A SIMPLE CONTINUOUS ASSAY FOR EPSP SYNTHASE FROM PLANT TISSUE.

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The discovery of glyphosate-resistant weeds naturally leads to investigations of the mechanism of resistance. The most common mechanism is target site modification where a variant of the herbicide-targeted enzyme has been selected in the surviving weed. Hence, determining the sensitivity of the enzyme to the herbicide can result in identifying the resistance mechanism. We present a simple method to assay EPSPS in a continuous phosphate release assay which allows an estimation of the of the inhibition constant for glyphosate by determining the I50. The assay is an adaptation of the commercial phosphate assay kit sold by Molecular Probes. The enzyme purine nucleotide phosphorylase (PNPase) scavenges phosphate to phosphorylyze the nucleoside bond of 2-amino, 6-mercapto, 7-methyl-purine riboside (MESG) to create an increase in absorbance at 360nm due to the release of the modified purine. Maintaining an excess of the coupling enzyme PNPase, allows the rate of phosphate produced in the EPSPS reaction to be determined.

The procedure for the extraction, concentration and stabilization of an EPSPS protein for enzyme assay is reported. We demonstrate that the EPSPS from Palmer amaranth (Amaranthus palmeri), resistant to glyphosate, is actually more sensitive to glyphosate then the very well studied *E. coli* EPSPS. We do not believe that glyphosate resistant Palmer amaranth is utilizing a modified EPSPS for the resistance mechanism.

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