View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by University of Saskatchewan's Research Archive

The Effect of Hydrogen Evolution from HUP$^-$ Field Pea Nodules on Nitrous Oxide Production

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

[1]Department of Soil Science, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada
[2]Agriculture and Agricultural Food Canada (AAFC), Saskatoon, SK, Canada

Key Words: legumes**,** *Rhizobium leguminosarum*, nitrous oxide, hydrogen, greenhouse gas

Introduction

Pulse crops refer to edible legumes such as lentil, field pea and chickpea. These three crops are important in Saskatchewan agriculture and this province in the largest exporter of field pea in Canada. One of the benefits of growing pulses in rotation is their ability to fix atmospheric nitrogen into plant available forms. This means that pulse crops often have lower fertilizer requirements than cereal crops. Pulses fix nitrogen by forming symbiotic relationships with rhizobia bacteria; the field pea compatible rhizobia is *Rhizobium leguminosarum*. Biological nitrogen fixation occurs through the nitrogenase reaction that converts atmospheric nitrogen into plant available forms. Hydrogen is produced as a byproduct of the nitrogenase reaction. Hydrogen production during biological nitrogen fixation is relatively energy intensive and accounts for approximately 5% of net photosynthesis (Dong and Layzell, 2001).

Some rhizobia possess the uptake hydrogenase (HUP) enzyme. Rhizobia that possess the HUP enzyme are referred to as HUP$^+$ (positive) and rhizobia that lack the enzyme are termed HUP$^-$ (negative). If the HUP enzyme is present (HUP$^+$), the hydrogen produced through biological nitrogen fixation is oxidized and the hydrogen is recycled. Under these conditions, no hydrogen leaves the nodule and much of the energy used to produce the hydrogen is recovered. If the HUP enzyme is not present, meaning the rhizobia are HUP$^-$, the hydrogen produced through biological nitrogen fixation diffuses out from the nodule into the surrounding soil. Once the hydrogen is in the soil, it is used up by hydrogen-oxidizing micoorgansisms within a few cm of the nodule causing an increase in oxygen consumption and carbon dioxide fixation (Dong and Layzell, 2001). These conditions may create hypoxic or anoxic zones in the rhizosphere that favour dentrification and nitrous oxide production (Golding and Dong, 2010).

Many studies have tried to quantify nitrous oxide emissions from legume crops because they are an important component of the nitrogen cycle. Biological nitrogen fixation may contribute to nitrous oxide emissions through a couple different pathways: nitrifcation or dentrification of nitrogen-rich plant residues or direct denitrifcation by certain rhizobia. However, to date, most studies have not focused on the HUP trait and the possible interactions of hydrogen in the soil. A recent study reported a tenfold increase in nitrous oxide emissions from soil that had been artificially treated with hydrogen at a rate similar to that evolved from HUP$^-$ soybean compared to soil that was treated with air. The system used involved pumping hydrogen gas or ambient air through bulk soil and measuring the nitrous oxide emissions from the soil. There were no plants involved in this experiment (Golding and Dong, 2010).

Nitrous oxide is a potent greenhouse gas with a much greater global warming potential than carbon dioxide. Globally, agriculture produces approximately 60% of nitrous oxide emissions, which are largely from nitrogen fertilizer use, crop residue decomposition and manure storage. Nitrous oxide can be produced through two microbial processes: nitrification and denitrification. It is still unknown where in the rhizosphere nitrous oxide may be produced and what processes may be spatially responsible.

Study Objectives

The objective of the two studies was to compare the effect of the HUP trait on nitrous oxide emissions from field pea. The first study was focused on measuring nitrous oxide emissions from roots and nodules in the absence of soil. The objectives of the study were to: determine the amount of hydrogen evolved from nodules, quantify nitrous oxide from pea roots, and determine if increased hydrogen around the nodules stimulated nitrous oxide production. The second study focused on measuring rhizosphere and surface soil nitrous oxide emissions from field pea. The objectives of the study were to determine the effect of hydrogen from HUP^- rhizobia on nitrous oxide emissions from soil and to compare nitrous oxide production in the rhizosphere to surface soil emissions.

