Determining DNA Sequence Variations Potentially Associated with Herbicide (RoundUp) Resistance in *Chenopodium ficifolium*, a Weedy Relative of Quinoa

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Introduction

The genus *Chenopodium* includes quinoa (*C. quinoa*), and the common weed lambsquarters (*C. album*) found across the United States. Many farmers use the herbicide Roundup® to quickly kill weeds such as *C. album* in an efficient manner. Frequent use of glyphosate, the active ingredient in most Roundup products, has resulted in human imposed selective pressure for glyphosate resistance in *C. album* and other weed populations throughout the United States.

Glyphosate works by inhibiting the EPSP synthase enzyme, which is the sixth step in the shikimate anabolic pathway, which is a process in plants that synthesizes aromatic amino acids that are essential for life. However, in plants the gene(s) for EPSP synthase production can mutate, changing the structure of EPSP synthase, and preventing glyphosate from effectively binding to the enzyme and disabling it. I am using *C. ficifolium*, a diploid relative of tetraploid quinoa and hexaploid *C. album*, as a model system in which to study the evolution of glyphosate resistance.

Glyphosate is also a subject of high controversy as it has been linked to cancer in people who have been exposed to it. As a result, Bayer (who owns the rights to glyphosate has experienced many lawsuits

Figure 1: Typically bought container of Roundup®.

Objectives

- Isolate a glyphosate resistant mutant or population of *C. ficifolium*.
- Establish differences in EPSP synthase gene sequence between resistant and susceptible populations.

Methods

- Two *Chenopodium ficifolium* accessions collected from Quebec City (QC) Canada, and Portsmouth (P) NH were grown in the UNH Macfarlane Greenhouses.
- Glyphosate tolerance differences were determined across populations.
- The EPSPS gene DNA was sequenced in both QC and P.
- Sequences were analyzed to identify possible causative differences between QC and P.
- Glyphosate application performed by Luke Hydock, licensed pesticide applicator.
- Safe handling of all research materials consistent with glyphosate label requirements.

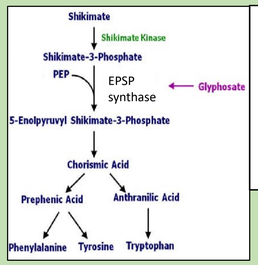
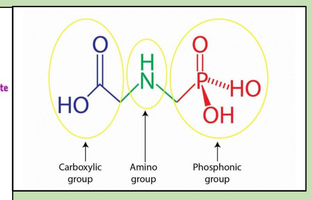


Figure 2: The shikimic anabolic pathway step that glyphosate inhibits.



Glyphosate molecule

Figure 3: Quebec City population (left side) next to a Portsmouth population (right side) one week after glyphosate application.

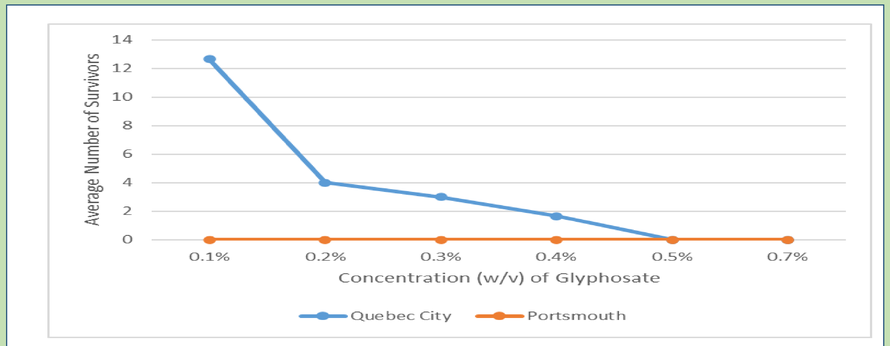


Figure 4: The average number of survivors between the Portsmouth population and the Quebec City population when dosed with the corresponding glyphosate concentrations.

Figure 5: The results of the genetic analyses in the *C. ficifolium* populations from Portsmouth (P) and Quebec City (QC). Both of these polymorphisms are in predicted regulatory elements of the promoter region of the EPSP synthase gene

Screening Results

- The Quebec City population displayed greater resistant to glyphosate than the Portsmouth population (Figure 4).
  - Portsmouth had a 100% mortality rate at the lowest concentration (0.1% w/v).
  - Quebec City was tolerant of concentrations up to 0.4% (w/v).

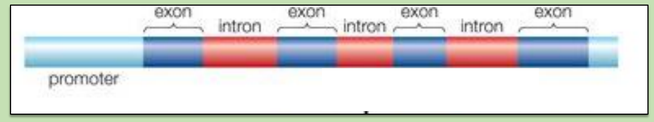


Figure 6: The general structure of a eukaryotic gene. The promoter regulates gene expression, and the exons contain the genetic code for the amino acid sequence of the encoded protein.

Genetic Analysis and Results

- From the DNA sequence data of the QC and P populations, three possible mechanisms of resistance were investigated:
  - EPSPS amino acid sequence variation
  - Polymorphisms in the EPSPS gene promoter region
  - Copy number variation creating over-expression of EPSPS in resistant populations
- There were no differences between QC and P in the predicted amino acid structure of the EPSP synthase enzymes, or in gene copy number.
- Two potential causative differences were identified in predicted regulatory sites in the promoter regions between both populations (Figure 5, red ovals).

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Macfarlane Greenhouse Staff

Future Directions

- Analyze a Mendelian F_2 to observe segregation and correlate glyphosate resistance to suspected resistant alleles.
- Compare glyphosate resistance in the tested Quebec City population to available populations from Switzerland and Florida.