



## Experimental and modelling approach to the legume-Rhizobium interaction: test of plant-host sanctions in co-inoculated plants with fixing and non-fixing strains

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## Experimental and modelling approach to the legume-*Rhizobium* interaction: test of plant-host sanctions in co-inoculated plants with fixing and non-fixing strains.

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### ABSTRACT

We tested the plant host sanction hypothesis using soybean plants co-inoculated with two rhizobial strains, a normally N<sub>2</sub> fixing strain and a mutant derivative that lacks nitrogenase activity but has the same nodulation abilities. We found no evidence of functioning plant host sanctions to cheater rhizobia based on nodular rhizobia viability in co-inoculated plants.

### INTRODUCTION

The origin and persistence in nature of symbiotic interactions is difficult to explain since the existence of exploitative, 'cheating' partners that could erode the interaction is common. Host sanctions against non N<sub>2</sub> fixing, cheating symbionts have been proposed as a force stabilizing mutualism in legume-*Rhizobium* symbiosis (Denison, 2000). Penalizations would include decreased nodular rhizobial viability and/or early nodule senescence in nodules occupied by cheating rhizobia.

We analyze the ecological and evolutionary stability of *Rhizobium*-legume symbiosis when "cheating" strains are present, using a combination of experiments and modelling. Co-occupation of the same nodule by strains with different fixation abilities is an important source of concern in cultivated legumes (Rolfe and Gresshoff, 1980). Effects of co-occupation of nodules by non-fixing rhizobia would be diluted by fixing rhizobia occupying the same nodule (Denison, 2000). We performed experiments with soybean plants co-inoculated with two rhizobial strains, a normally N<sub>2</sub> fixing strain and an isogenic non-fixing, cheating mutant derivative that lacks nitrogenase activity but has the same nodulation abilities.

### MATERIAL AND METHODS

**Bacterial strains and inoculum preparation.** Mutant derivatives BJD321 (*nopB-lacZ-uidA*, Zehner *et al.*, 2008) and A3 (*nifH::Tn5*, Nod<sup>+</sup> Fix<sup>-</sup>; Hahn *et al.*, 1984) strains derived from *Bradyrhizobium japonicum* USDA110 were grown in PSY medium (Regensburger and Hennecke, 1983) and used for inoculations (10<sup>9</sup> cells/ml). Co-inocula were prepared by mixing bacterial solutions containing similar number of colony forming units (CFUs). Strain A3 lacks nitrogenase activity but shows similar infection and nodule formation levels respect to USDA110 and BJD321.

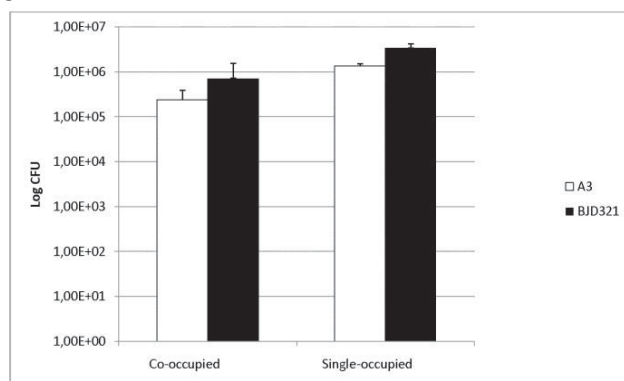
**Plant experimental setting.** Seeds of soybean (*Glycine max*) cv. Williams were surface-sterilized, germinated and sowed in Leonard jar assemblies. Seedlings (2/jar) were inoculated independently with 1 ml of a bacterial suspension made from cultures of each BJD321, A3 and a mixture of each strain. Jars were periodically supplied with a sterile N<sub>2</sub>-free nutrient solution. Plants were placed in a growth chamber under 600  $\mu\text{Em}^{-2} \text{s}^{-1}$  photosynthetically active radiation, at 25/18 °C day/night temperature, and 16/8 h photoperiod. Four weeks after inoculation nodules of each plant were collected.

**Determination of nodule occupancy and viable rhizobial counts.** Collected nodules were individually surface sterilized using Cl<sub>2</sub>Hg (2.5%), manually crushed, homogenized and

resuspended in Tris-HCL mannitol buffer containing. Each crushed nodule was smeared on PSY plates supplemented with selective antibiotics depending on the strain to determine if the nodule was occupied by BJD321, A3 or both strains. To determine rhizobial viability, appropriate serial dilutions from each nodule of another set of homogenized nodules were plated (three replicates per dilution) in PSY supplemented with selective antibiotics depending on the strain. Plates were incubated at 28 °C for a week or until no further growth was detected, and CFUs were counted. Plant dry weight and N content were determined in plants that had been heated at 60 °C for 48 h.

## RESULTS AND DISCUSSION

Plants with all nodules occupied by non-fixing rhizobia were not able of maintaining good vegetative conditions as plants with co-occupied or exclusively occupied nodules with fixing rhizobia, and ultimately they died due to N starvation about 5 weeks after inoculation. Thus, comparisons were performed using plants co-inoculated and plants inoculated only with strain BJD321. In co-inoculated plants nodule co-occupation did not differ (36.35 % BJD321, 33.32 % BJD321 and 27.28 % A3,  $\chi^2 = 6.00$ ,  $p = 0.199$ ,  $n = 66$ ). Co-inoculated plants and plants only inoculated with strain BJD321 did not differ in dry weight (Kolmogorov-Smirnov  $Z = 0.707$ ,  $p = 0.699$ ,  $n = 6$ ). Total plant N did also not differ between treatments (Kolmogorov-Smirnov  $Z = 1.299$ ,  $p = 0.068$ ,  $n = 6$ ). Nodule mass did not differ between co-inoculated plants and plants inoculated with BJD321 only ( $\chi^2 = 1.66$ ,  $p = 0.56$ ,  $n = 66$ ). Number of CFUs did not differ between co-occupied nodules and BJD321 or A3 single-occupied nodules in co-inoculated plants (Figure 1).



**Figure 1.** Number of CFUs in co-occupied nodules or single-occupied nodules in co-inoculated plants.

No evidence of functioning plant host sanctions to cheater rhizobia based on nodular rhizobia viability in co-inoculated plants was found. These experimental results will be incorporated to the mathematical model (Marco *et al.*, 2009) to check for plant population persistence in presence of cheating rhizobia.

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## REFERENCES

- Denison, R.F. (2000). *Amer. Nat.* 156: 567-576.
- Hahn, M., *et al.* (1984). *Plant Mol. Biol.* 3:159-168.
- Marco, D.E., *et al.* (2009). *J. Theor. Biol.* 259: 423-433.
- Regensburger, B., and Hennecke, H. (1983). *Arch. Microbiol.* 135: 103-109.
- Rolfe, B.G., and Gresshoff, P.M., 1980. *Austr. J. Biol. Sci.* 33, 491-504.
- Zehner, S., *et al.* (2008). *Mol. Plant Microbe Interact.* 21: 1087-1093.