Does Hydrogen Evolution From HUP$^-$ Field Pea Nodules Stimulate Nitrous Oxide Production?

M. Sather[1], R. Farrell[1], J.D. Knight[1], R. Lemke[2], A. Bedard-Haughn[1], S. Siciliano[1]

[1] Department of Soil Science, University of Saskatchewan, Saskatoon, SK, S7N 5A8
[2] Agriculture and Agricultural Food Canada (AAFC), Saskatoon, SK, S7N 0X2

Key Words: field pea, biological nitrogen fixation, *Rhizobium*, hydrogenase uptake

Introduction

Legumes, such as *Pisum sativum* (field pea), can fix atmospheric N_2 into plant available forms through symbiosis with *Rhizobium* bacteria. During biological nitrogen fixation (BNF), hydrogen gas (H_2) is produced as a byproduct (Dong and Layzell, 2001). If the legume-*Rhizobium* symbiosis possesses the hydrogenase uptake enzyme (HUP^+), the H_2 gas produced can be recovered. However, if the HUP enzyme is lacking (HUP^-), the H_2 gas produced through BNF diffuses into the rhizosphere (Dong and Layzell, 2001). Hydrogen gas may cause conditions in the rhizosphere to change in a way that favours nitrous oxide (N_2O) production. These changes could include enhancing O_2 consumption and increasing CO_2 fixation; both of these conditions may favour denitrification and increase the potential for N_2O production (Golding and Dong, 2010).

Nitrous oxide is a greenhouse gas that has a negative impact on the atmosphere by increasing radiative forcing and catalyzing ozone destruction. The majority of N_2O emissions in Canada are from agricultural sources; however, it is still unknown where much of the N_2O is produced in the soil-plant system (Golding and Dong, 2010).

The objectives of this preliminary study were to analyze *Rhizobium leguminosarum* strains to identify HUP^+ and HUP^- strains using field pea nodules. And secondly, to analyze H_2 and N_2O concentrations from pea roots and nodules grown with different *R. leguminosarum* strains as inoculants to determine if HUP^- symbiosis, in the absence of soil, produces greater concentrations of H_2 and N_2O.

Materials and Methods

The first experiment used the methylene blue reduction assay to determine the HUP status of field pea nodules inoculated with various strains of *R. leguminosarum* (Lambert et al., 1985). The four *Rhizobium* strains selected for this experiment were 128C52, 128C79, 128C53, and PJB5J1. Strains 128C53 and PJB5J1 are largely isogenic except for the HUP enzyme. Pea plants were grown in sterile sand for four weeks with N-free nutrient media. The assay was conducted on nodules from the pea plants.

The second experiment conducted was focused on the H_2 and N_2O concentrations produced from pea roots. Pea seeds were inoculated with one of six inoculation treatments. The treatments included the four *Rhizobium* strains from the first experiment (128C52, 128C79, 128C53 and

PJB5J1) and two control treatments, B151 (non-nodulating *Rhizobium* strain, largely isogenic with 128C53 and PJB5J1) and sterile water. Pea plants were grown for four weeks in sterile sand with N-free nutrient media. After four weeks, roots were washed and then sealed in glass media jars with lids fitted with septa for gas sampling. Half of the replicates for each treatment were given an ambient air atmosphere, the other half of the replicates had 120 ppm H_2 injected into the jar to simulate a pre-existing H_2 atmosphere in the rhizosphere. Gas samples were collected every 30 minutes for a total of 120 minutes. The atmosphere was replaced in the jar after each sampling. Gas samples were analyzed for H_2 and N_2O concentrations.

Results

The methylene blue reduction assay measures the HUP status of legume nodules (Lambert et al. 1985). Nodules that are HUP^+ are able to reduce the methylene blue solution, which results in white discolouration around the nodules (Figure 1). Nodules that are HUP^- are not able to reduce the methylene blue and undergo no visible colour change (Figure 1). Strains 128C52 and 128C53 underwent discolouration and are HUP^+ *R. leguminosarum* strains. Strains 128C79 and PJB5J1 are did not change colour and are HUP^- *R. leguminosarum* strains.

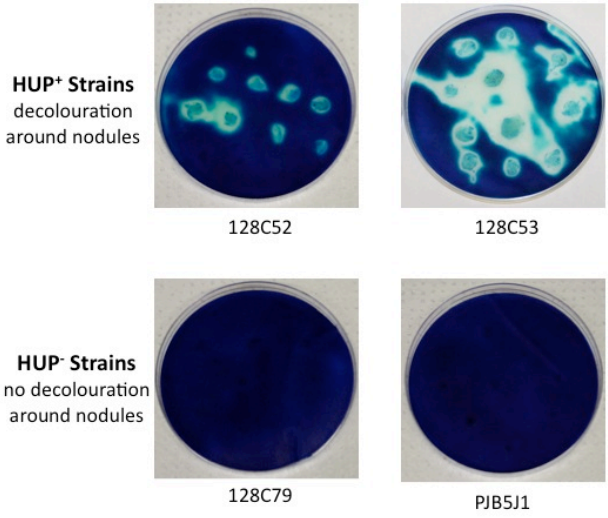

Figure 1. Results of the methylene blue reduction assay.

Hydrogen concentrations from the six *R. leguminosarum* treatments under an ambient atmosphere were significantly different from one another (Figure 2). However, the H_2 results from the six treatments were not significantly different from one another under the elevated H_2 (120 ppm) atmosphere (Figure 3).

