the nodules of the PI genotypes contained USDA 123.
- **Keyser and Cregan (1987): 20 field isolates of serogroup 123 strains tested for nodulation of the two USDA 123-restricting PI genotypes and Williams**
  - The nodulation of only 4 of the field isolates were restricted by the PI genotypes these were serogroup 123 strains.
  - The strains that were not restricted were determined to be USDA 127 and USDA 129 serotypes – members of “serocluster 123”(as defined by Schmidt et al. (1986).

Background Information

Identification of soybean genotypes that restrict the nodulation of previously unrestricted "serocluster" 123 *B. japonicum* strains

- **Cregan, Keyser & Sadowsky (1989): 850 soybean cultivars and Plant Introductions were screened in the greenhouse for nodulation with serocluster 123 strain MN1-1c (serotype 127).**
  - Two genotypes (PI417566 and PI283326) were identified that restricted nodulation of strain MN1-1c.
  - The PI genotypes also restricted the nodulation of inoculant quality strains USDA 110 and USDA 138. PI283326 restricted the nodulation of a number of other strains.
  - In competition studies with MN1-1c vs. inoculant quality strain CB1809, over 90% of the nodules on the control genotype , Williams, were occupied with MN1-1c while less than 10% with CB1809. 70% of the nodules on PI417566 were occupied by CB1809.

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Background Information

Observations and questions raised by previous research regarding soybean host controlled strain-specific nodulation restriction of *Bradyrhizobium* strains

- There are apparently a number or perhaps many different genes that control strain specific nodulation restriction: *Rj*2, *Rj*3, *Rj*4, strain USDA 123 specific nodulation restriction, strain MN1-c specific nodulation restriction
- Is there really any relationship between *B. japonicum* serology and nodulation restriction?
- What strains/serotypes actually form nodules when genotypes with various nodulation restricting characteristics are grown in soils in which soybeans are normally grown?

Multilocus Sequence Typing (MLST) to Identify Genotypes of *Bradyrhizobium* and to Identify Nodule Occupants

van Berkum *et al*. 2012. MPMI 25:321-330

- **Based upon the sequence analysis of seven chromosomal loci in 190 soybean nodulating strains as well as Rhizobia and Bradyrhizobia of other legumes from the USDA National Rhizobium Resource Collection**
- **The seven chromosomal loci: *asd, gapA, gyrB, ilvI, lepA, mdh and purC***
- **The sequence of an average of 425 bp from each of 7 chromosomal genes are used to define the allele present in each *Bradyrhizobium* strain**
- **A combination of the alleles for each of the seven gene fragments is used to define the allelic profile or "GT" of each strain**

Multi-location Field Trials to Analyze Soybean Host Control of Nodulation by Strains of *Bradyrhizobium*

- **Four locations: Illinois, North Carolina and two Maryland locations (Beltsville and Princess Anne)**
- **Five soybean genotypes: Williams 82, Bragg, PI 371607, Peking and Evans in Randomized Complete Block experiments with 4 replications**
- **The root systems of 4 plants from each rep removed from the soil at 60 days after emergence and washed to remove soil, stored frozen and sent to Beltsville.**
- **Nodules removed from plants and bacteroids recovered from a random sample of 96 nodules per genotype-rep combination**
- **Genomic DNA isolated from each bacteroid and MLST applied based upon the sequence analysis of gene fragments from *gapA*, *gyrB* and *mdh***

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Nodule occupancy of *B. japonicum* vs. *B. elkanii*

Highly significant (< 0.0001) differences in the frequencies of *B. japonicum* and *B. elkanii* at the three locations:

	% Nodule Occupancy		
	Beltsville	Illinois	N. Carolina
B. japonicum	78.8 a	85.8 a	28.7 b
B. elkanii	21.2 b	14.2 b	71.3 a

No significant differences among genotypes in the frequencies of *B. japonicum* and *B. elkanii* at the three locations:

	B. japonicum % Nodule Occupancy			*B. elkanii* % Nodule Occupancy		
	Beltsville	Illinois	N. Carolina	Beltsville	Illinois	N. Carolina
Williams 82	84.9 a	89.5 a	31.1 a	15.1 a	10.5 a	68.9 a
Bragg	81.6 a	88.4 a	25.5 a	18.4 a	11.6 a	74.5 a
PI 371607	69.8 a	79.5 a	29.6 a	30.2 a	20.5 a	70.4 a

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Nodule occupancy of *B. japonicum* strains at the Beltsville and Illinois locations

- There were no PI 371607 nodules occupied by *B. japonicum* USDA 123.
- No significant differences among genotypes for nodule occupancy by inoculant quality strains USDA 110 or USDA

	Beltsville			Illinois		
	% of *B. japonicum* nodules occupied by			% of *B. japonicum* nodules occupied by		
	USDA 123	USDA 110	USDA 6	USDA 123	USDA 110	USDA 6
Williams 82	4.9 a	44.2 a	17.8 a	26.0 a	38.0 a	26.6 a
Bragg	2.6 a	72.9 a	20.6 a	33.3 a	33.3 a	36.3 a
PI 371607	0.0 a	74.7 a	6.3 a	0.0 b	53.8 a	12.0 a

