The Effects of Glyphosate on the Growth and Viability of *Azotobacter vinelandii*

Abstract

This experiment tests the effects of the herbicide glyphosate, commonly found in RoundUp, on important nitrogen fixing bacteria. These bacteria provide many benefits to the environment as well as to domestic plantlife. With increases in overall use of pesticides it's important to know how they interact with other organsism. The glyphosate had a significant impact on *Azotobacter vinelandii*'s growth, keeping the concentration of the bacteria at around the same level as when the media was first inoculated. This shows that the glyphosate is, even in lower concentrations, capable of inhibiting the growth and reproduction of soil bacteria species.

Hypothesis

The glyphosate will inhibit *A. vinelandii*'s growth in an agitated liquid media, with higher concentrations showing a slower growth rate and less overall reproduction.

Introduction

Azotobacter vinelandii belongs to a group of soil dwelling bacteria known for their ability to take gaseous nitrogen from the atmosphere and convert it into ammonia, a process known as nitrogen fixation. This conversion is essential for growth in plants, being the second most important nutrient directly after water [1]. *Az. vinelandii* is considered a model species for these nitrogen fixing bacteria due to its nearly global presence and adaptability, living freely in the soil as opposed to many other nitrogen fixers that live in the rhizosphere and symbiotically live with the plants [2]. As such, they are much better at withstanding changes in their environment.

Glyphosate is a nonspecific herbicide, meaning it affects a wide range of organisms, commonly found in the herbicide product RoundUp. It works by blocking the enzyme EPSP synthase which is an important enzyme for the synthesis of the amino acids tyrosine, tryptophan, and phenylalanine [3]. This enzyme isn't just found in plants though, and is common in many bacteria and fungi as well. With pesticide use on the rise, it is important to know how the chemicals being used interact with the environment as well as the plants that it's being applied to.

This experiment tested to see whether or not *Az. vinelandii* was capable of surviving in an environment where it is exposed to various levels of glyphosate significantly lower than the concentration found in RoundUp.

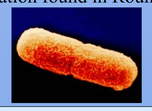

Figure 1- *Azotobacter vinelandii* is a rod shaped, gram-negative bacteria that lives freely in the soil and fixes nitrogen into the soil through the use of specialized enzymes called nitrogenases.

Materials

- *Azotobacter vinelandii* culture (Ward Science)
- Ashby's Sucrose Broth (From TOHS Lab)
- Micropipettes (From TOHS Lab)
- Activated Carbon (Sigma-Aldrich)
- Litmus paper (From TOHS Lab)
- Micropipette tips (From TOHS Lab)
- Incubator (Boekel from TOHS Lab)
- Fume Hood (Fisher Hamilton from TOHS Lab)
- Glyphosate (Sigma Aldrich)

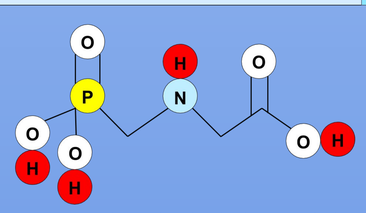

Figure 2- The molecule glyphosate mimics the shape of EPSP synthase's secondary substrate phosphoenol pyruvate.

Methods

1. Twelve 50 mL cultures
2. Six groups, two cultures for each:
   - Control of only media
   - Control with bacteria
   - 5,10,50,100 ppm glyphosate
3. Grown for 5 days at 25°
   - Orbital shaker at 100 rpm
4. 2 mL sample taken
   - 0 hours
   - 20 hours
   - 24 hours
   - 48 hours
   - 120 hours
5. Placed into cuvette and inserted into spectrophotometer at 600 nm and zeroed against DI water
6. Measured optical density
7. Cuvette removed and litmus paper dipped in
8. Rinsed with 70% alcohol
   - Cultures bleached after use

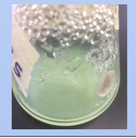

Figure 3- A standing culture of *Az. vinelandii* after being incubated for a week.

