Investigation of potential glufosinate-degrading ability of N-fixing soil bacteria

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ABSTRACT

Glufosinate has been used as a broad-spectrum herbicide to combat glyphosate (Roundup)-resistant plants since its introduction in 1993. Application of this herbicide has increased across the Midwest and Southern regions of the U.S. in the past decade, leading to increased soil contamination. Therefore, residual glufosinate in soil could change microbial composition and activities in the rhizosphere. *Bradyrhizobium japonicum* is a nitrogen-fixing soil bacterium that forms a symbiotic relationship with soybeans. Here, we test *B. japonicum* for its ability to thrive in a medium with higher concentrations of glufosinate-ammonium (95%). We used microliter plates to examine the growth of *B. japonicum* in the concentration range of 20-200 µM glufosinate. This study was done over a period of 48 h with monitoring the growth by measuring $O.D._{600\ nm}$ values every 2 h. The results show no significant and visible reduction in bacterial growth in the presence of higher concentrations of glufosinate up to 200 µM. This study suggests a potential tolerance of *B. japonicum* against glufosinate, which leads to further investigation on the herbicide biodegradation ability of the bacteria both in vitro and in planta.

INTRODUCTION

Bioremediation is a promising topic of research in the quest to develop environmentally sustainable methods to clean contaminated water, freshen the soil, and purify the atmosphere. It is important to identify microbes within the soil that can degrade common herbicides, such as glufosinate-ammonium, to rid the earth of potentially toxic chemicals (Fig. 1).

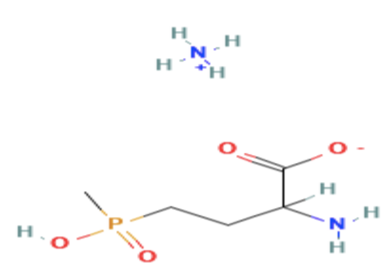

Figure 1. Chemical structure of glufosinate-ammonium

Commercial glufosinate-ammonium consists of a racemic mixture of the D- and L- forms of the phosphinothricin, a chemical metabolized by *Streptomycin* sp. ([3]). The herbicide acts as a competitive inhibitor for the enzyme glutamine synthetase within a broad range of weeds[1]. Research has shown that uptake of glufosinate into plant cells is mostly driven by diffusion rather than through a specific membrane protein channel[2]. Increased usage of glufosinate across the country has been a consequence of an expansion of glyphosate-resistant weeds (Fig. 2).

