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MISSISSIPPI SOYBEAN PROMOTION BOARD
PROJECT NO. 18-2016 (YEAR 1)
2016 ANNUAL REPORT

TITLE: 2,4-D and Dicamba Resistant Soybeans: Stewardship and Testing

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OBJECTIVES

1. Develop and validate analytical testing methods using FT-IR technology to differentiate 2,4 D and dicamba herbicides formulations.

2. Determine how to best sample soybean plants for the detection of auxin herbicides.

3. Work with the Bureau of Plant Industry and MSU Extension agents to participate in a growers educational program and design an off target field sampling program for best practices and fundamental integrated pest management.

BACKGROUND

Bio-technology companies have developed cultivars of corn, soybean, and cotton with transgenic resistances to the synthetic auxin herbicides 2,4-D and dicamba, which were slated for release in MS for the 2015 and 2016 growing seasons. The introduction of new herbicide tolerant soybeans may provide many benefits for producers such as alternative control options for resistant weed species, decreased costs, and different modes of action. Along with these benefits, the use of auxin-containing herbicides may also increase concern for issues such as herbicide drift, volatilization, and tank contamination.

To combat these concerns, Monsanto in collaboration with BASF (Dicamba-BAPMA) and Dow AgroSciences (2,4-D-choline), have developed new formulations that are less prone to volatilization and drift. Additionally these companies have created product stewardship programs. The Mississippi State Chemical Laboratory (MSCL) analyzes drift samples each year for dicamba and 2,4-D; however, current testing methods cannot differentiate between the amine, ester, or choline formulations. Therefore, it is imperative that we develop new analytical and sampling methods to ensure an effective stewardship program.

PROGRESS/ACTIVITY

Objective 1. Coupling FT-IR spectra to principal component analysis, we have been able to separate and identify soybeans that were treated with dicamba-BAPMA and traditional dicamba formulations. In 1996, soybeans were the first glyphosate-resistant (GR) crop to be released in the United States. Over time, weeds have become more tolerant of glyphosate, leading to GR weeds. As of 2011, 21 weed species, including rigid ryegrass, horseweed, ragweed, and water hemp, have developed resistance to glyphosate

The popularity of dicamba (3,6-dichloro-o-anisic acid) and 2,4-D (2,4-Dichlorophenoxyacetic acid) as herbicides has increased in order to address the growing issue of GR weeds. Dicamba and 2,4-D are synthetic auxins that act similarly to the natural hormone indole-3-acetic acid (IAA), which is the regulator of several plant functions. Soybean (*Glycine max*) and cotton (*Gossypium hirsutum*) have 2,4-D and dicamba resistant varieties that are commercially available. 2,4-D resistant-crops are still vulnerable to dicamba, and dicamba-resistant crops may be susceptible to 2,4-D. Drift is a well-known issue with the application of both of these herbicides. Some formulations of 2,4-D and dicamba are prominently known for volatilization drift after application. This is due to a combination of a high vapor pressure of the herbicides and high temperatures and wind speeds during application. Companies such as Monsanto and BASF have developed lower volatile versions of dicamba (Engenia) and 2,4-D (2,4-D Choline) in hopes to reduce the amount of drift that occurs. Product Stewardships programs are essential to ensure their proper use.

Figure1. Dicamba Drift Injury on Soybeans

Figure 2. Thermo Nicolet FTIR 6700 Spectrometer

In order to simulate drift, soybean plants in the R3 stage were sprayed with a 1/64X rate of Banvel (dimethylamine salt of dicamba), Clarity (diglycolamine salt of dicamba), MON76980 (diglycolamine salt of dicamba), Engenia (BAMPA salt of dicamba), and Roundup, with untreated soybeans used as a control. The plants were collected immediately and at 1, 3, 7, 14, and 28 days after application and placed in a freezer at -80°C. The samples were ground with a mortar and pestle using liquid nitrogen and analyzed using a Thermo Nicolet 6700 FT-IR spectrometer with a liquid nitrogen-cooled MCT high-D detector, a KBr beam splitter, and the Smart ARK accessory. Approximately 1 gram of sample was used for each analysis. The ZnSe horizontal attenuated total reflectance crystal had an angle of incidence of 60° that allows for 10 reflections of infrared light through the crystal with a total of 64 scans per spectra. The spectra were collected from 4000 to 650 cm^{-1}. Spectra were baseline corrected, normalized to the area under the curve, and converted to the first derivative using the Savitzky-Golay algorithm using OMNIC and the Unscrambler X software.

