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The Effect of Celite Formulated Rhizobium Rubi AT3-4RS/6 and Tryptophan on Velvetleaf Plant Growth

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Abstract

Rhizobium rubi AT3-4RS/6 and tryptophan may be useful in replacing chemical herbicides as biological control agents (Kennedy et al., 1990). Previous research has shown that *Rhizobium rubi* AT3-4RS/6 produces IAA-like compounds that are deleterious to weed growth. In this project *R. rubi* AT3-4RS/6 will be formulated in Celite, a granular, diatomaceous earth carrier. The purpose of this research is to analyze if tryptophan influences *R. rubi* AT3-4RS/6 populations in the velveltleaf rhizosphere, and if this colonization is associated with reduced root weight and shoot length. The experiment design consists of five soil treatments (bacteria+tryptophan+celite, tryptophan+celite, bacteria+celite, celite alone, and soil alone) with 10 velvetleaf plants each. The decreasing trend of the root weights, shoot lengths, and bacterial colony counts of the trptophan and *R. rubi* AT3-4RS/6 treatments will be recorded and analyzed using two statistical tests (t-test, and ANOVA). The results showed that for all three independent trials there was significant variance of the shoot lengths and root weights. And the average bacterial population of both trials for the tryptophan and non-tryptophan treatment was 1.26×10^{10} cfu/g dry root and 1.02×10^{11} cfu/g dry root respectively.

Introduction

This research project stems from the idea of replacing chemical herbicides with biological control agents. The emergence of chemical-resistant weeds has greatly motivated agricultural science to find an alternative to chemical herbicides (Tranel and Wright, 2002). One method that is being investigated is the use of rhizobacteria. Rhizobacteria are root-colonizing bacteria that form associations with plants. The goals of this research are to 1.) further investigate *Rhizobium rubi* AT3-4RS/6 as a biological control agent by analyzing if tryptophan influences *R. rubi* AT3-4RS/6 populations in the rhizosphere, 2.) assess if this colonization is associated with reduced root weight and shoot length, and 3.) to test the use of Celite as a medium for bacterial formulation. Since past research examined the effect of *R. rubi* on plant growth using a liquid cell suspension, the use of a non-liquid application method of the control agent is desirable. Celite, a fine granular diatomaceous earth carrier, has been found to be compatible with bacteria (Slininger et al 2010).

Tryptophan was used in this study because it serves as a precursor in indole acetic acid (IAA) synthesis, an auxin class hormone. Auxin is usually known for its plant growth promotion but can also serve to inhibit plant growth depending on the concentration (Lambrecht et al 2000, Parsello-Cartiaux et al 2003). Past research has shown that *R. rubi* along with trytophan produces a large amount of IAA-like compounds and wasdeleterious to the velvetleaf plant (Brubaker and Zdor, 2009). Velvetleaf is a weed that originated from southern Asia but has become a problem in North America to major crops such as corn, cotton, and soybean. It grows very rapidly and has a tall height and therefore blocks sunlight penetration to the crop plants (Anonymous 2011).

The first null hypothesis is that there will be no variance of root weight and shoot length between the different treatments. And the second null hypothesis is that there will be no significant difference between the average bacterial populations of the tryptophan+bacteria treatment and non-tryptophan+bacteria treatment.

Methodology

The procedure consists of four major parts: preparation of bacterial formulations, planting and growing the velvetleaf plants, harvesting the plants, and analysis of plant growth and bacterial root colonization. Five separate treatments were tested and three independent trials were done and data was recorded and analyzed for each trial.

Preparation of Bacterial Formulations

R. rubi was inoculated into tryptic soy broth (TSB) and cultured with shaking at 28^{o} C for 2 days. After two days cells were collected via centrifugation and resuspended in 2 mL sterile TSB. The purity of this suspension was assessed using gram staining and streak plating.

To create the Celite formulations, 12.5 g Celite, the cell suspension, and (for one treatment) 125 mg of tryptophan were mixed together using the blender. After blending, the product was dried overnight at 28^{o} C. After drying the powder was transferred to 50-mL centrifuge tubes and refrigerated overnight.

In order to measure the level of *R. rubi* cells in the forumulations, serial dilutions were performed using 0.1% peptone which were then spread plated on half-strength tryptic soy agar in triplicate. Calculations were done to determine the average cfu/gram in the Celite powder.

Planting

The soil used in this study was obtained from the Andrews University Dairy farm and was autoclaved for 1 hour. The following day, the soil was autoclaved a second time for one hour. Each soil treatment consisted of 15 g of autoclaved dry soil, celite formulations of bacteria 10^9 cfu, and 2.25 distilled H_20 (autoclaved) in a 17mm by 120mm high polypropylene tube (10 tubes per treatment were prepared). One pregerminated velvetleaf seed was placed into each tube and was grown under lights for 4 weeks and watered every other day with sterile dH_2O. Seeds were surface disinfested in 50% bleach (5 min.) followed by 50% ethanol (5 min.) and thoroughly rinsed before pregermination on 1% water agar for 20-24 hours at 28^o C.