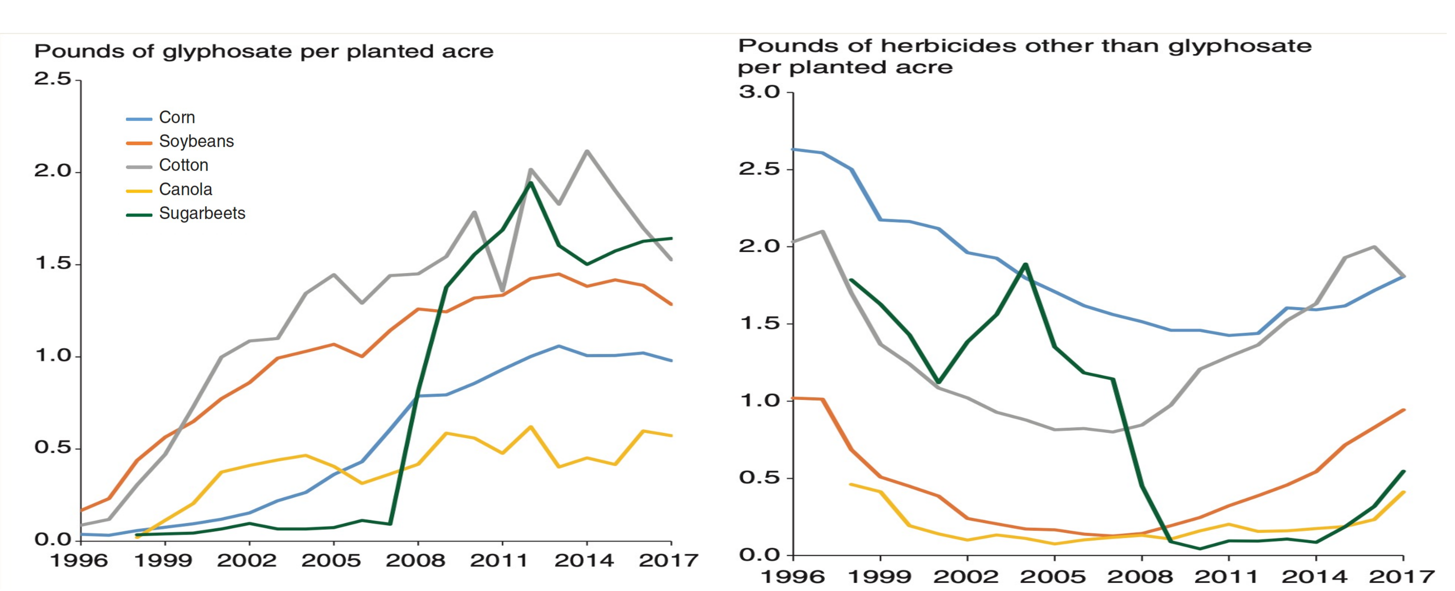

Figure 2. Comparative representation of glyphosate and non-glyphosate herbicides over the years from 1996 to 2017 (source: USDA, Economic research service, ers.usda.gov)

OBJECTIVE: To display the potential of N-fixing soil bacterium *B. japonicum* as a bioremediation microbe in glufosinate-containing media

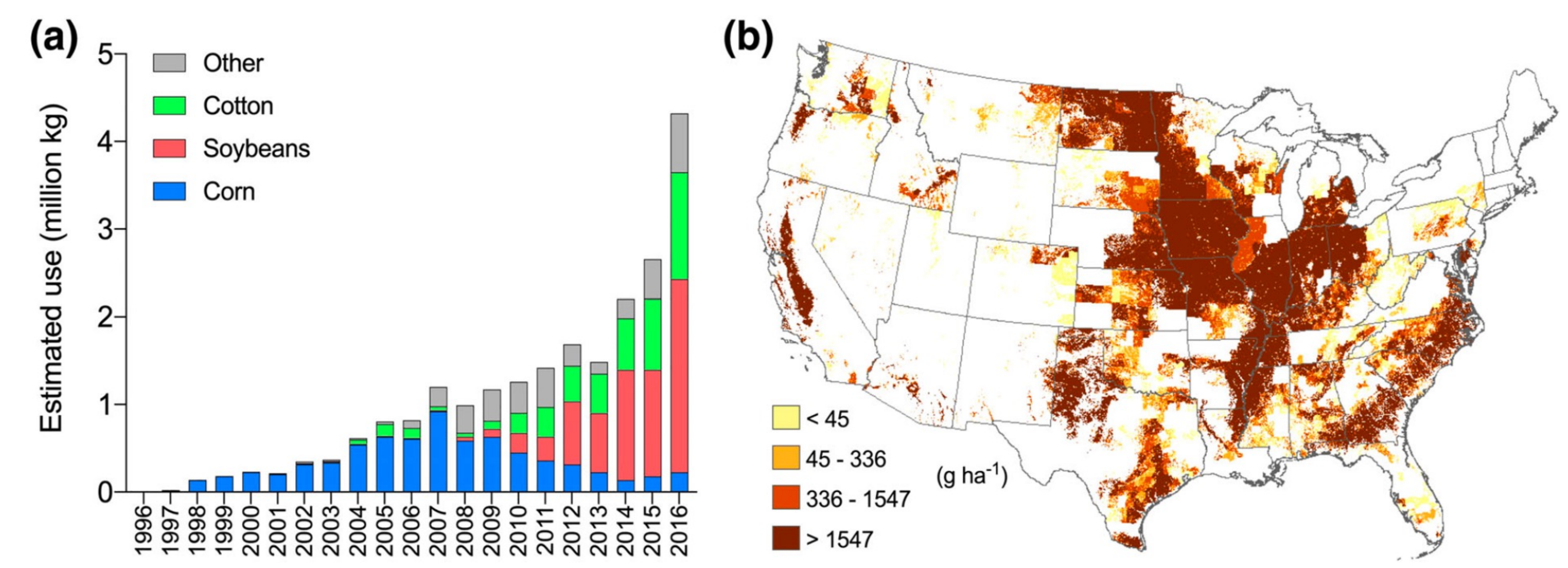

Figure 3. Estimated glufosinate usage from 1996 through 2016 in the US, here, 'other' denotes vineyard, orchard, wheat, rice, pasture, non-agricultural lands, and vegetables[3].

