EVALUATION OF SPECTROPHOTOMETRIC METHODS FOR SHIKIMIC ACID DETERMINATION IN PLANTS. Ian A. Zelaya and Micheal D.K. Owen, Graduate Research Assistant and Professor, Department of Agronomy, Iowa State University, Ames, IA 50011.

Glyphosate inhibits 3-phosphoshikimate 1-carboxyvinyl transferase (EPSPS; EC 2.5.1.19), causing an accumulation of shikimic acid in protoplasts of plant cells. Thus, shikimic acid accumulation is an indirect estimate of EPSPS inhibition by glyphosate, and in a pragmatic sense, represents a method to ascertain glyphosate drift to non-target plants, differentiate between injury attributable to EPSPS and non-EPSPS inhibiting herbicides, and corroborate glyphosate resistance in plants.

Spectrophotometric methods are based on the oxidation of shikimic acid with periodate to form *trans*-2-pentene-1,5,-dialdehyde-3-carboxylic acid, followed by alkalinization and optical density (OD) detection at 380 nm. No direct comparison of the sensitivity and reproducibility of methods for shikimic acid determination exists in the literature. In addition, reported methods use diverse plant sources with different moisture contents. A standardization of methods is reported, comparing the reproducibility and respective strengths and limitations of the methods.

Glyphosate-resistant 'Asgrow AG2901' and susceptible 'Asgrow A2833' soybean (*Glycine max* (L.) Merr.) and glyphosate resistant 'DeKalb 545' and susceptible 'Garst 8550' maize (*Zea mays* L) were treated with 0.83 kg ae ha^{-1} glyphosate when soybean (V2-V3) or maize (V3-V4) reached 10-12 cm tall. Visual herbicide injury, plant height, and plant samples were collected prior to treatment (PT) and five, 12, 24, 72, and 192 h after treatment (HAT). Plant height was determined by measuring the distance from the soils surface to the apex of soybean or the collar of the utmost expanded leaf in maize. Shikimic acid was determined in actively growing tissues, thus sampling comprised removing the apex and the youngest fully-expanded trifoliate in soybean and the basal 3-cm of the coleoptile in maize. Plant samples were placed in a plastic bag iced, and immediately chilled to –10 C until utilized. After storage in –10 C, samples consigned for dry tissue determination were dried at 35 C for 48 h. Shikimic acid extraction and determination followed the protocols developed by Cromartie and Polge (2000, *WSSA Abs* 40:12; Spec #1) and Singh and Shaner (1998 *Weed Technol* 12:527; Spec #2); determinations were conducted at –4 C and ambient temperature.

Oxidation by periodic acid changed the absorbance maxima of shikimic acid standards and plant extracts from 280 nm to 380 nm. Similar absorption spectra were obtained from *Sorghum bicolor* (L.) Moench, *Ambrosia trifida* L., *Eriochloa villosa* (Thunb.) Kunth, and *Helianthus annuus* L. extracts spiked with known concentrations of shikimic acid, suggesting that the methods are specific to this aromatic compound. Extinction of the chromophore was temperature and concentration dependant and followed a sigmoidal and standard decay model for Spec #1 and Spec #2, respectively. The –4 C treatment increased the chromophore half-life ($t_{½}$) by 2.0-2.3 fold for Spec #1 and 1.6-2.0 for Spec #2. Glyphosate at the sprayed rates abolished plant growth in susceptible varieties; statistical differences ($\alpha \leq 0.05$) were obtained after 24 HAT for soybean and 72 HAT for maize. Visual herbicide injury was apparent at 72 HAT and near plant death occurred at 192 HAT for either susceptible crop variety.

Accumulation of shikimic acid was apparent after 5 h of treatment and increased exponentially until the last evaluation time. While both methods effectively quantified shikimic acid in soybean or maize tissues, and their estimations correlated ($r^2 = 0.96$; $P \leq 0.01$), Spec #1 estimated more shikimic acid in identical samples than Spec #2. Untreated and treated glyphosate-resistant soybean contained 0.0 to 0.4 μmol ml^{-1} shikimic acid per g of tissue, suggesting that the turnover rate of the shikimic acid pathway in these plants was comparable. Both methods estimated more shikimic acid in soybean apices than maize coleoptile tissues, however a better correlation was obtained with maize ($r^2 = 0.95$; $P \leq 0.01$) than soybean ($r^2 = 0.90$; $P \leq 0.01$) samples. As expected, almost twice as much shikimic acid was detected in dry tissues compared to wet tissues of soybean and corn.

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The pattern of shikimic acid accumulation was consistent with the principle that glyphosate inhibits EPSPS; susceptible plants accumulated more of the unphosphorylated substrate of EPSPS than resistant plants. Methods were specific for shikimic acid and under the extraction protocol evaluated, permitted quantification of shikimic acid from plant sources. In addition, the methods were inexpensive and quick to perform. Future work will focus on evaluating the extraction and HPLC techniques reported Lydon and Duke (1988 *J Agric Food Chem* 36:813).