

SEQUENCING AND COMPARING REGIONS OF THE EPSPS GENE IN WEED SPECIES. Aaron L. Waltz, Don J. Lee, Alex R. Martin, and Fred W. Roeth, Graduate Research Assistant, Professor, Professor, and Professor, Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68583-0915.

Glyphosate is rapidly becoming the most widely used pesticide in US crop production history. In large part, the widespread adoption of glyphosate for weed management is due to its broad-spectrum efficacy and to the commercialization of glyphosate-resistant crop cultivars. Virtually all agricultural fields are infested by several weed species and glyphosate is active on most green plants making it a powerful weed management tool when used in conjunction with glyphosate-resistant crop cultivars. Glyphosate was used on over 70% of the 70,000,000 acres of soybean produced in the U. S. in 2001 (National Agricultural Statistics Service 2001). Limited availability of adapted glyphosate-resistant corn hybrids has limited glyphosate use in corn to date. However, with the increase in availability of glyphosate-resistant corn hybrids, it is not unreasonable to project that 70% of the 80,000,000 acres of corn produced in the U. S. will be treated with glyphosate in a matter of a few years. Since much of the corn and soybeans are normally grown in rotation with each other there could be over 100,000,000 acres treated with glyphosate for weed control year after year. Such reliance on a single pesticide for multi-species pest control is unprecedented in US history and potentially sets the stage for an increase in glyphosate-resistant or tolerant weed species.

A number of plant and bacterial species have been engineered or selected for tolerance/resistance to glyphosate, and at least total eight glyphosate-resistant biotypes of four weed species now exist. One of these biotypes, a glyphosate-resistant goosegrass, has one single amino acid modification in common with commercially available, glyphosate-resistant corn hybrids. Some weed populations are naturally more tolerant of glyphosate as well (e.g. morningglory species).

The goal of this research is to amplify and compare regions of the EPSPS DNA sequence within weed species. Primers were developed for PCR by aligning several EPSPS sequences available through GenBank. Highly conserved base pair regions within this enzyme make this a possibility. Four forward and four reverse primers were initially selected. Amplified products from Arabidopsis, barnyardgrass, common lambsquarters, maize, redroot pigweed, shattercane, and velvetleaf were run on an agarose gel, excised, purified, and sent to Davis Sequencing (Davis, CA) for sequencing reactions. So far, the sequences obtained are very noisy and unclear. It appears that at least one of the primers has multiple targets within the Arabidopsis genome. One possibility from here is to design longer primers that will be more specific for the EPSPS gene sequence.

From the gene sequence of the EPSPS gene within important weed species, many things are possible. The gene sequences themselves may give us information about the EPSPS enzyme across species. Primers for the EPSPS gene sequence that work across multiple species would be beneficial in the process of surveying weed populations. Research into EPSPS gene families and copy number would be aided by this information as well. Another possibility is the use of EPSPS primers to utilize the power of PCR to examine EPSPS mRNA expression.

The ultimate goal of weed science research is to help producers effectively manage harmful weed populations. Understanding the basic biology of weed species is a fundamental step in developing more effective weed management programs. Understanding the variation in the EPSPS gene in and among weed species in relation to plant response to glyphosate will provide the foundation required to predict the species most likely to be successful in a glyphosate-based system and to develop effective IWM (Integrated Weed Management) programs. It is a virtual certainty that more biotypes with lessened sensitivity to glyphosate will surface as the intense use of glyphosate continues. Characterizing the diversity in the EPSPS gene in weed populations will serve as the foundation for proactive preemptive weed management programs.