Effect of Nutrient Supplements on Cowpea Nodulation

Sallee Reynolds

Plant Nutrition - PSB 6430

Fall Semester 2005

Abstract

Little is known about ideal conditions for initiation of biological nitrogen fixation. It has been shown that field cropping history, nitrogen availability, and soil condition play a major role in nodulation. It is thought that some nitrogen increases nodulation but that too much inhibits it. This study examined the nodulation response of cow pea (*Vigna unguiculata)* to nutrient supplement. Plants were grown in tap water, tap water with a 10% concentration of slow release fertilizer, and Peter's Complete Nutrient Solution. Groups of those grown in nutrient solution were moved to tap water after 35, and 45 days. Plants were destructively harvested at 35, 45 and 55 days after planting. On a logarithmic scale, the data for number of nodules formed were normal and showed a statistical difference between the number of nodules per harvest (p=.018) but did not show statistical difference between nutrient levels (p=.08). The weight data for this project showed normal data that was statistically different between harvests (p=.003) but not statistically different for nutrient levels (p=.268). These non-significant results could have been caused by a confounding factor that the plants watered with nutrient solution were compromised due to drought stress experienced at two different times during the study.

Introduction

In biological nitrogen fixation (BNF), plants provide the carbon for the nitrogen-fixing bacteria and a place for the oxygen-sensitive nitrogenase enzyme that is required to convert dinitrogen to ammonium. This conversion can account for 7 to 20% of the annual input of N in systems like short-grass prairie (Lauenroth and Dodd, 1979). Legumes are capable of making this bacterial association. Although nodulation, the first step of the BNF process, is still not well understood, since the first part of the 20th century it has been fairly well-accepted that the most extensive nodulation takes place under nitrogen limiting conditions. It is now well accepted that some N fertilizer in irrigated systems along with rhizobial inoculation results in the highest yields (Vargas et al., 2000).

Vargas el al. (2000) showed that the response of legumes to inoculation in irrigated systems depends on N fertilization and cropping history. They showed that the application of N fertilizer had no effect on early formation of nodules but decreased the number of nodules at 40 days after emergence in soils that had a history of legume cropping at 40 and 60 kg N ha^{-1}. N fertilizer also increased N in both shoot and dry matter of plants at these levels.

Seguin et al. (2001) found that rhizobial inoculation increased nodulation in Kura Clover and that response to inoculation increased with time. They found that nodulation was promoted by some N fertilization in two out of three sites. They conclude that at low initial soil N levels, moderate N fertilization stimulates nodulation. It is important to note that statistically there were two- and three-way interactions between inoculation, N fertilization and sampling date.

A study by Qasim et al. (2001) in Pakistan produced non-significant results in a study that examined the response of soybean toward inoculant and NPK fertilizer. The only statistically significant result they found was seed yield and weight, which was highest at factory-recommend levels of both fertilizer and inoculant

Plants limit nodule number through a regulatory mechanism that favors older nodules and suppresses younger ones (Stougaard, 2000). Parsons et al. (1993) suggested that reduced nitrogen compounds, such as amino acids, found in the phloem of the lower leaves inhibit nodule growth and activity. It has also been suggested that carbohydrate deprivation or ethylene could actually be the controlling mechanisms. Regulation is thought to be an active process (Caetano-Anolles and Bauer, 1988).

With the current trend of more environmentally sustainable agriculture practice it is important to understand how to best manipulate BNF to decrease N inputs and maximize yield. This study will examine nodulation of cow-pea in fertilized and unfertilized conditions and then examine how nodulation changes as fertilization is taken away.

Objective

This objective of this study is to examine the effect of fertilization on the quick-nodulating legume cow-pea (*Vigna unguiculata*).

Hypothesis

Nodulation will take place more quickly in plants grown in unfertilized conditions than plants that have been grown in fertilized conditions which are then removed from fertilizer.

