POTENTIAL FOR SELECTION OF GLYPHOSATE RESISTANCE IN *AMARANTHUS TUBERCULATUS* (MQ. EX DC) J.D. SAUER). Ian A. Zelaya and Micheal D.K. Owen, Graduate Research Assistant and Professor, Department of Agronomy, Iowa State University, Ames, IA 50011.

Despite the prolonged use of glyphosate worldwide, the evolution of resistance in weeds is an uncommon event. The low probability for resistance may be attributed to the limited metabolism of glyphosate in plants, the short-half life in the environment and unique biochemical characteristics of the herbicide. Some reported cases of resistant weeds evolved after prolonged selection pressure by glyphosate, thus suggesting that the frequency of resistant individuals within the populations are extremely low or that the event causes a physiological penalty to the plant. Point mutations in 3-phosphoshikimate 1-carboxyvinyltransferase (EPSPS; 2.5.1.19) associated with an increased dissociation constant for glyphosate ($K_i^{glyphosate}$), can result in a loss of kinetic efficiency of the enzyme.

One species resistant to glyphosate has been reported to date in the US. However, the adoption of glyphosate resistant crops may increase the selection pressure and isolate genotypes with an increased fitness to glyphosate. Midwest producers have reported inconsistent control of *Amaranthus tuberculatus* (Mq. ex DC) J.D. Sauer with glyphosate in glyphosate-resistant crops. Investigation of reports in Everly and Badger, Iowa suggested that *A. tuberculatus* plants were differentially affected by glyphosate. Plant and seed samples were collected at the Everly location to assess the potential for selection of glyphosate resistance.

Comparisons of the Everly, Iowa *A. tuberculatus* and a pristine population from Paint Creek, Ohio suggested that the Everly biotype was more variable to glyphosate than the unselected material. Thus, the Everly population was subjected to a divergent recurrent selection, where resistant and susceptible plants were identified though a seedling assay. Shikimic acid determinations and seedling and whole plants dose responses were performed after every cycle of selection. This approach resulted in a 1.7 and 3.5 fold increase in population divergence in the first (F1) and second (F2) filial generations, respectively. However, significant segregation for glyphosate efficacy was still apparent in the selected material. While the selection method has increased the frequency of resistant individuals within the population, no homozygous-stable lines have been isolated. This limited segregation suggested that the response to glyphosate observed in *A. tuberculatus* may be determined by a polygenic event or incomplete dominance. A single nuclear gene inherited in an incompletely-dominant manner is associated with glyphosate resistance in *Lolium rigidum* Gaudin. Other reported cases of glyphosate resistance required several years and cycles of selection to evolve. We speculate that resistance will evolve in *A. tuberculatus* if similar selection pressures are enforced on the populations.

Presently, we are attempting to reduce the genotypic variability through a three-level selection scheme where plants are characterized by their response to glyphosate at the seedling and whole-plant levels and confirmed by their shikimic acid accumulation pattern. We plan to investigate possible resistance mechanism in asexually-propagated resistant, susceptible, and pristine *A. tuberculatus* plantlets. Methods have been optimized to assess *in vivo* differences in absorption and translocation of glyphosate and degradation to the major metabolite α-aminomethylphosphonic acid (AMPA). In addition, attempts will be made to sequence *EPSPS* and determine whether polymorphism of the gene is associated with the observed phenotype. We constructed an *EPSPS* contig from 25 genes from 14 plant species and identified four regions of nucleotide conservation suitable for primer development. Genomic DNA was amplified by polymerase chain reaction (PCR) and products cloned in a vector for direct nucleotide sequencing. This approach enabled cloning a 798 bp fragment with 78% and 92% homology at the nucleotide and amino acid levels to the canola (*Brassica napus* L.) *EPSPS*, respectively. This putative *A. tuberculatus EPSPS* fragment lies at position 1795 and encompasses

introns #4 and #5 of *B. napus EPSPS*. Characterization of the mechanism(s) of glyphosate resistance may be important in developing strategies to mitigate potential future problems.

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