Materials and Methods

Six treatments were used for both studies. They included five *Rhizobium leguminosarum* strains with known HUP statuses; as well sterilized water was used as a control. The HUP^+ rhizobia strains used in the studies were 128C52 and 128C53. The HUP^- strains used were 128C79 and PJB5J1. A non-nodulating rhizobial strain (B151) was also used as a second control treatment.

First study

Pea plants were inoculated with the rhizobial treatments and grown in Leonard jars for four weeks. Leonard jars are used to grow plants without soil; they utilize a reservoir filled with a nutrient solution that can be wicked up into sand that acts as the growth medium. The entire Leonard jar is autoclaved beforehand. After four weeks, the roots were harvested, washed and sealed in media jars that could be gas sampled. Each set of roots was sampled at 30, 60, 90 and 120 minutes after sealing. All gas samples were analyzed for hydrogen and nitrous oxide. Half the replicates of each treatment had additional hydrogen injected into the jars to create an elevated hydrogen atmosphere in the media jars. This was done to see if increased hydrogen around the nodules, similar to a HUP^- system would stimulate nitrous oxide production in the absence of soil.

Second study

The second study was a greenhouse pot study. Soil was collected from the Agriculture and Agricultural Food Canada research station in Swift Current, SK. The soil was from a long-term, wheat-fallow rotation with no history of legumes in rotation. This was done to minimize the number of indigenous rhizobia present in the soil. The same six rhizobial treatments were used to inoculate pea seeds as were used in the first study. The rhizosphere gas samples were

collected from a coil of silicone tubing installed within the rooting zone of the pot. Gas samples were collected from two gas sampling ports installed in the side of the pot. Surface gas samples were collected using a lid that fit the top of the pot that sealed around the stem of the plant.

Results

Hydrogen results

The hydrogen results from the first study showed the HUP$^-$ treatments produced significantly greater amounts of hydrogen than the other treatments (Figure 1).

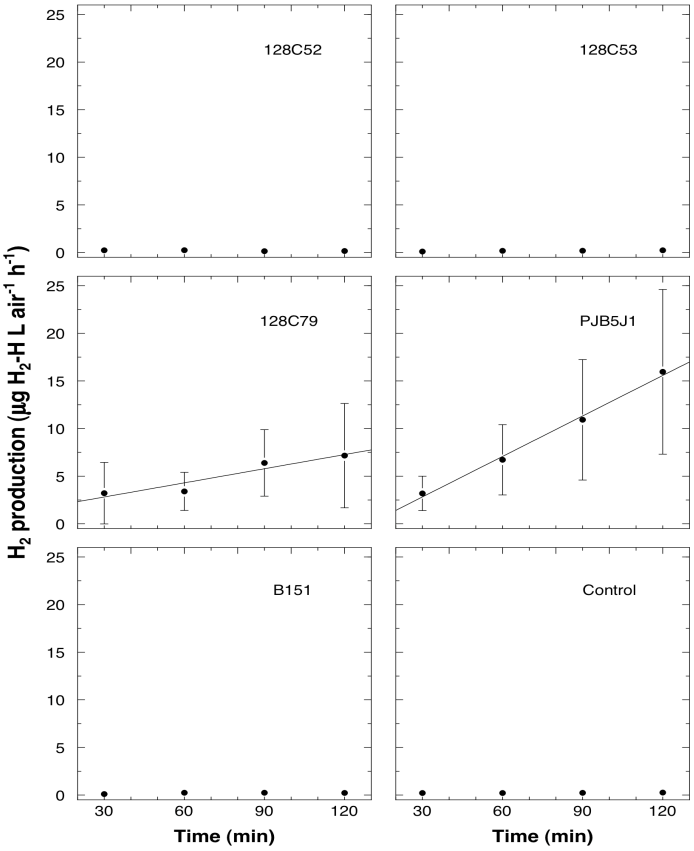

Figure 1. Hydrogen production from roots and nodules in media jars in ambient air (n= 4).