Methods and Materials

Experiments were initiated in October 2005 at Logan, UT. Growing media used in the study was Turface. Average daily and nightly temperatures were 25/20 C respectively. The relative humidity was approximately 40% at mid day and 70% at night. The photosynthetic photon flux is estimated to be 12 moles m^{-2} day^{-1} from the sun and an additional 15 moles m^{-2} day^{-1} from supplemental HPS lamps for a total of 27 moles m^{-2} day^{-1}.

Seeds were germinated in Petri-dishes on filter paper. At germination, seeds were transferred to 46-cm (18 in) PVC columns and inoculated. Plants were watered 20 times daily for 30 seconds each watering session.

At planting, individuals were placed in one of 5 nutrient conditions; tap water, 10% of the factory recommended amount of a 3-4 month slow-release fertilizer, Peter's Complete Nutrient Solution (H3), Peter's Complete Nutrient

Solution that would be moved to tap water after 35 days (H1), and Peter's Complete Nutrient Solution that would be moved to tap water after 45 days (H2). Seven days after planting seedlings that had not survived were replaced with newly germinated seeds.

The study consisted of three harvests; 35 days after planting (DAP), 45 DAP and 55 DAP. At harvest, plant roots were washed, nodules were counted, removed from roots and weighed.

Nodule number and weight data were then analyzed using the SAS program for statistical analysis using a complete block design with each harvest designated as a block and each of the nutrient levels a treatment.

Results

It was found that in both harvest 1 and 2 the plants in H1 had the heaviest nodules while in harvest 3 the plants in H2 had the heaviest nodules.

The plants receiving tap water had the most nodules at the first harvest, H1 had the most nodules at the second harvest and H2 had the most nodules at the third harvest.

Further results of this study are summarized in Tables 1, 2 and 3 and Graphs 1 and 2.

Discussion

On a logarithmic scale, the data for number of nodules were normal and showed statistical difference between the number of nodules per harvest ($p=.018$) but did not show statistical difference between nutrient levels ($p=.08$).

The weight data showed normal data that was statistically different between harvests ($p=.003$) but not statistically different for nutrient levels ($p=.268$).

There are compounding factors to this project. At two different times the plants that were watered with Peter's complete solution were drought stressed. This lead the plants grown on Peter's solution to be smaller and less developed with smaller root systems than their tap-water equivalents. (See pictures 1-4)

It was observed that the plants grown on nutrient solution had nodules more evenly distributed in the root zone than did those grown on tap-water. The tap watered plants and those grown with the 10% concentration of slow release fertilizer seemed to concentrate their nodules near the surface. (See pictures 5 and 6)

Though the results were inconclusive, I do think that there is merit in the study. I think that further studies should cover the same nutrient conditions but should have more replicates. I saw such disparity between plants in the same nutrient condition that I think more replicates would show more statistical difference. From visual observations, I think there is something about plants that are germinated and grown for 35 days with fertilizer and are then moved to tap water. I think that it would be interesting to see in a further study not only the difference of nodule number and weight, but plant color, above ground biomass and seed production.

Table 1: Average number and weight for each of the nutrient treatments in Harvest 1

Treatment	Average Number	Average Weight (mg)	Total Weight (g)
Water	113	4.6	.520
House 1	33	12.9	.455
House 2	23	8.0	.175
House 3	23	8.0	.175
Slow Release	74	4.9	.360