Results

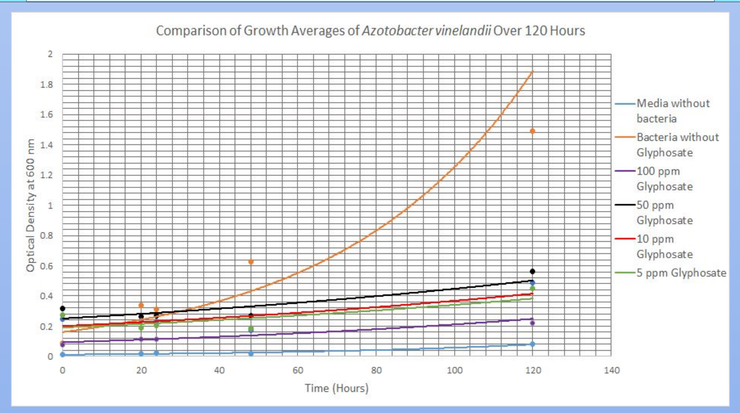

Figure 4- The growth of *Az. vinelandii* after 120 hours of incubation by measuring the optical density of the media. Displays the various concentrations of glyphosate (5, 10, 50, 100 ppm) and the two controls of purely a bacteria and broth culture and a purely broth culture. Unpaired t-test $p<0.05$.

Table 1- This table displays the pH of the media during the course of incubation.

Culture	0 hours	20 hours	24 hour	48 hours	120 hours
Media without bacteria	7.0	7.0	7.0	7.0	7.0
Bacteria without Glyphosate	7.0	7.0	7.0	7.0	7.0
5 ppm Glyphosate	7.0	7.0	7.0	7.0	7.0
10 ppm Glyphosate	7.0	7.0	7.0	7.0	7.0
50 ppm Glyphosate	7.0	7.0	7.0	7.0	7.0
100 ppm Glyphosate	7.0	7.0	7.0	7.0	7.0

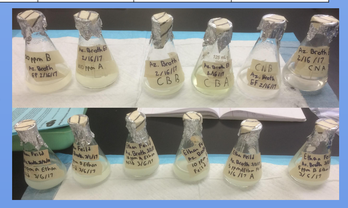

Figure 5- The twelve cultures five days post inoculation. Note the different shades of green.
Top L-R: 100 ppm, bacteria, media
Bottom: 50, 10, 5 ppm

Discussion

The results display that the control bacteria were able to grow much better than any of the glyphosate exposed cultures. As shown in **Figure 4,** the four treatments' growth rates remained relatively similar to one another with some differences in between but all were much lower than the bacterial control. The experimental cultures were far less dense than the control after 120 hours, meaning that the glyphosate was able to severely inhibit the bacteria's ability to grow and reproduce. The most likely cause of their slower growth is either the inability of the bacteria to produce aromatic amino acids or damage caused to the cell wall or the membrane by the glyphosate. Death by a drastic change in pH can be ruled out since the media remained mostly neutral within less than 0.5 of a pH of 7 (Ashby's Sucrose has a pH of 7.4±0.2).

As *Az. vinelandii*'s growth was inhibited, the assumption can be made that other soil living bacterium are vulnerable to glyphosate as well, even in concentrations smaller than that of what's commercially available for household and agricultural use. Soil and water surrounding where the pesticides were sprayed are likely to experience reduced microbial growth as well, though to a lesser degree, meaning that more than just the immediate area of application is at risk.

With the death of many important microorganisms plants will have less nitrogen to absorb and beneficial growth hormones secreted from the rhizosphere like auxins and cytokinins [4]. As such, fertilizer is needed to make up the deficit. Fertilizer is harmful to produce and can sometimes contain pesticides of their own, furthering the damage done to the natural microbial organisms living in the area. Additionally, fertilizer has been shown to be less effective at promoting plant growth and yield in the case of crops.

A possible way to help improve the diversity and health of soil would be to use more selective pesticides, ones that tend to target only specific pest species, or even to avoid using pesticides at all. By doing so the microorganisms, plants, and by extension the rest of the organisms that depend on them would benefit from a larger amount of nitrogen being added into the ecosystem.

Conclusion

Glyphosate does inhibit *Azotobacter vinelandii's* growth in a liquid culture by blocking the production of aromatic amino acids.

Further Work

There are a number of possible ways that the research could be continued, such as by testing a wider range of glyphosate concentrations, other pesticides, or by examining possible ways to induce glyphosate resistance to *Azotobacter vinelandii*.

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References

[1]Huhe, B. S. (2014). Effect of Abandonment on Diversity and Abundance of Free-Living Nitrogen-Fixing Bacteria and Total Bacteria in the Cropland Soils of Hulun Buir, Inner Mongolia. *PLoS ONE, 9*(9), 1-11.

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[4] Huhe, B. S. (2014). Effect of Abandonment on Diversity and Abundance of Free-Living Nitrogen-Fixing Bacteria and Total Bacteria in the Cropland Soils of Hulun Buir, Inner Mongolia. *PLoS ONE, 9*(9), 1-11.