Nitrous oxide results

No nitrous oxide was produced from roots and nodules under ambient air or the hydrogen-enriched air in the first study. There were no treatment differences detected between any of the HUP treatments.

In the second study, the nitrous oxide results from the rhizosphere and surface showed a high degree of correlation meaning the method used to measure the rhizosphere gas concentrations was successful (Figure 2). As well, the HUP$^-$ treatments showed the least variability and the control treatments showed the highest level of variability (Figure 2). The

control treatments were nodulated by indigenous rhizobia present in the soil; however, the number of nodules was small compared to the inoculated treatments.

The cumulative nitrous oxide results show that the HUP^- treatments produced the least amount of nitrous oxide over the growing season, and the HUP^+ treatments produced more than the HUP^- treatments, but less than the control treatments (Figure 3). The control treatments produced the highest amount of nitrous oxide of all the treatments; however the treatment differences were not significant.

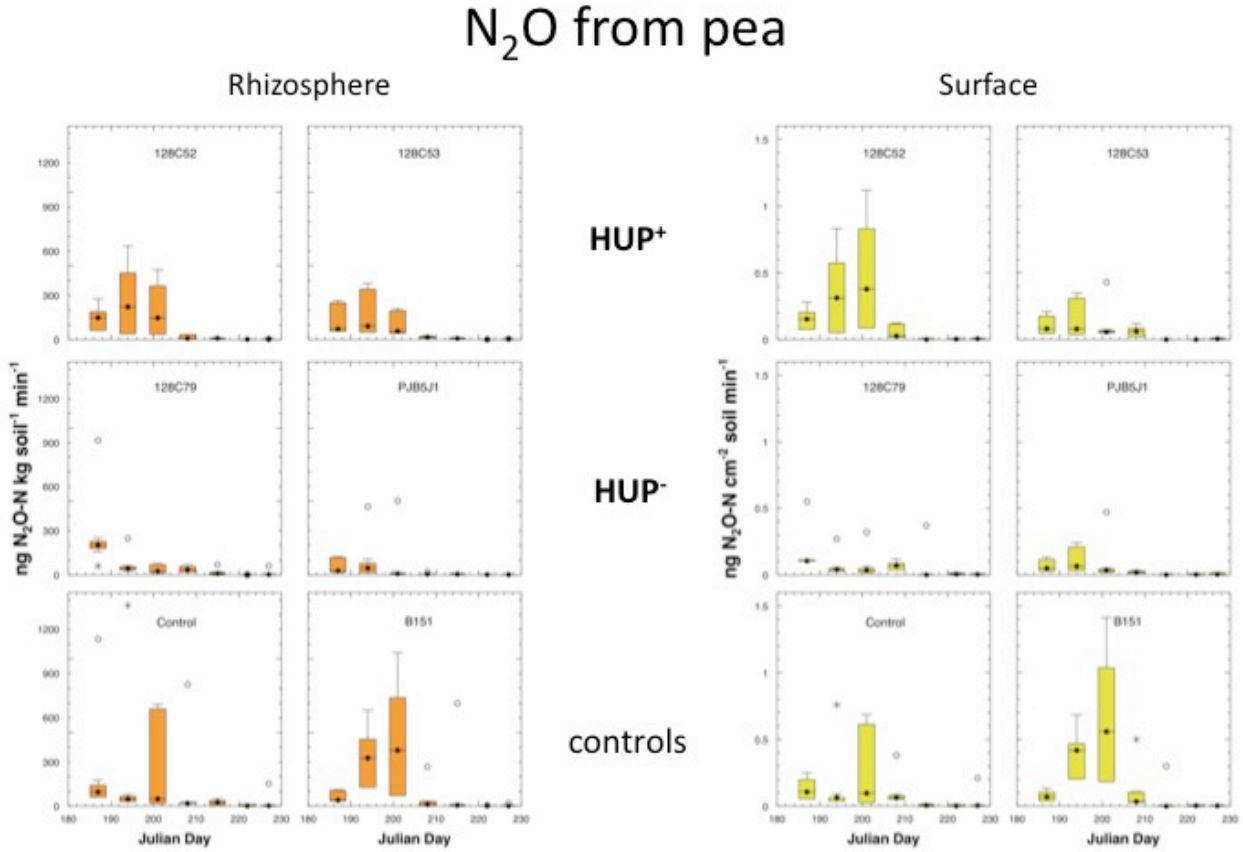