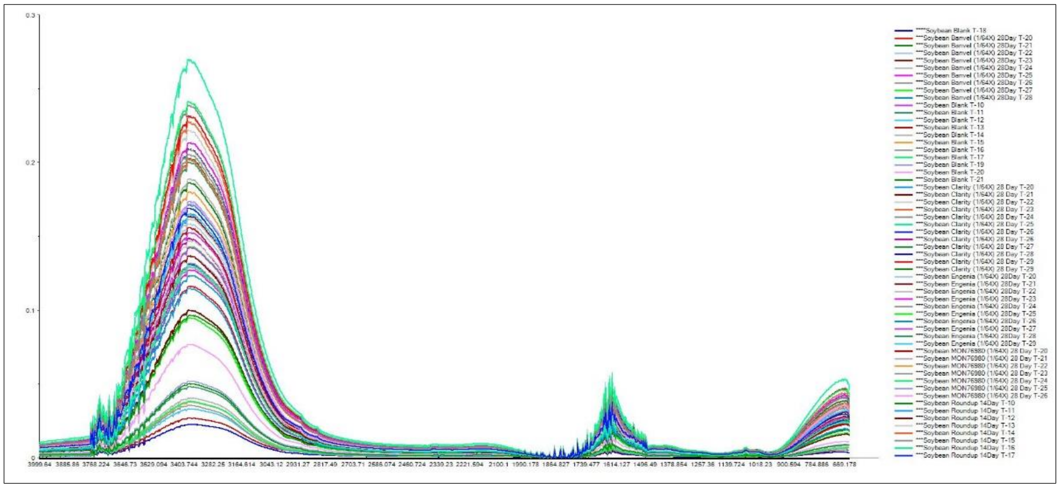

Figure 3. Infrared Spectrum of Dicamba and 2,4-D Applied Soybean Samples from 4000 to 650cm^{-1}

PCA (Principal Component Analysis) was used first in order to reduce the dimensionality of the data. PCA discovers the relationships between the samples and the variables to determine similarities between the samples. The first seven principal components accounted for 94% of the explained variance between samples. Then LDA (Linear Discriminant Analysis) was used to develop a model for classification of unknown samples. LDA is a supervised, classification method known to produce the optimal linear function used to transform the data into a lower-dimensional classification space. It is commonly used in facial recognition systems. Using these statistical techniques, the various herbicides were successfully differentiated up to 28 days after application in soybean plants with 93.1% accuracy using linear transformation and 100% accuracy using quadratic transformation.

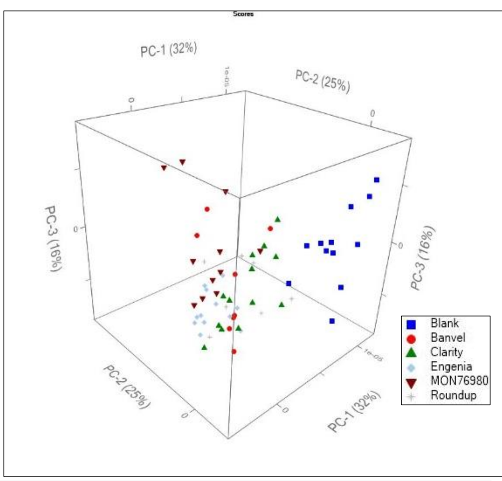

Figure 4. PCA of Dicamba Applied Soybeans 14 days after application

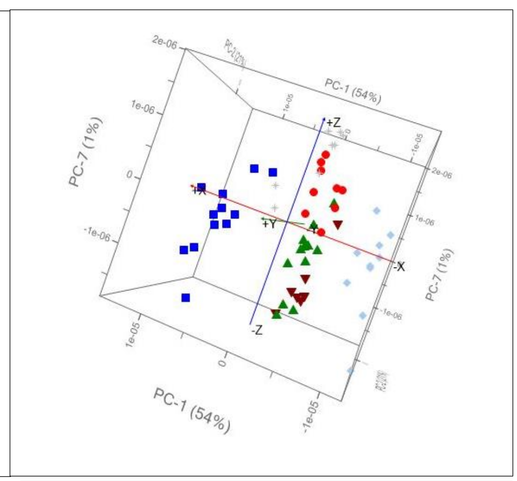

Figure 5. PCA of Dicamba Applied Soybeans 28 days after application

Linear transformation estimates one covariance matrix for all classes, while quadratic transformation measures one covariance matrix for each class. The only samples that were not accurately categorized when using a linear transformation were two MON76980 applied plants which were classified as Clarity applied plants and three Clarity applied plants were labeled as MON76980 applied plants. This could be because both MON76980 and Clarity are the diglycolamine salt form (DGA) of dicamba. In soybean plants collected 14 days or less after herbicide application, the model can discern with 96.15% accuracy. At 28 days, the model can successfully differentiate formulations with 93.1% accuracy.

