

# Examining 14C Glyphosate Translocation to Classify Glyphosate Resistant Weeds

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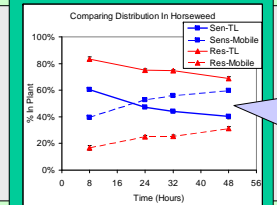


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## Abstract

The mechanism used by a glyphosate-resistant weed is always an essential discussion. Do you think its target-site? is probably the most common question. The facts seem to say it usually is not, and if it is, like for ryegrass and goosegrass, the resistance is of very low magnitude (2-3X) and comes with a reproductive penalty. Everyone seems to know that glyphosate is rarely metabolized so the second question is "What is the mechanism of resistance?" We have developed a scheme to classify resistant weeds into these three principal categories and have implemented the protocol against *Amaranthus palmeri* (Palmer pigweed) using mare's tail as a standard for the exclusion mechanism (restricted glyphosate delivery). Our data supports the premise of the scheme in mare's tail but, it shows that the resistance mechanism in Palmer pigweed requires a new hypothesis since translocation is the same in R and S lines

What we have seen before *Amaranthus sp.*, The Exclusion mechanism in Mare's tail was revealed by less translocation and less shikimate formation.



8 24 32 48 Hours **5ug dose**

## Methods

- 14C Glyphosate as 0.5 µL drops on oldest leaf or 2 leaves, 10 drops, formulated with UltraMax at use the rate applied to 4-6 plants, 4-5 leaf stage.
- Treated Leaves(TL) removed and washed at time (Tn).
- Plant dissected, above the TL (apex and stem), below TL (to soil) and roots.
- Fresh weights taken, extracted with 0.1N Sulfuric in 2 cycles of freeze thaw and counted and sampled for HPLC.
- HPLC, C18 µ-Bondapak, 1mL/min, 6mM Phosphoric acid for Shikimate at A220nm.



*Amaranthus palmeri*, Palmer pigweed was from Dr. Stanley Culpepper, Macon, GA  
*Amaranthus rudis*, Waterhemp was from Dr. Keven Bradley, NWMO1.

## Hypothesis

- Premise is: That glyphosate resistance is confirmed in Shikimate/Glyphosate ratio where  $R \neq S$  (if they are equal then there is NO resistance)
- A single common mechanism is a significant concern, so can we characterize the mechanism in a simple experiment?
- 14C glyphosate translocation, allows for Metabolism check, examination of translocation efficiency, determination of shikimate/glyphosate ratio, all in one.
- We presumed translocation would always be less (exclusion like mare's tail) or more translocated like Target site mutants allow (weak Roundup Ready).

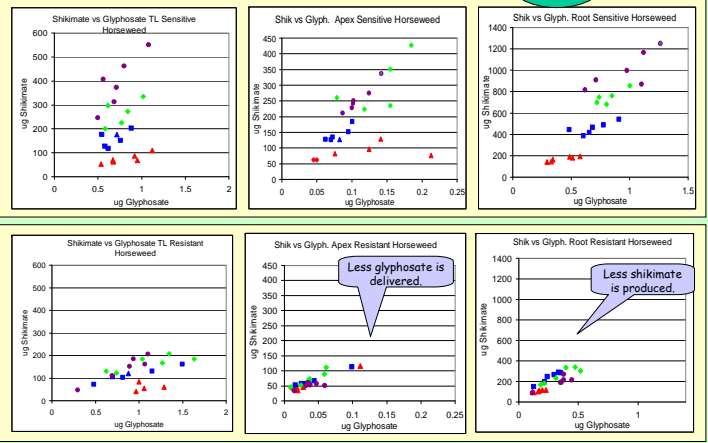
Based on results in RR soy we predicted translocation efficiencies relative to dose, We expected opposite trends with increasing concentration

## CONCLUSIONS

1. Suspected glyphosate resistant *Amaranthus sp.* (*palmerii* and *rudis*) are glyphosate resistant, less shikimate is produced per unit glyphosate.
2. 14C-Glyphosate translocation is the same in Resistant and Sensitive Palmer pigweed and waterhemp.
3. No significant change in translocation efficiency over a factor 20 fold in glyphosate concentration, but still very little toxicity observed. (Means we need a higher dose.)

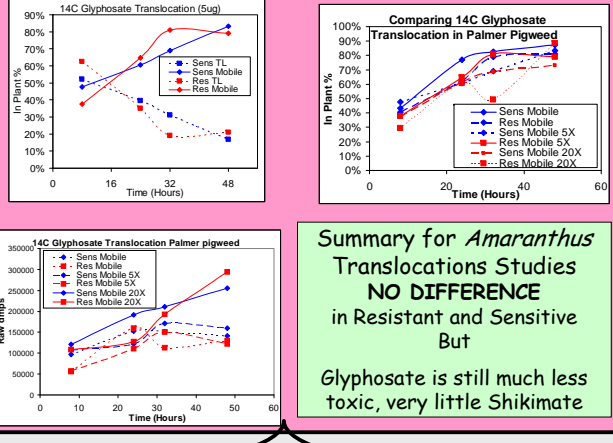
## New Hypothesis

- 1.) Translocation is a "red herring", because translocation efficiency is primarily dependent on how sucrose loads the phloem, symplastic or apoplastic.
  - Symplastic loading allows for immediate feed back of sucrose respiration from sink tissue, hence "self-limitation" of glyphosate is quickly evident.
  - Apoplastic loading is 'dis-connected' from the rate of respiration and sucrose metabolism and so may not respond as quickly to glyphosate toxicity.
- 2.) Exclusion mechanism can still operate in a "apoplastic" loading plant, eg. *Amaranthus*(?)
- 3.) Re-Focus on whether glyphosate is active in the chloroplast or not
  - If not then must be a Target -Site mutant or a chloroplast exclusion system.
  - Need to assay EPSPS to find out.
  - If it is active then must be a vacuolar exclusion mechanism.

