Plants Topic: **The Mechanisms of Weed Resistance to Glyphosate**

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Glyphosate (**N**-phosphonomethylglycine), is common an herbicide that inhibits the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Scientists have used CRISPR/Cas9 to make crops like rice resistant to glyphosate and even though this has a small success rate this resistance is passed down the next generation after many iterations of DNA shuffling. (1,2) Once an herbicide, like glyphosate, is distributed to a population of weeds, like rygrass, there is small chance that those weeds will survive and reproduce. (3) Over many cycles of reproduction ryegrass will produce offspring that gradually apply a "weak" mechanism to become more resistant to glyphosate and distribute glyphosate all the way to its leaf tips instead of letting it stay in the roots. (4) In ryegrass, there is also a "strong" resistance mechanism that results in a nucleotide mutation in the EPSPS gene at position 301 that changes cytosine to thymine. This resulted in a change in the amino acid code from proline to serine. (5) This is a problem because this means that glyphosate would become useless as an herbicide. Luckily, researcher have found that is not the case. In plants like ryegrass, there are many factors (e.g. height, seed production) that determine fitness that are affected once the plant becomes resistant to glyphosate. This results in the glyphosate sensitive biotype to have a competitive advantage over the resistance ryegrass. (6)

References

Article below was found using Pub Med search (Glyphosate AND CRISPR):

1. Gene replacements and insertions in rice by intron targeting using CRISPR-Cas9.
    a. Jun Li, Xiangbing Meng, Yuan Zong, Kunling Chen, Huawei Zhang, Jinxing Liu, Jiayang Li, Caixia Gao Nat Plants. 2016; 2: 16139. Published online 2016 Sep 12. doi: 10.1038/nplants.2016.139

   This is a study done by researchers in china who used CRISPR/Cas9 who achieved gene replacement in endogenous gene EPSPS at a 2.0% success rate in rice plants. This is intended to make the plants resistant to glyphosate (herbicidal ingredient in Round Up) and it was successfully passed down to the next generation. This technique can be used in other plants too.

   Articles below were referenced in:
   Pollegioni, L., Schonbrunn, E., & Siehl, D. (2011). Molecular basis of glyphosate resistance-different approaches through protein engineering. *The FEBS journal, 278*(16), 2753–2766. doi:10.1111/j.1742-4658.2011.08214.x

2. Castle LA, Siehl DL, Gorton R, Patten PA, Chen YH, Bertain S, Cho HJ, Duck N, Wong J, Liu D, Lassner MW. Discovery and directed evolution of a glyphosate tolerance gene. Science. 2004;304:1151–1154.

   This article discusses the use of glyphosate N-acetylation and how plants don't become tolerant to glyphosate after many iterations of DNA shuffling. This was true in the case of four different types of plants and proves that glyphosate acetylation provides an alternative strategy for supporting glyphosate use in crops.

   The article below was referenced in:
   Powles S. B. Evolved glyphosate-resistant weeds around the world: lessons to be learnt. Pest Manag. Sci. 2008, 64, 360–365.

3. Broster, JC and Pratley, JE, A decade of monitoring herbicide resistance in *Lolium rigidum* in Australia. *Aust J Exp Agric* **46**: 1151– 1160 (2006).
   The research done in this article is self-explanatory based on the title. They first found that the species of grass was resistant to at least one herbicide in South Wales. They were then tasked with surveying the same grass in Australia to see if they had become resistant also. They defined each herbicide as a group with glyphosate being labeled as "Group M" and it had the lowest level of resistance found. They also mentioned that the environmental factors in Australia as well as cultural practices played a role in their results.

4. Powles, SB, Lorraine-Colwill, DF, Dellow, JJ and Preston, C, Evolved resistance to glyphosate in rigid ryegrass (*Lolium rigidum)* in Australia. *Weed Sci* **46**: 604– 607 (1998).
   The researchers in this article found that a population of rigid rye grass had become resistant to glyphosate after 15 year of successful use. They found that the resistance has increased to about 7-to-11 fold and concluded that there needs to change in the patterns of the glyphosate usage.

The articles below were referenced in:
Powles, S., & Preston, C. (2006). Evolved Glyphosate Resistance in Plants: Biochemical and Genetic Basis of Resistance. *Weed Technology, 20*(2), 282-289. doi:10.1614/WT-04-142R.1

5. Simarmata, M., & Penner, D. (2008). The Basis for Glyphosate Resistance in Rigid Ryegrass (Lolium rigidum) from California. *Weed Science, 56*(2), 181-188. Retrieved from http://www.jstor.org/stable/25148501
   The researchers collected the most glyphosate resistant rigid ryegrass plants from northern California and the most sensitive. They found that the resistant plants had become tolerant to glyphosate by a nucleotide mutation in the EPSPS gene at position 301 that changed cytosine to thymine. This resulted in a change in the amino acid code from proline to serine.

6. Fernández-Moreno, P. T., Alcántara-de la Cruz, R., Smeda, R. J., & De Prado, R. (2017). Differential Resistance Mechanisms to Glyphosate Result in Fitness Cost for *Lolium perenne* and *L. multiflorum*. *Frontiers in plant science*, *8*, 1796. doi:10.3389/fpls.2017.01796
   This research shows the same change in amino acid from proline to serine of the glyphosate resistant ryegrass. They observed the reproductive fitness of the wild type and resistant biotype and found that when there is no selective pressure the R biotype is at a competitive disadvantage. This is because there is are difference in their fitness like height and seed production.