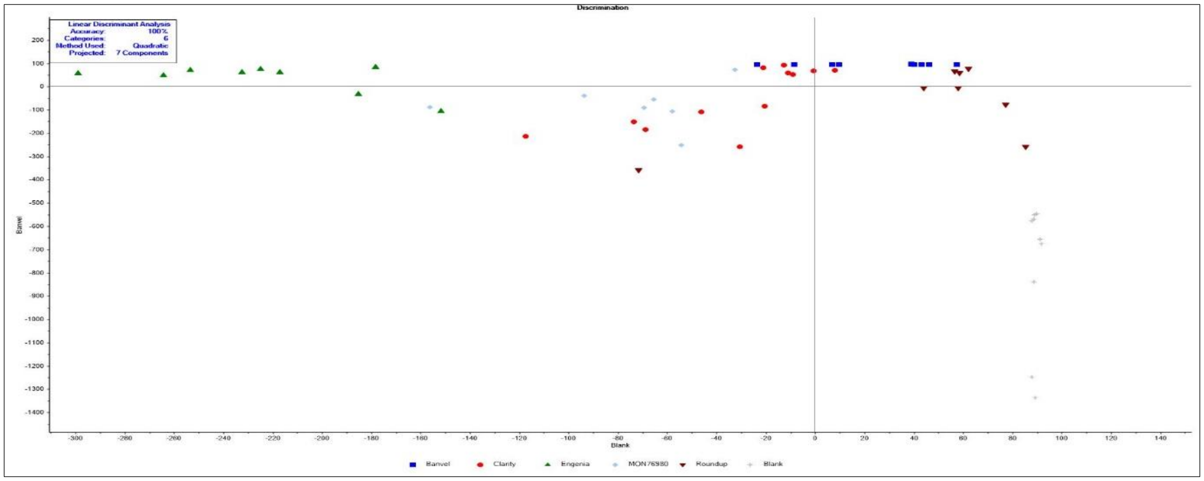

Figure 6. Linear Discriminant Analysis of dicamba applied soybeans 28 days after application

A promising model has been developed that can determine which type of dicamba was applied to soybeans up to 28 days after application. In year two, more samples will be analyzed, and in order to increase the precision of the model, unknowns will need to be run to increase the robustness of the model. Cotton samples with various 2,4-D herbicides applied are in the process of being analyzed in order to design a similar model. These models will hopefully allow us to determine unknown herbicide applications due to drift in crops to help solve and ultimately diminish drift cases.

Confusion Matrix	Blank (Actual)	Banvel (Actual)	Clarity (Actual)	Engenia (Actual)	MON76980 (Actual)	Roundup (Actual)
Blank (Predicted)	12	0	0	0	0	0
Banvel (Predicted)	0	9	0	0	0	0
Clarity (Predicted)	0	0	12	0	0	0
Engenia (Predicted)	0	0	0	10	0	0
MON76980 (Predicted)	0	0	0	0	7	0
Roundup (Predicted)	0	0	0	0	0	8

Objective 2. We have begun analysis of greenhouse plants treated and sampled by various stratification methods to determine which portions of the plant contain the highest concentrations of auxin herbicides. Plant samples were segmented into three sections—upper, middle, and lower foliar parts for analysis. We are comparing LC/MS detection to the FTIR method. All data are being processed. Additionally, year two field studies have been planned to provide plants for additional analysis.

Objective 3. Through our routine meetings with BPI and Extension Faculty, we are developing a sampling protocol.

IMPACTS

The expected outcome of this study will be the development FT-IR methodologies for identifying low volatility 2,4-D and dicamba formulations, as well as design and participate in a stewardship program so farmers can provide effective weed management, improve farm productivity, and maintain environmental conservation. This technology could enhance the position of Mississippi as an agricultural leader by exhibiting agricultural responsibility.

END PRODUCTS

Alice Hason, Cedric Reid, Dan Reynolds, Darrell Sparks, Ashli Brown (2017). Promoting Agricultural Stewardship Through Identification of Synthetic Auxins. Undergraduate Student Research Symposium, Mississippi State University, Mississippi State, MS (April 13).

Cedric Reid, Daniel Reynolds, Darrell Sparks, Ashli Brown (2017). FT-IR Method Development of a Unique Tool to Identify Drift Agents. GSA Graduate Student Research Symposium, Mississippi State University, Mississippi State, MS (April 2).

Ashley Meredith, Cedric Reid, Curtis Atkinson, Dan Reynolds, Scott Boone, Darrell Sparks, Ashli Brown (2016). Development of a FT-IR Testing Method for 2,4-D and Dicamba Resistant Soybeans. Society of Environmental Toxicology and Chemistry North America 37th Annual Meeting. Orlando, FL (November 6-10).

Cedric Reid, Gary Cundiff, Daniel Reynolds, Darrell Sparks, Ashli Brown (2016). Development of FTIR Method to Identify Herbicides and Their Low Volatile Counterparts. FPRW. St. Pete Beach, FL (July 18, 2016).

Additionally, this work will be featured as chapter in Cedric Reid's Dissertation. His expected graduation will be December 2017.

GRAPHICS:

Picture 1. Mr. Cedric Reid analyzing soybean samples using FT-IR.