ONGOING INVESTIGATIONS INTO GLYPHOSATE RESISTANT HORSEWEED: RESISTANCE MECHANISM STUDIES. Gregory R. Heck, Sophia Y. Chen, Tommy Chiu, Paul Feng, Jintai Huang, Chris S. Hubmeier, Youlin Qi, R. Douglas Sammons, Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, MO 63198.

Genetic studies have continued on glyphosate resistant horseweed (*Conyza canadensis*) isolated from the Delmarva region. Reciprocal F1 crosses between sensitive (S) and resistant (R) biotypes were monitored for growth reduction over a glyphosate rate titration. Relative to the R parent, both crosses transmit the trait equally and in dominant fashion revealing a nuclear-encoded basis for resistance. F2 analysis of 200 individuals/rate (using a titration series, 0.42 – 3.38 kg ae/ha) showed a 3:1 segregation (R:S) indicating a single dominant locus accounts for the majority of resistance. F1 backcrosses [S x (S x R)] with 50 individuals/rate confirmed a single dominant locus with a 1:1 segregation ratio.

Analysis of the S biotype showed additional genetic variation within the sensitivity of individuals from this biotype. An approximately 2X difference in sensitivity was apparent in the S1 (most sensitive lineage, controlled at 0.25X field rate or 0.21 kg ae/ha) vs. S2 (less sensitive lineage, controlled at 0.5X) lineages. This differential in response could be transmitted in a dominant or semi-dominant fashion to F1 progeny (S1 x S2). F2 progeny are being examined and crosses to the R parent (S2 x R) for allelism testing have been completed.

Three EPSPS genes have been isolated from horseweed, EPSPS 1-3. An active site variant, P106T (proline typically found at the 106 position is substituted with threonine), in EPSPS-3 was identified in both S and R biotypes from Delaware. This variant would be predicted to have some glyphosate resistance based on biochemical studies of petunia and maize EPSPS active site mutants, however, it has not been characterized in vitro. Surveys of biotypes across the country (including: R biotype from DE and TN; and S biotypes from CA, DE, IA, MI, MO, NC, and WA) all had the EPSPS-3 gene bearing P106T. This form of EPSPS is thus a feature of the species and not unique to resistant biotypes (a novel observation for the well-conserved EPSPS active site of higher plants). In addition, an isolate from Walla Walla, WA also had a M104L change (i.e. M104L and P106T both in EPSPS-3) relative to other horseweed isolates. We are currently expressing EPSPS-3 for in vitro biochemical characterization. It is unclear how EPSPS-3 could contribute significantly to resistance since it is found in both biotypes. Minimally, EPSPS-3 cannot function independently from the genetically defined locus to convey resistance.

Previously, we had shown that resistance does not appear to be based on differential glyphosate uptake, glyphosate metabolism, differential gene expression of EPSPS, or amplification of EPSPS genes. One physiological distinction noted was differential translocation of glyphosate to roots. We confirmed this using a sub-lethal rate (0.026 kg ae/ha to minimize glyphosate toxicity effects on transport) of ^{14}C-glyphosate applied with a track sprayer. Although comparable amounts of glyphosate entered leaves, S lineages translocated approximately 2-fold more glyphosate to roots relative to R lineages. Even though we do not know how this differential is translated to the larger differential in whole plant resistance of R vs. S biotypes, it is a signature feature imparted by the resistance locus. Analysis of glyphosate resistant horseweed is continuing to fully understand the mechanism of glyphosate resistance.