

CHARACTERIZATION OF THE MECHANISM OF GLYPHOSATE RESISTANCE IN HORSEWEED (*Conyza canadensis*). Greg Heck, R. Douglas Sammons, Paul Feng, Greg Bunkers, Minhien Tran, Murtaza Alibhai, Youlin Qi, Stanislaw Flasinski, Marian Malven and Chris Hubmeier, Molecular Biologists and Physiologists – Agronomic Traits. Monsanto Company, 700 Chesterfield Parkway West, Chesterfield, Missouri 63017.

Conyza canadensis (horseweed, maretail, ERICA) is a winter or summer annual indigenous to North America that is readily dispersed and is now represented in both the New and Old World in a wide variety of agricultural systems. The appearance of glyphosate resistant horseweed in 2001 represented the first dicot species to be recognized as resistant to this herbicide. We have undertaken a series of studies to determine how this weed achieves resistance. We have previously established that a single dominant genetic locus accounts for biotypes with a glyphosate LD₅₀ of 7-8X of the 1X use rate (0.84 kg ae ha⁻¹). We have also demonstrated that the mechanism is not due to metabolism of glyphosate.

Examination of the EPSPS genes of horseweed as candidates for the resistance locus has shown that three genes are present in the genome. Two of these genes (EPSPS1 and EPSPS2) are likely to be found in all members of the Asteraceae since we have cloned putative orthologs in close relatives such as annual fleabane (*Erigeron annuus*) and more distantly related willowleaf sunflower (*Helianthus salicifolius*). Horseweed EPSPS3 likely appeared in the genome after a gene duplication event of EPSPS2. Since that duplication, EPSPS3 has acquired a number of codon changes leading to several amino acid differences relative to EPSPS2. These changes, e.g. Q181V, disrupt catalytic activity and make EPSPS3 non-functional as demonstrated in biochemical assays, relegating EPSPS3 to pseudogene status. This observation, coupled with the lack of any functionally resistant EPSPS sequences, the absence of EPSPS gene amplification, and lack of increased EPSPS gene expression amongst the biotypes, eliminated EPSPS loci as causative agents for resistance in horseweed.

Although overall uptake is similar between susceptible and resistant biotypes, mechanistic work has focused on the observation of reduced glyphosate translocation in the resistant biotype. This characteristic is operational at the site of application and is manifest in reduced glyphosate translocation to crown and root. Work is ongoing to isolate the resistance locus.

We have surveyed geographically distinct biotypes and found reduced translocation was correlated with the resistance phenotype in the greenhouse and transmits genetically in crosses between susceptible and resistant biotypes. Reduced translocation, a lower shikimate:glyphosate ratio in the resistant biotype, and a sensitive target enzyme indicate a mechanism that functions to “shield” the EPSPS target from the full glyphosate dose normally experienced in a susceptible individual. The genetic relationship between the geographically distinct susceptible and resistant horseweed accessions analyzed in the greenhouse was examined by inter-MITE (Minature Inverted Transposable Elements) polymorphisms. Broad geographical and known genetic relationships were associated by this technique. Several distinct clades were evident in the data. At this point, we cannot determine if resistant populations potentially spread from a common source population or arose by independent origin in multiple populations. Cloning and sequencing of the resistance locus will be needed to resolve the origins of resistance in these populations.