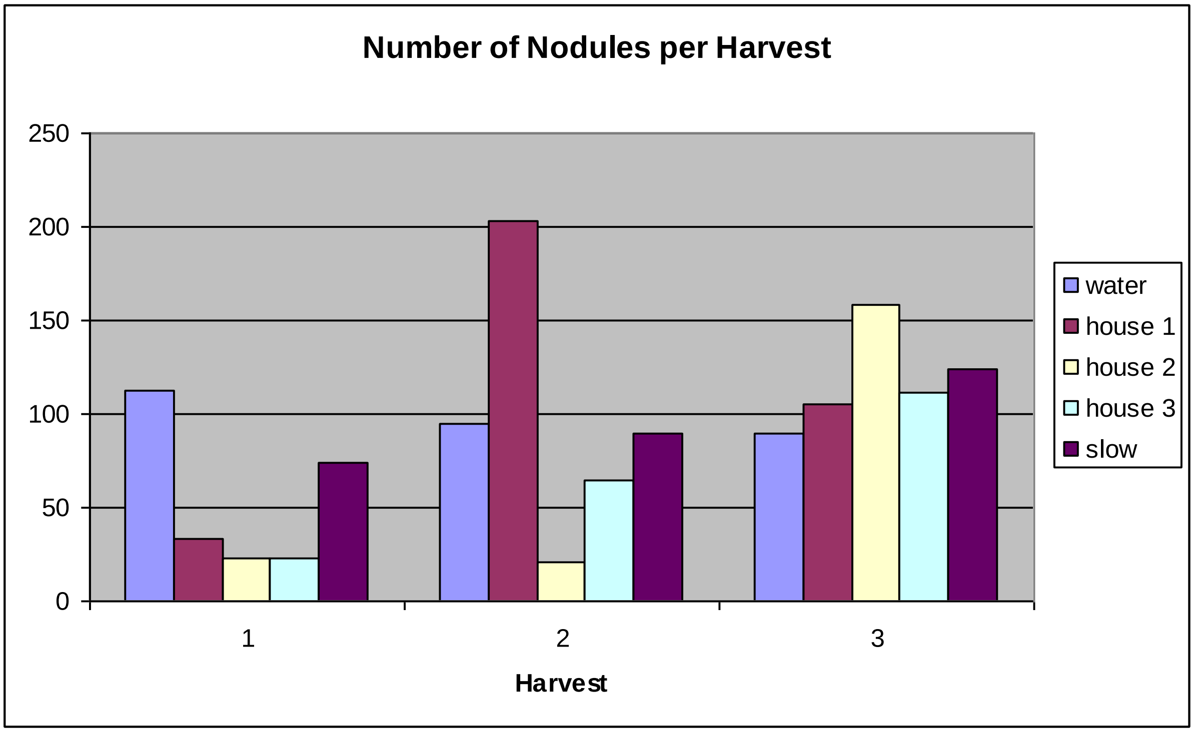
Table 2:
Average number and weight for each of the nutrient treatments in Harvest 2

Treatment	Average Number	Average Weight (mg)	Total Weight (g)
Water	95	4.40	.891
House 1	203	14.3	2.907
House 2	21	10.8	.219
House 3	64.5	7.2	.484
Slow Release	89.5	13.5	1.025

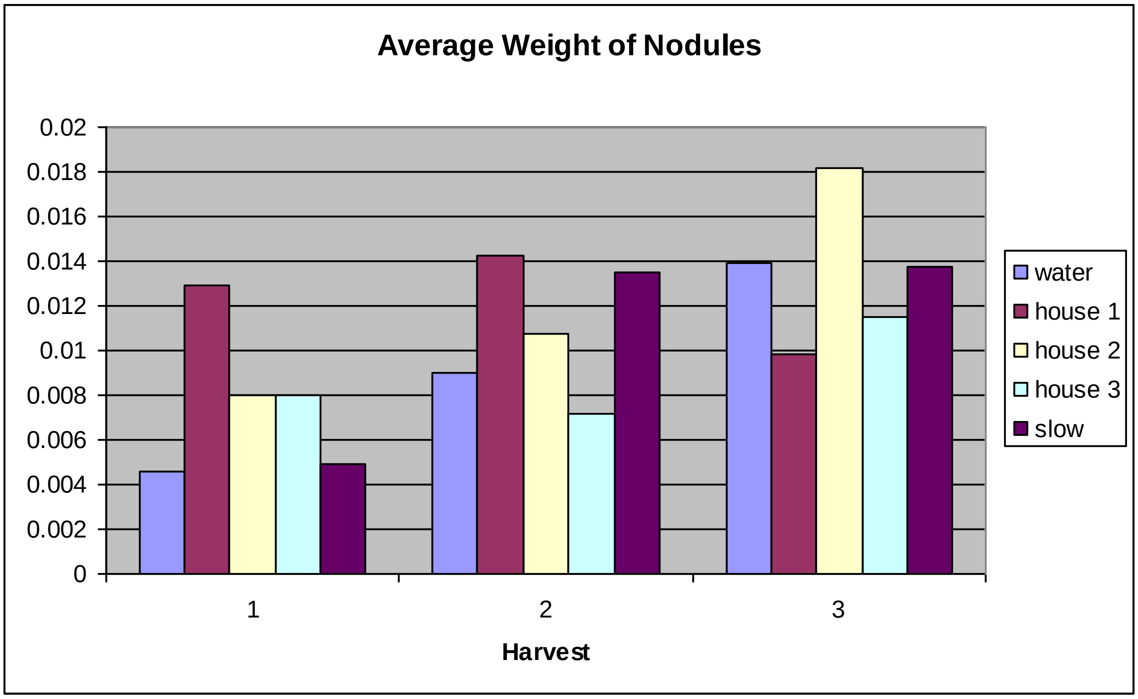
Table 3:
Average number and weight for each of the nutrient treatments in Harvest 3

