

## Jonathon J. Ahn & Robert Zdor

<sup>1</sup>Biology Department & Biology Program, Andrews University

jonathoa@andrews.edu

J.N. Andrews  
Honors Program

### 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 velvetleaf 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 tryptophan and *R. rubi* AT3-4RS/6 treatments will be recorded and analyzed using two statistical tests (t-test, and ANOVA)

### Methodology

#### Preparation of Bacterial Formulations

- Made 5 separate treatments: one containing the bacteria, tryptophan, and celite, one containing just bacteria and celite, one containing just tryptophan and celite, one containing just celite, and one with just soil.
- Incorporated approximately  $10 \times 10^9$  cfu *Rhizobium rubi* in the treatments after culturing in tryptic soy broth by blending into mixture with soil, hyflo celite, and for one treatment 0.125 mg of tryptophan.

#### Planting/growing

- Transferred the soil treatments to ten, 10-mL test tubes for each treatment.
- Planted a surface-disinfested velvetleaf seed in each test tube
- Plants grown for a month under lights and were watered every other day.

### Methodology cont.

#### Harvesting/Analysis

- Recorded number of plants alive per treatment
- Extracted the plants from the tubes and removed the roots
- Roots were processed in groups of 3.
- Bacterial recovery scheme to assess root colonization: serial dilution and spread plating of root washings for each treatment.
- Root weight/shoot length/bacterial count for each treatment was recorded and analyzed.



Figure 1. Velvetleaf plant in test tube at 2 weeks.

### Results

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

The t-Test for Trial #1 of the bacterial population between the tryptophan and non-tryptophan treatments showed a significant difference ( $t=5.86, df=11, p=0.00005$ ) with the tryptophan bacterial count mean at 48 and the non-tryptophan bacterial count mean at 175 and for Trial #2 it also showed a significant difference ( $t=12.29, df=10, p=2.24e-7$ ) with the tryptophan bacterial count mean at 21 and the non-tryptophan bacterial count at 133.

### Results cont.

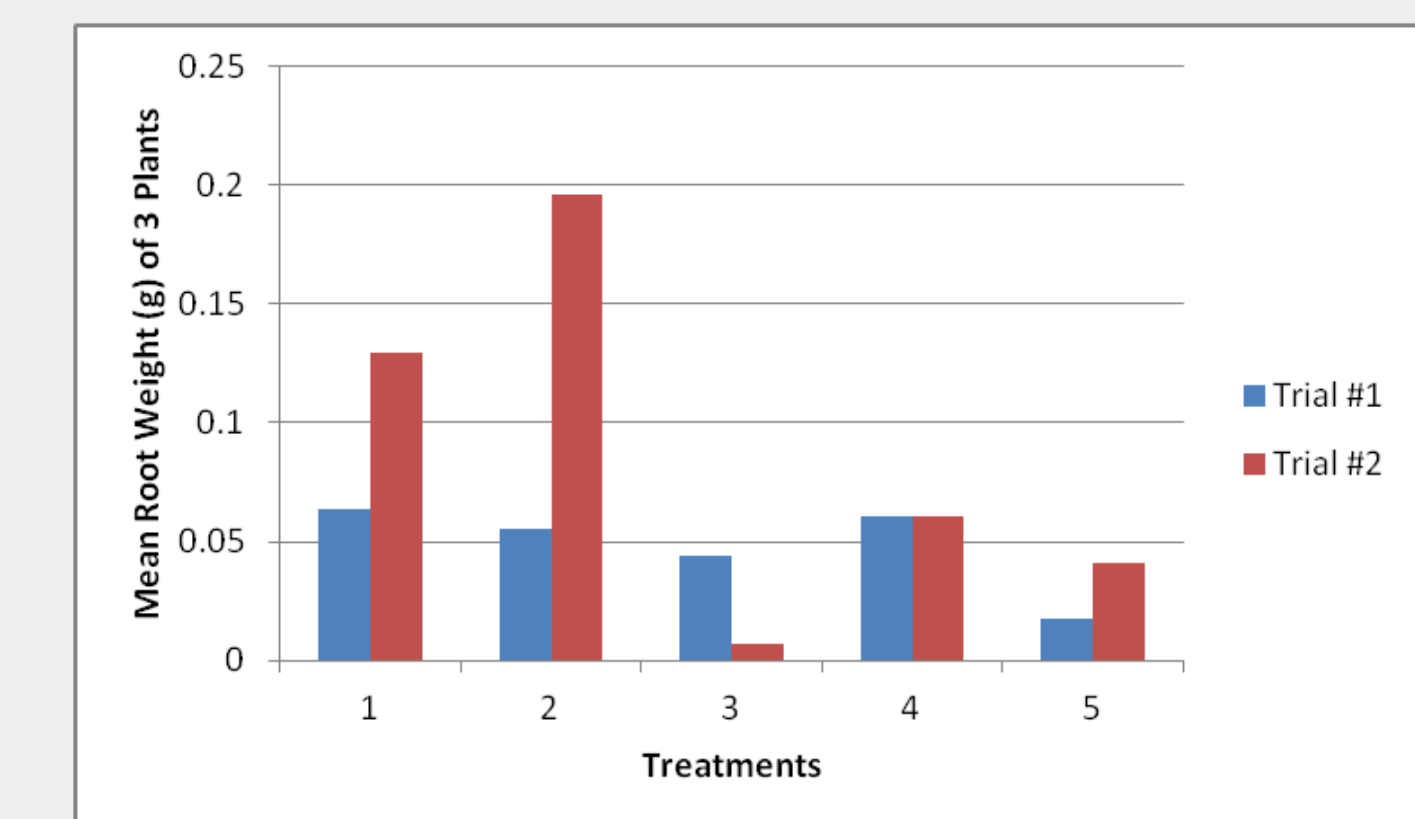


Figure 2. Average root weights of 3 groups of roots, with 3 individual roots in 1 group, for each of the five treatments. \*Treatments: 1-no soil amendment; 2-celite; 3-celite, bacteria, tryptophan; 4-celite and bacteria; and 5- celite and tryptophan