Harvesting

Plants were harvested by cutting each tube in half and the roots and shoots were separated, with the roots being processed in groups of three. Shoot lengths were recorded in centimeters and the roots were processed in groups of three. The roots were vortexed in the 50-mL centrifuge tubes containing 20 mL of 0.1% peptone for 2 minutes to remove any bacteria from the roots. The fresh weight of the roots was recorded and the dry weight determined after drying at 80^o C for 24 hours. The 20 mL root wash was serially diluted in 0.1% peptone and spread plated on half-strength TSA containing 100 µg rifampicin/mL to determine levels of viable *R. rubi* cells on the root. The average cfu/g dry root was calculated for treatments where plants were inoculated with *R. rubi*.

Analysis

An ANOVA test was done on the shoot lengths and root weight to see if there was significant variance between the treatment groups for all three independent trials and a t-

Test was done to test if there was a significant difference of the average cfu/gram dry weight between the tryptophan+bacteria treatment and non-tryptophan+bacteria treatment on the first two trials.

Results

The level of *R. rubi* AT3-4RS/6 obtained for Trial #1 for the tryptophan and non-tryptophan treatment was 1.77 x 10^{10} cfu/g dry root and 1.01 x10^{11} cfu/g dry root respectively. And for Trial #2 the level of *R. rubi* AT3-4RS/6 for the tryptophan and non-tryptophan treatment was 4.64 x10^{10} cfu/g dry root and 6.52 x 10^{10} cfu/g dry root respectively.

For the first, second, and third independent trial, the ANOVA test on the dry root weights between the treatments indicated that there was significant variance between the treatments (F=4.667,df=14, p=0.02197; F=8.359, df=14, p=0.00313; F=5.929, df=13, p=0.128 respectively). And the ANOVA test on the shoot lengths between the treatments also showed that there was significant variance (F=23.937, df=44, p=3.578e-10; F=12.315, df=44, p=1.28e-6 ; F=76.3, df=41, p=2.33e-17 respectively).

The t-Test for Trial #1 of the bacterial population between the tryptophan and non-tryptophan treatments did not show significant difference (t=1.95, df=2, p=0.0953). Trial #2 also showed that there was not a significant difference (t=1.91, df=2, p=0.098).

The trends for the shoot length and root weight show a significant variance between the treatments, with bacteria+tryptophan treatment (#3) and tryptophan treatment (#5) showing the greatest decrease in growth, supporting the findings that *Rhizobium rubi* and

tryptophan produce IAA-like compounds that inhibit growth (Brubaker and Zdor, 2009). The results of treatment 5 suggest that tryptophan alone seems to reduce plant growth.

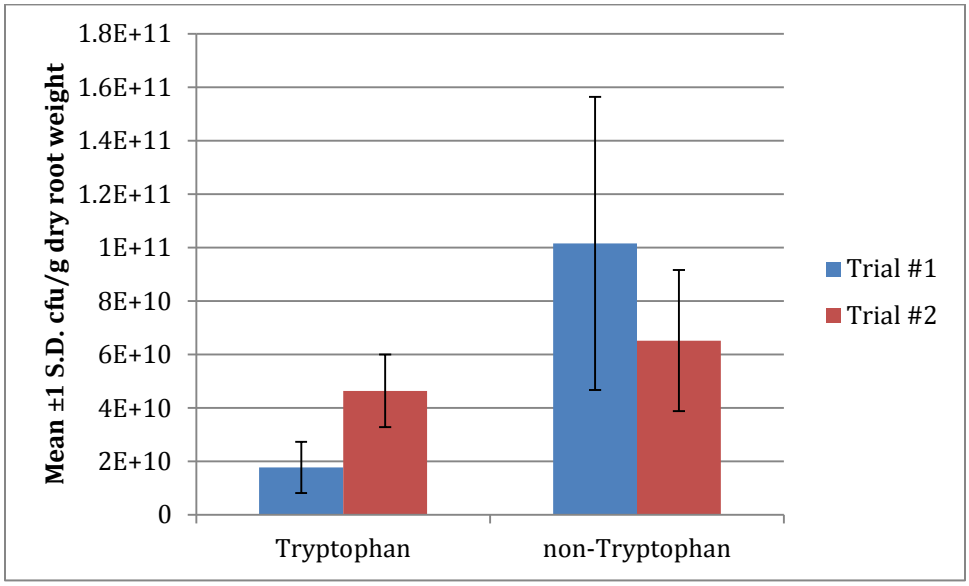

Figure 1. Populations of *R. rubi* AT3-4RS/6 on velvetleaf roots grown in the presence of Celite with or without tryptophan. Each bar is the average of 3 root aggregates (3 root systems/aggregate).

