ANALYZING GENE EXPRESSION AT THE PROTEIN LEVEL: USING PROTEOMICS TECHNIQUES TO INVESTIGATE HERBICIDE SAFENER MECHANISM OF ACTION. Dean E. Riechers and Qin Zhang, Assistant Professor and Graduate Research Assistant, Department of Crop Sciences, University of Illinois, Urbana, IL 61801.

New molecular techniques are currently available that allow researchers to analyze global effects of treatments (such as herbicides or herbicide safeners) on gene expression patterns and profiles in plants. Examples of such techniques that are used as tools for gene discovery are cDNA microarrays (for genomics and functional genomics) and two-dimensional electrophoresis coupled with mass spectrometry (proteomics). One advantage of using these techniques is that they allow researchers to observe changes in gene expression for entire subsets of genes or proteins that are expressed under the given experimental condition.

Proteomics techniques are currently being utilized in our laboratory to examine all of the proteins that are expressed in the coleoptile of *Triticum tauschii* (a diploid wheat species with the D genome) seedlings, with and without herbicide safener treatment. Proteins are extracted from isolated coleoptiles, analyzed on two-dimensional protein electrophoresis gels (resolved by isoelectric point in the first dimension, and molecular mass in the second dimension), and the identity of individual protein spots are determined by mass spectrometry of peptide fragments derived from protease-digested proteins in the gel. Mass fingerprints that are generated are used to search the GenBank database for matches with corresponding peptide fragment masses from proteins in the existing database (usually deduced protein sequences translated from gene sequences). In addition to mass spectral analysis of individual proteins from the gel, we have also performed two-dimensional immunoblots probed with two different glutathione *S*-transferase (GST) antisera. Results to date show that the majority of safener-induced proteins are either phi or tau class GSTs, which vary greatly in both isoelectric point (pI 5 to 7) and molecular mass (24 to 29 kDa). Other safener upregulated proteins have also been identified that are not GSTs, but may also be involved in the safener response/herbicide detoxification pathway in wheat coleoptiles. Mass spectrometry data and immunoblot analyses have also indicated that there may be significant amounts of post-translational modification of GST proteins occurring in response to safener treatment. Future studies in our laboratory will examine protein profiles generated from *Triticum tauschii* coleoptiles that have been treated with herbicide (dimethenamid) only, herbicide safener only, and the herbicide plus safener together. We anticipate that these experiments will help us to better understand the entire herbicide detoxification pathway in wheat, and its biochemical components.

For molecular studies aimed at determining the regulation of gene expression in response to a given treatment, the ability to detect post-translational modification of proteins is a distinct advantage of proteomics techniques. Conversely, decreased sensitivity (relative to mRNA profiling with microarrays), difficulty in extracting integral membrane proteins, and the inability to identify proteins of very low abundance with current detection methods might be considered disadvantages of proteomics techniques. These drawbacks will hopefully be addressed and resolved once improvements are made with current proteomic technologies.