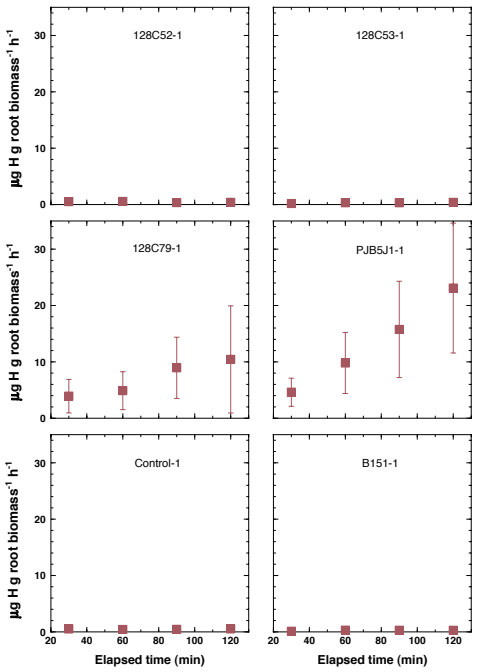

Figure 2. Hydrogen gas concentrations for roots and nodules under ambient atmosphere conditions ($P \leq 0.05$, n=4).

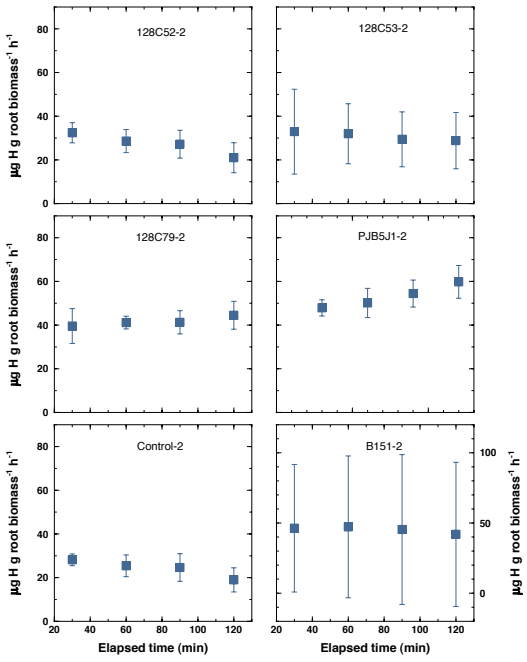

Figure 3. Hydrogen gas concentrations from six inoculation treatments under elevated H_2 atmosphere ($P \leq 0.05$, n=4).

Nitrous oxide emissions were also measured in conjunction with the H_2 concentrations from the six inoculation treatments under both the ambient air atmosphere and the elevated H_2 atmosphere. The N_2O concentrations under ambient atmosphere were not distinguishable from ambient N_2O concentrations (results not shown). The N_2O results under an elevated H_2 atmosphere had no significant differences between treatments (Figure 4).

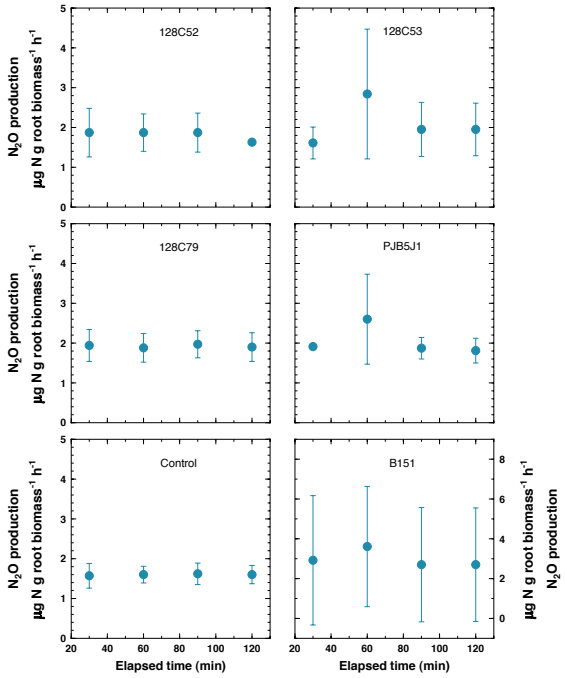

Figure 4. Nitrous oxide concentrations from roots and nodules under an elevated H_2 atmosphere ($P \leq 0.05$, n=4).

Discussion

The methylene reduction assay was successful at identifying HUP^- and HUP^+ *R. leguminosarum* strains. These four strains along with the two control treatments, B151 and Control (sterile water) form the six inoculation treatments that were used in the second experiment and which will be used in future experiments as well.

Under ambient air conditions, H_2 concentrations from128C79 and PJB5J1 were significantly different from one another and the other four treatments. The results confirm that theses strains are HUP^- because they are producing H_2 and not recycling it. The differences in treatment were not observed under the elevated H_2 atmosphere, differences were possibly masked by the artificially elevated H_2 atmosphere background levels.

There were no significant differences in the N_2O concentrations measured under either atmosphere; this is an anticipated result because there is no direct biological pathway between H_2 production and N_2O production in legume BNF.

Conclusion

In the absence of soil, H_2 production from HUP^- nodules does not stimulate N_2O production. The next step in this process is to conduct a similar experiment with soil present in the rhizosphere to see whether or not H_2 creates conditions in the rhizosphere that favour denitrification and N_2O production.

References

Dong, Z. and D.B. Layzell. 2001. H_2 oxidation, O_2 uptake and CO_2 fixation in hydrogen treated soils. Plant and Soil 229: 1-12.

Golding, A. and Z. Dong. 2010. Hydrogen production by nitrogenase as a potential crop rotation benefit. Environ. Chem. Lett. 8: 101-121.

Lambert, G.R., F.J. Hanus, S.A. Russell and H.J. Evans. 1985. Determination of the Hydrogenase Status of Individual Legume Nodules by a Methylene Blue Reduction Assay. App. And Environ. Micro. 50: 537-539.