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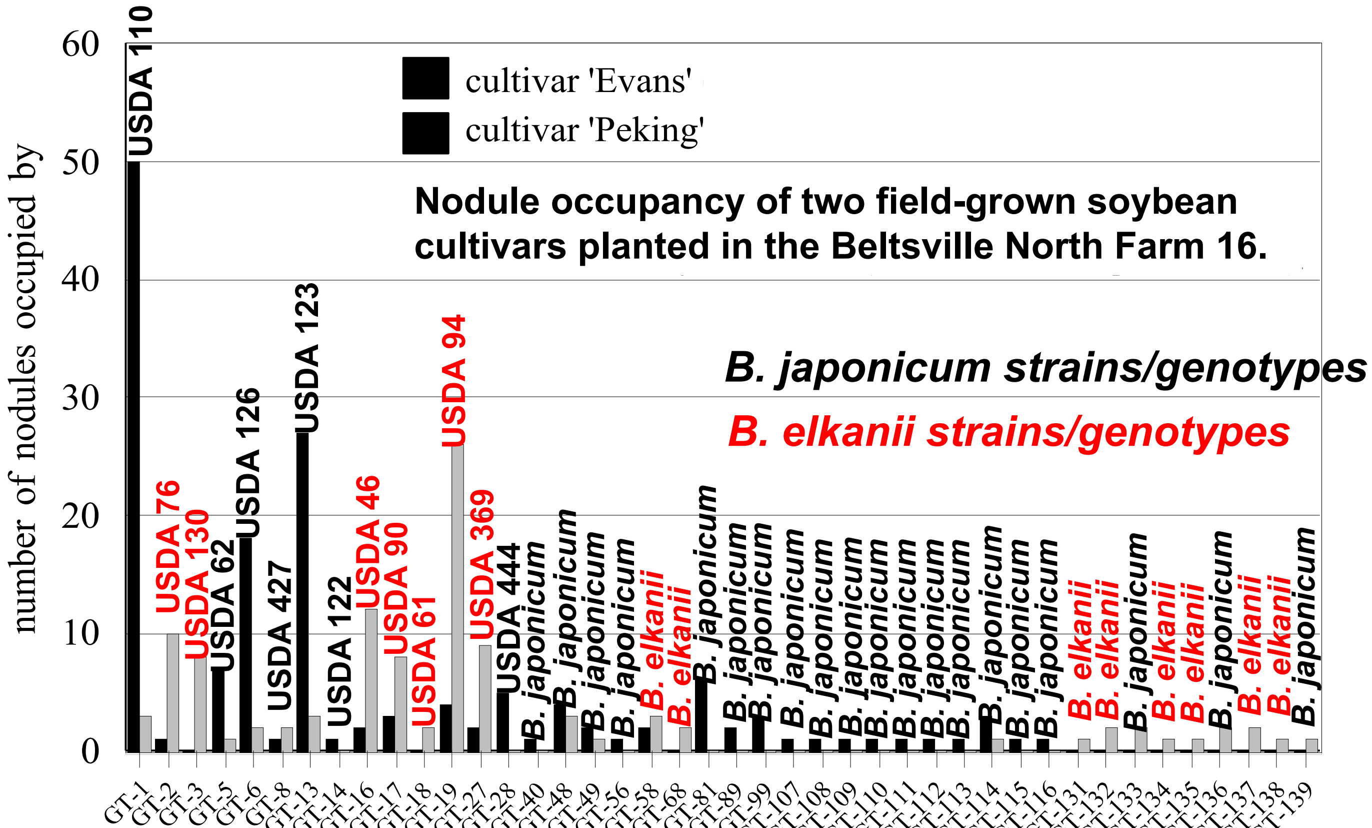
The impact of soybean genotype on nodule occupancy by *B. japonicum* vs. *B. elkanii*



	% *B.j*	% *B.e*
Evans	92.2 a	7.8 b
Peking	27.3 b	72.7 a

Future research to understand and use soybean genetic control of nodulation to maximize the efficiency of N2 fixation and enhance soybean yield

- **Multi-Locus Sequence Typing will allow a better understanding of the relationship between *B. japonicum* serology and restricted nodulation, assuming there is one.**
- **There is clearly much soybean genetic variation that impacts nodulation by a diversity of different *B. japonicum* genotypes/serotypes.**
- **The genetic control of genotype/serotype nodulation restriction can be determined.**
- **The molecular basis of host controlled nodulation restriction can be determined.**
- **With this knowledge can a novel plant-*Bradyrhizobium* recognition system be engineered that will only allow nodulation by a specific *Bradyrhizobium* with high symbiotic capacity?**
- **Will the reduction of nodulation by *B. elkanii* in favor of *B. japonicum* have a positive impact on N2 fixation and seed yield?**