COMPARING SHIKIMATE PRODUCTION IN GLYPHOSATE RESISTANT WEEDS. R. Douglas Sammons, Amanda Ohs, Robert Eilers, and William Gruenloh. Monsanto Co. 700 Chesterfield Parkway West, Chesterfield, MO 63017.

The conclusion that a particular weed is resistant to glyphosate is important to a weed management program. A definitive procedure is recommended at www.weedscience.com however, the steps outlined there can take considerably more time then a current weed problem allows for a best weed control option. Several reports (Shaner et al. 2005, Koger et al. 2005) describe a quick determination of shikimate in leaf samples as a means to identify glyphosate resistance. The idea being that surviving weeds might be quickly assayed to determine if they are glyphosate resistant so an appropriate follow-up treatment can be prescribed. The assay reported is fairly simple using a colorimetric assay developed by Cromartie and Polge 2002. The method relies on glyphosate inhibition to create high levels of shikimate (or not for resistant plants) which are extracted and then quantified by chemical conversion to a unique chromophore absorbing at 382 nm. We were interested in whether this method would work for weeds that are weakly resistant to glyphosate e.g., goosegrass (Malaysia) or Italian ryegrass (Chile) where control can be achieved with 2-3 times the label rate of glyphosate (1.6-2.4 kg ae ha^{-1}). Secondly, we wanted to see if the method was generally applicable to various species where the resistance level is higher e.g. in the 6-10 times the labeled rate range (4.8-8 kg ae ha^{-1}). Finally, we examined glyphosate dose and shikimate production with respect to time and confirmed all colorimetric measurements by HPLC analysis directly (Lydon and Duke 1988). The HPLC method isolates shikimate from the crude extract directly as a single compound identifiable by its UV spectrum at 210-240 nm.

The colorimetric assay gave mixed results compared to the HPLC method. In a well behaved system, for example horseweed, the colorimetric assay was similar to the HPLC result in the range up to 2.0 absorbance units. Some plant extracts however, were not well behaved. For example; Chilean Italian ryegrass (and goosegrass too) had variable glyphosate induced shikimate production which was not stable with time for several dose regimes in the 1-100 mg glyphosate ae L^{-1}. Italian ryegrass had a colorimetric impurity that was significant enough to prevent simple blank subtraction and there was also significant shikimate present in untreated plants. *Amaranthus* samples were variable with some plants containing a colorimetric impurity and others did not while background shikimate could be found in some untreated plants. This variability of shikimate background in untreated control plants did not correlate to glyphosate sensitivity or resistance.

In conclusion, the colorimetric assay can be used on some species if several precautions are observed. There are important variables in the glyphosate incubation with respect to buffer where 2-(4-morpholino)-ethane sulfonate (MES) made resistant horseweed leaf dics behave as if they were sensitive. Whereas the recommended ammonium phosphate buffer made all leaf samples more tolerant to glyphosate. Further, the fold resistance and dose interact with time (or light quality) so that incubation period must be lengthened to 48 hours for more certain results. With respect to shikimate analysis; species with high backgrounds of a colorimetric contaminant or background shikimate will be problematic. In these studies Italian ryegrass from Chile (2-3X label rate) and Brasil (6-8X label rate) could not be definitively labeled resistant or sensitive. Secondly, *A. palmerii* contained shikimate in untreated plants making a definitive test difficult. Thirdly some species, Johnsongrass (Argentina) had variable background shikimate and/or colorimetric contaminants such that individual plants were unique making it difficult to compare plants and determine glyphosate sensitivity.

2006 North Central Weed Science Society Proceedings 61:63.

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