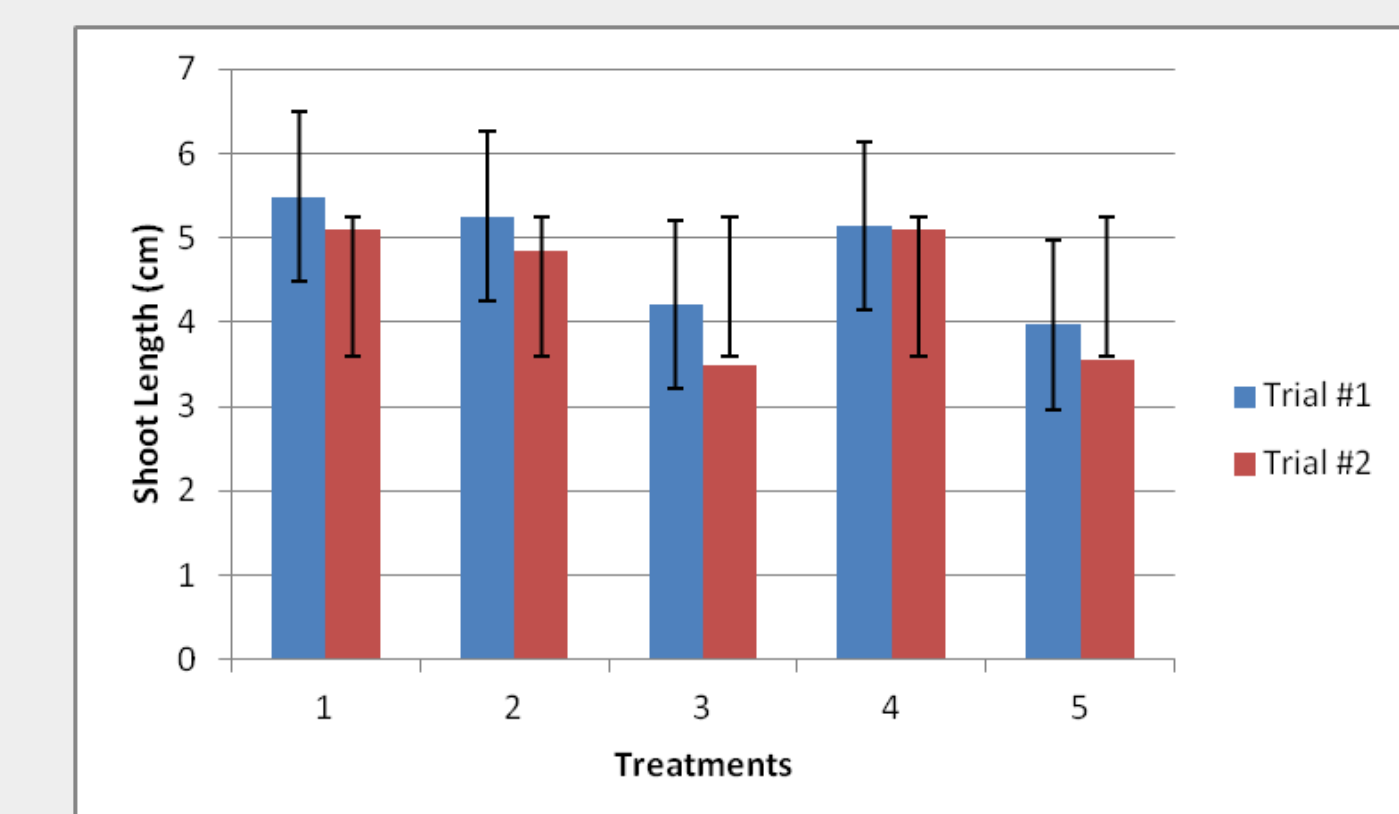


Figure 2. Average shoot lengths (cm) of 9 plants for each of the five treatments with the blue and red bars representing Trial #1 and Trial #2 respectively. \*Treatments: 1-no soil amendment; 2-celite; 3-celite, bacteria, tryptophan; 4-celite and bacteria; and 5- celite and tryptophan

### Conclusion

The experiment has confirmed the use of celite as a viable medium for bacterial treatments and the results show a significant variance between the treatments, with treatments #3 and #5 showing the greatest decrease in growth supporting the findings of *Rhizobium rubi* and tryptophan producing IAA-like compounds that inhibit growth (Brubaker and Zdor, 2009). The results of treatment 5 suggest that tryptophan alone seems to reduce plant growth. There was also a significant difference in bacterial count between the tryptophan and non-tryptophan treatments with higher bacterial count in the non-tryptophan.

#### Literature Cited

- Brubaker, H. and R. Zdor. (2009). "Velvetleaf Sensitivity to IAA and Rhizobacteria that produce IAA-like compounds." Honors.
- Kennedy, A.C., F.L. Young, L.F. Elliot, and C.L. Douglas. "Rhizobacteria Suppressive to the Weed Downy Brome." Soil Science Society of America Journal 55.3 (1990): 722-727. Print.