MATERIALS AND METHODS

Growth Curve Experiment

Grow starter culture of B. *japonicum and make glufosinate concentrations* → Construct randomized map of 96 well plate → Inoculate 96 well plate with controls, starter culture and glufosinate solutions → Start growth curve experiment in plate reader → Collect data after 48 h and analyze results

Data Analysis

- Growth curve data analyzed using Microsoft Excel and R packages growthcurver and ggplot2

Kirby-Bauer Assay

Streak AG plates with starter B. japonicum culture and make glufosinate concentrations → Inoculate sterile punch paper with control and glufosinate solutions → Place punch paper onto media within specific regions → Incubate at 30°C and interpret results after growth

RESULTS

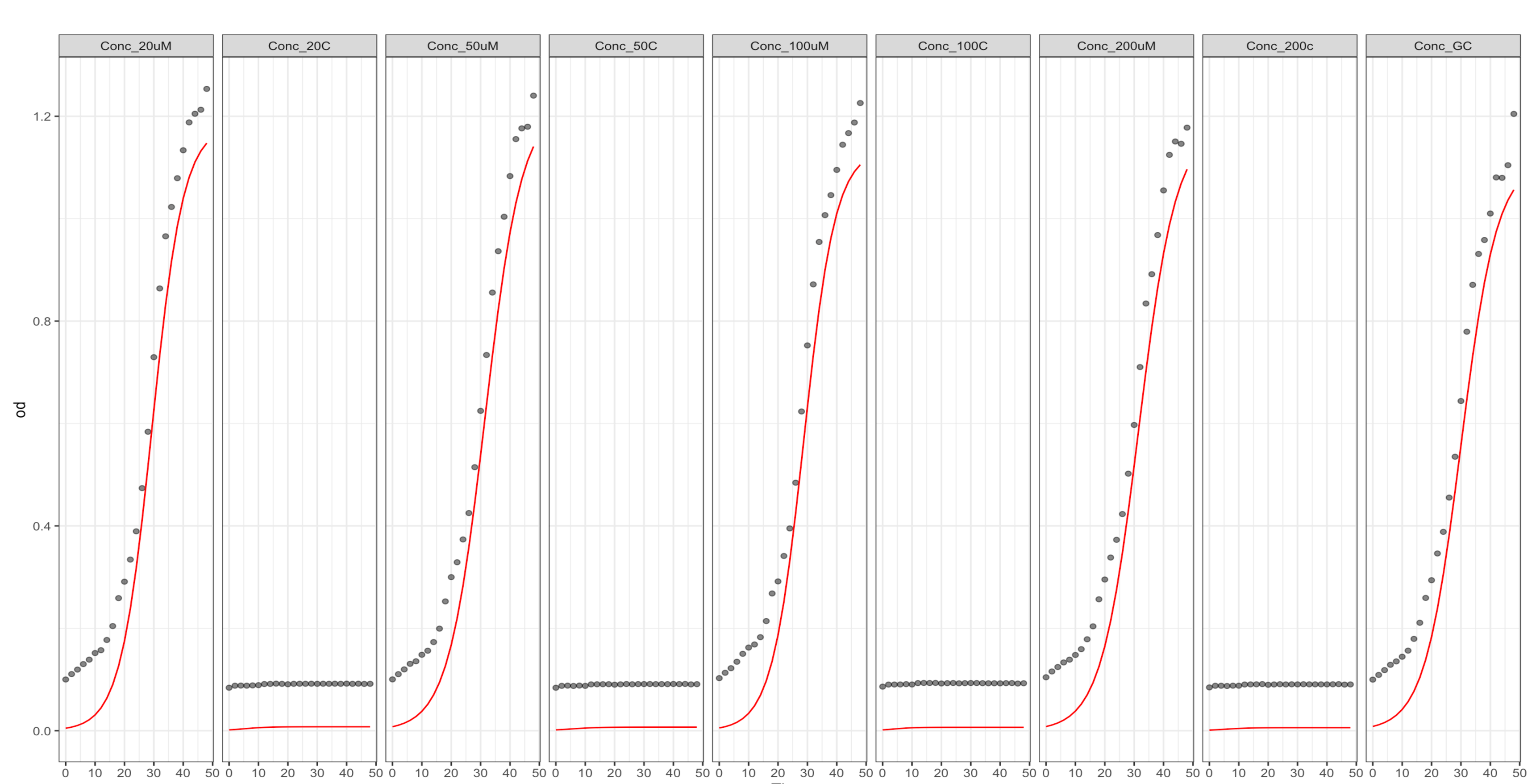

Figure 4. Graphical representation of the growth of *B. japonicum* USDA110 in 4 different concentrations of glufosinate (20, 50, 100, and 200 µM).
* C indicates no bacterial culture, while GC indicates bacterial culture without glufosinate.

