Characterising glyphosate resistance in *Amaranthus palmeri*

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Summary The unit toxicity of glyphosate is correlated to the shikimate to glyphosate ratio (S/G). Comparing the S/G values for mobilised glyphosate in resistant (R) and sensitive (S) plants is decisive evidence of resistance. The distinctly different values for S/G in glyphosate-resistant *Amaranthus palmeri* Wats., confirmed resistance. Further, we found equivalent glyphosate translocation in R and S lines suggesting translocation efficiency alone does not characterise the resistance mechanism.

Glyphosate inhibition of the shikimate biosynthetic pathway results in accumulation of shikimate and thereby serves to measure glyphosate toxicity (Amrhein *et al*. 1980). The S/G ratio then allows for comparisons of glyphosate toxicity and to decisively identify resistant plants. We designed whole plant translocation experiments where phloem mobilised glyphosate could be examined for toxicity in sink tissues (Feng *et al*. 2004). The movement of glyphosate to sink tissues ensured that only symplast-active glyphosate was being evaluated to characterise the resistance mechanism.

The use of ^{14}C glyphosate as described by Feng *et al*. 2004 allows a careful inventory of uptake, mobilisation and quantification of glyphosate concentration in sink tissues. Hence, the potency of glyphosate could be measured by quantifying with HPLC the shikimate in the corresponding tissues (Lydon and Duke 1988). Because the comparison of R and S plants at toxic levels of glyphosate can invoke disproportionate levels of toxicity and very different toxic effects, very low doses of glyphosate are used to observe the nascent resistance mechanism. Palmer pigweed obtained from Dr S. Culpepper, Macon Georgia was treated at the five leaf stage with 1, 5 or 20 µg of glyphosate in ten 1 µL drops on the first leaf in 5–6 replicates. Plants were harvested at 8, 24, 32 and 48 hours by washing the treated leaf (TL) and dividing the plant into above and below the treated leaf and root portions. Fresh weights were obtained and tissues extracted with 0.2N sulphuric acid in two freeze thaw cycles. Extracts were evaluated by HPLC for shikimate and counted for ^{14}C-glyphosate. Overall a statistically equal average amount of glyphosate was found at each dose and time point in each tissue for R and S Palmer pigweed. In the R biotype only background shikimate was measured at the 1 and 5 µg doses. The 20 µg dose treatment showed substantially lower S/G for the R biotype with statistically equivalent amounts of glyphosate (Table 1).

Table 1. Comparing S/G in R and S Palmer pigweed.

48 hours	S		R	
	G µM	S/G	G µM	S/G
Above TL	25 ± 6	340 ± 60	60 ± 30	5 ± 3
Roots	80 ± 7	115 ± 14	80 ± 5	15 ± 12

± standard error of the mean.

The equivalence of the mobilised glyphosate result for R Palmer pigweed contrasts to the restricted translocation of glyphosate in R *Conyza canadensis* Cronq., (Feng *et al*. 2004). We have also shown that R and S Palmer pigweed EPSPS's are equally sensitive to glyphosate ruling out an altered target site. The difference in glyphosate translocation efficiency in the two weeds might arise from species differences in phloem loading and assimilate flow if the phloem source-sink feedback mechanisms are different. Glyphosate toxicity does not limit its phloem transport in *Amaranthus palmeri*. The resistance mechanism in horseweed and Palmer pigweed might still be the same.

Keywords Glyphosate-resistance, shikimate, EPSPS, *Amaranthus palmeri*, Palmer pigweed.

REFERENCES

Amrhein, N., Deus, B., Gehrke, P., Hollander, H., Schulz, A. and Steinrucken, H.C. (1980). Glyphosate inhibits chorismate formation in vivo and in vitro. *Plant Physiology* 65, 97.

Feng, P.C.C., Tran, M., Chiu, T., Sammons, R.D., Heck, G.R. and CaJacob, C.A. (2004). Investigations into glyphosate-resistant horseweed (*Conyza canadensis*): retention, uptake, translocation, and metabolism. *Weed Science* 52, 498-505.

Lydon, J. and Duke, S.O. (1988). Glyphosate induction of elevated levels of hydroxybenzoic acids in higher plants. *Journal of Agricultural and Food Chemistry* 36, 813-18.