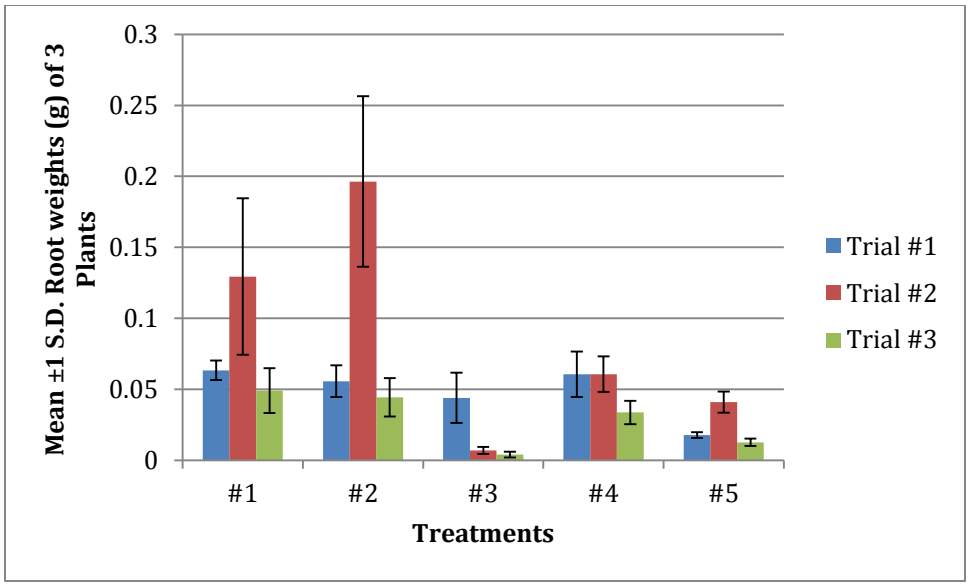

Figure 2. Average root weights of 3 root aggregates, (3 individual roots in 1 aggregate) for each of the five treatments. *Treatments: 1=no soil amendment; 2=celite; 3=celite+bacteria+tryptophan; 4=celite+bacteria; and 5=celite+tryptophan

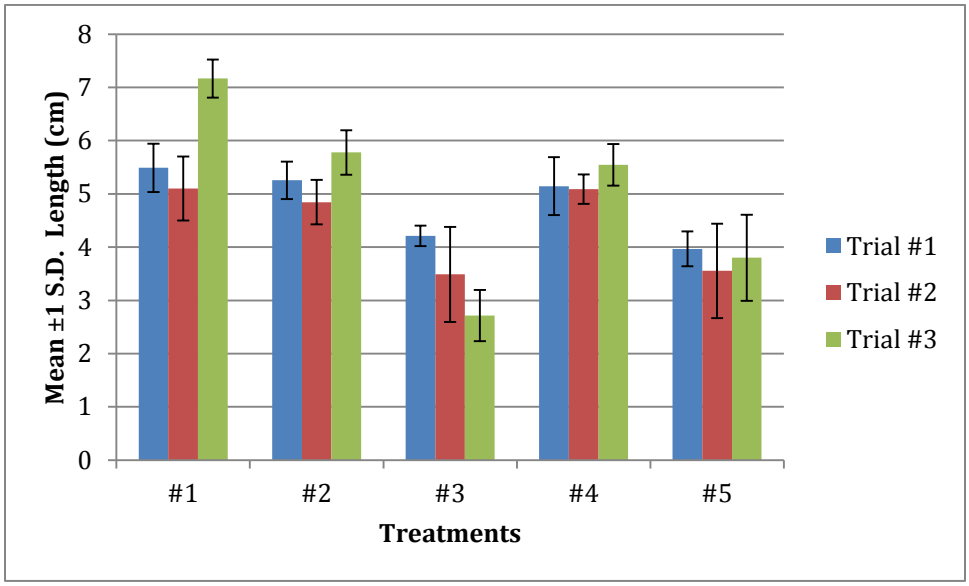

Figure 3. Average shoot lengths (cm) of 9 plants for each of the five treatments. *Treatments: 1=no soil amendment; 2=celite; 3=celite+bacteria+tryptophan; 4=celite+bacteria; and 5=celite+tryptophan

Conclusion

The results showing the levels of *R. rubi* AT3-4RS/6 present confirm the use of Celite as a viable medium for bacterial treatments (Slininger et al 2010). When statistical tests were done on the independent trials to determine if there was an influence of tryptophan on the *R. rubi* populations it was not significant but when the data was compiled from the first two trials, the difference became significant ($t=2.25$, $df=5$, $p=0.037$). A possible explanation for the difference is due to the small sample size of the data being analyzed in the independent trials and therefore increasing the sample size would help clarify the effect of tryptophan on bacterial population. Another possible explanation is that tryptophan has no effect on the population size of *R. rubi* but influences the rate of IAA-like compounds produced by the bacteria. Previous literature has shown that for other bacterial isolates (Azotobacter sp. and Pseudomonas sp.) in the presence of tryptophan produce high levels of IAA that inhibited plant growth (Ahmad et al 2005). This literature also supports our results for the variance between the treatments, with the treatments containing tryptophan and bacteria+tryptophan showing the largest decrease in both shoot length and root weight. Therefore we reject our first null hypothesis because there was significant variance of the shoot lengths and root weights between the treatments but fail to reject our second hypothesis because there was no significant difference in the average between the tryptophan and non-tryptophan treatments. Beneficial future research would be to determine if *R. rubi* and tryptophan's negative effect is selective only towards the velvetleaf weed.

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