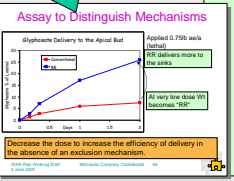


Less glyphosate is delivered.

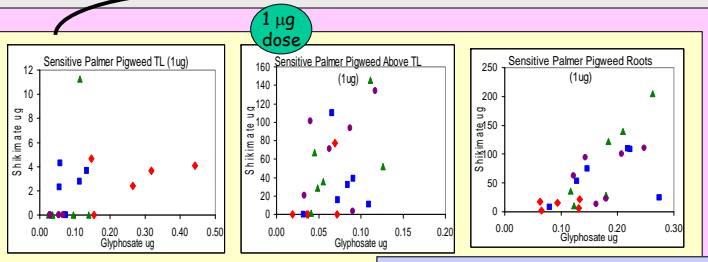
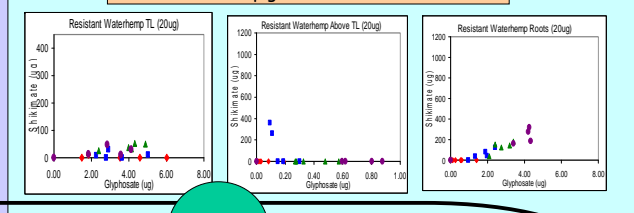
Less shikimate is produced.



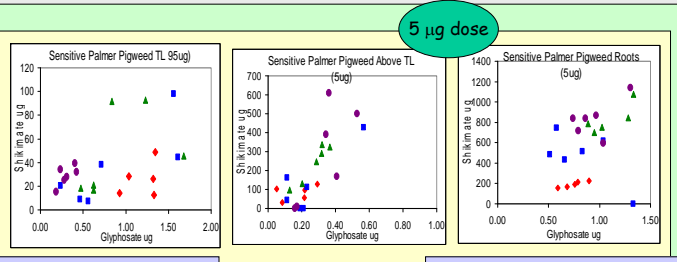
Summary for *Amaranthus* Translocations Studies NO DIFFERENCE in Resistant and Sensitive But Glyphosate is still much less toxic, very little Shikimate



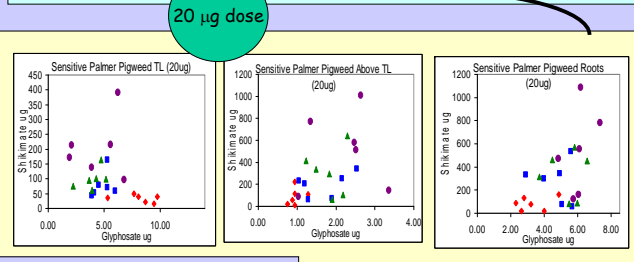
## Resistant Waterhemp (NWMO1) Behaves like Palmer pigweed from Macon



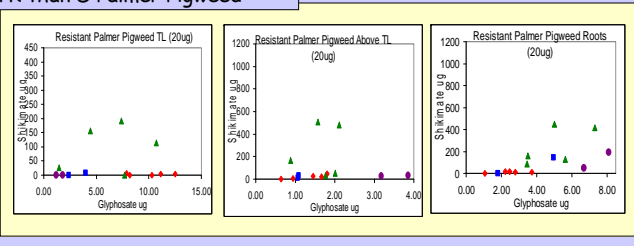
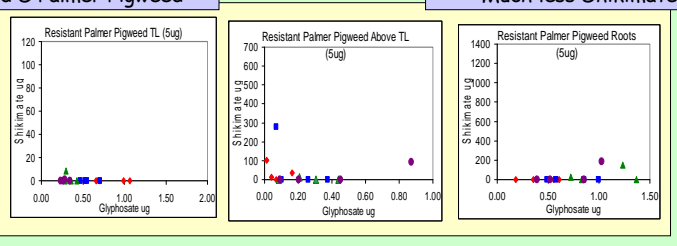
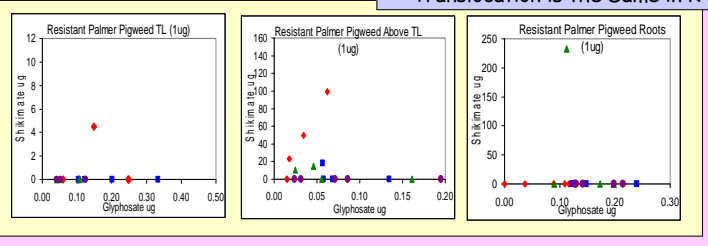
**1 µg dose**



**5 µg dose**



**20 µg dose**



Translocation is the Same in R and S Palmer Pigweed

Much less Shikimate in R than S Palmer Pigweed