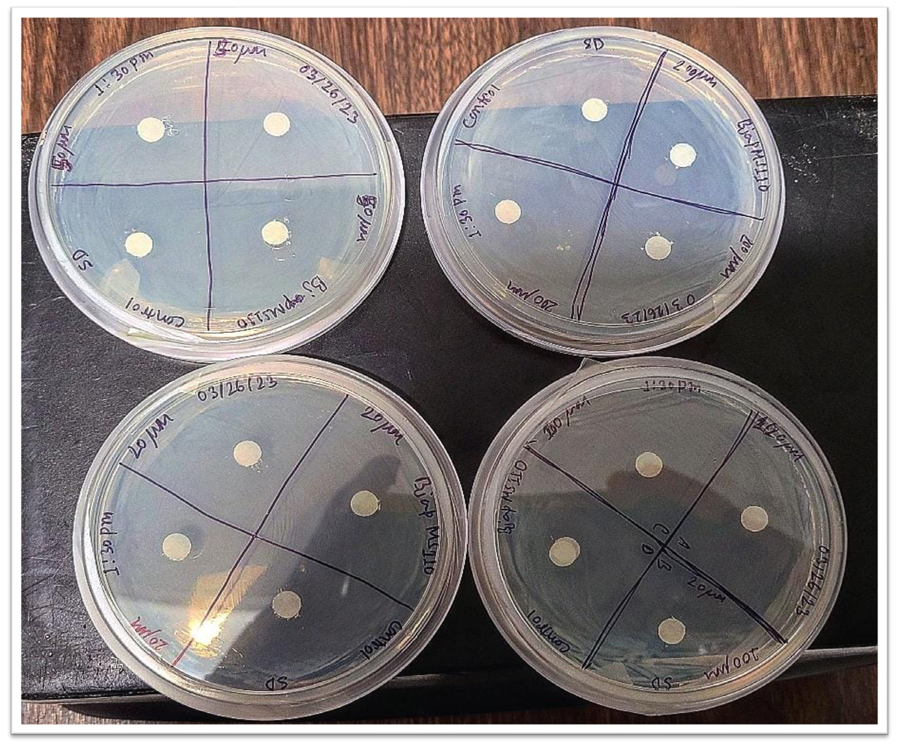

Figure 5. Pictorial representation of Kirby-Bauer assay performed with four different glufosinate concentrations (20, 50, 100, and 200 µM) on AG plates.

Table 1. Ecological characteristics of the population.

Sample (Concentration)	Carrying capacity	Initial population size (OD_{600nm})	Growth rate	Inflection point
Control	1.113	0.008	0.163	29.987
20 µM	1.183	0.005	0.187	29.379
50 µM	1.222	0.008	0.160	31.496
100 µM	1.137	0.005	0.185	28.793
200 µM	1.179	0.008	0.157	31.557

CONCLUSIONS

- The growth study experiments and the Kirby-Bauer assay of *B. japonicum* USDA110 shows tolerance to high concentrations of glufosinate-ammonium.

FUTURE WORK

- Growth study using higher concentrations of glufosinate-ammonium to examine the MIC (minimum inhibitory concentration) values of the herbicide.

REFERENCES

1. Gaines TA., Duke SO., Morran S., Rigon CAG., Tranel PJ., Küpper A., and Dayan FE. (2020). Mechanisms of evolved herbicide resistance. J Biol Chem. 295(30): 10307-10330.
2. Takano HK., Beffa R., Preston C., Westra P., and Dayan FE. (2020). Physiological Factors Affecting Uptake and Translocation of Glufosinate. J Agric Food Chem. 68(10): 3026-3032.
3. Takano HK. And Dayan FE. (2020). Glufosinate-ammonium: a review of the current state of knowledge. Pest Manag Sci. 76: 3911-3925.