Treatment	Average Number	Average Weight (mg)	Total Weight (g)
Water	90	13.9	1.3
House 1	105	9.9	1.0675
House 2	158.5	18.2	2.64
House 3	111	11.5	1.3396
Slow Release	123.5	13.7	1.676

Graph 1:
Number of nodules per harvest shown graphically where water is the pure tap water treatment, house 1 is the Peter's complete nutrient solution that were moved to tap after 35 days, house 2 is the Peter's complete solution that were moved after 45 days, house 3 is the Peter's solution and slow is the 10% factory recommended concentration of slow-release fertilizer.



Graph 2:
Average weight of nodules per harvest shown graphically where water is the pure tap water treatment, house 1 is the Peter's complete nutrient solution that were moved to tap after 35 days, house 2 is the Peter's complete solution that were moved after 45 days, house 3 is the Peter's solution and slow is the 10% factory recommended concentration of slow-release fertilizer.



Picture 1:
Plants after 55 days in the order H3, H2, H1, 10% slow-release fertilizer and tap water.

Picture 2: Plants and roots after 55 days in the order H3, H2, H1, 10% slow release fertilizer and tap water.

Picture 3:
Plants after 45 days in the order tap water, slow-release fertilizer H3

Picture 4:
Comparison of H1 and H2 after 45 days

Picture 5:
Nodules 10-cm from the surface in H1 after 45 days

Picture 6:
Nodules at the surface of a tap-water grown plant after 45 days

Work Cited

Qasim, M., Fazal Nawab, Himayatullah and Rahim Din. 2001. Response of Inoculum and Fertilizers on Nodulation and Economic Yield of Soybean Cultivars. Online Journal of Biological Sciences 1(2): 76-77

Stougaard, Jens. 2000. Regulators and Regulation of Legume Root Nodule Development. Plant Physiology. October 2000 Vol. 124 531-540

Vargas, Milton A.T., Ieda C. Mendes, 2000 Reponse of field-grown bean (Phaseolus vulgaris L.) to Rhizobiem inoculation and nitrogen fertilization in two Cerrados soils. Biology of Fertile Soils 32:228-233

Lauenroth, WK. and JL Dodd. 1979. Response of Native Grassland Leguem to Water and Nitrogen Treatments. Journal of Range Management 32(4)

Seguin, Philippe, Craig Sheaffer, Nancy Ehlke, Michael Russelle, and Peter H Graham. 2001. Nitrogen Fertilization and Rhizobial Inoculation Effects on Kura Clover Growth. Agronomy Journal Vol. 93 1262-1268

Parsons, R., A. Stanforth, J.A. Raven and J.I. Sprent. 1993. Nodule Growth and activity may be regulated by a feedback mechanism involving phloem nitrogen. Plant Cell and Environment 16 125-136

Caetano-Anolles, G. and W.D. Bauer. 1988. Feedback regulation of nodule formation in Alfalfa. Planta 546-557