Figure 2. Box and whisker plots showing the median nitrous oxide concentration (black dot) and the spread and variability of the data (size of the box) in the rhizosphere and surface gas samples.

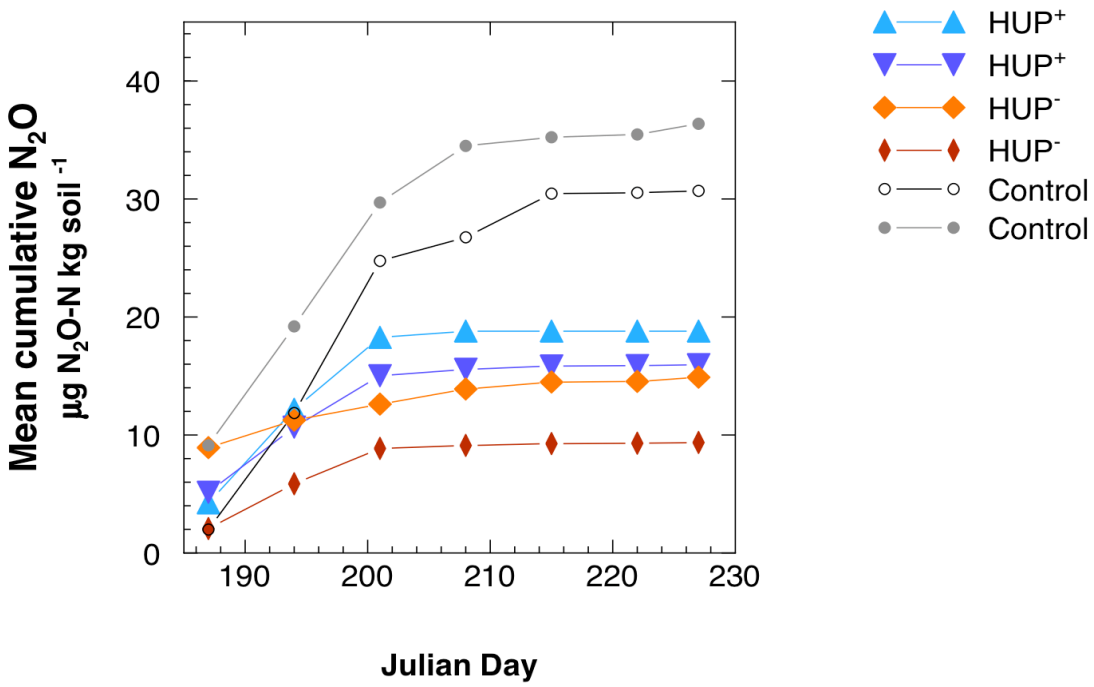

Figure 3. Cumulative nitrous oxide concentrations from soil over the growing period of the pea plants.

Discussion

The first study showed that HUP^- strains produced significantly higher amounts of hydrogen compared to the HUP^+ and control treatments. However, in the absence of soil, roots and nodules did not produce nitrous oxide. In the second study, nitrous oxide production in HUP^- treatments was lower than HUP^+ and control treatments showing the opposite results to the artificial hydrogen soil treatment, suggesting that artificially treating bulk soil may be a poor model for the natural system because it does not include interactions with plant roots and nodules. The results of these two studies could be used to better quantify nitrous oxide from field pea and influence inoculant formulations.

References

Dong, Z. and D.B. Layzell. 2001. H_2 oxidation, O_2 uptake and CO_2 fixation in hydrogen treated soils. Plant 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.