MISSISSIPPI SOYBEAN PROMOTION BOARD
PROJECT NO. 18-2017 (YEAR 2)
2017 ANNUAL REPORT

TITLE: 2,4 D and Dicamba Resistant Soybeans: Stewardship and Testing (Proj. No. 18-2017, BROWN-MSU)

INVESTIGATORS:
Ashli Brown, Associate Professor/MS State Chemist
abrown@bch.msstate.edu

Daniel B. Reynolds, Professor of Weed Science &
dreynolds@pss.msstate.edu

Darrell Sparks, Assistant Professor
dsparks@bch.msstate.edu

BACKGROUND

Injury and yield loss in sensitive cotton and soybeans can occur from exposure to dilute concentrations of 2,4-D and dicamba. The availability of older formulations not labeled for use in new weed control systems complicates crop injury diagnosis. Crop response from an event involving a legally applied auxin herbicide does not differ visually from that of older, non-labeled herbicides.

Fourier-Transform Infrared spectroscopy (FTIR) is an accurate and inexpensive way to analyze samples for the presence of different chemical functional groups. FTIR has great potential to differentiate plant tissue damaged by herbicides with identical active ingredients that differ only in the molecular structure of the additives they are formulated with.

An experiment was conducted to develop a FTIR method to identify various auxin herbicides present in cotton and soybean tissue damaged by a dilute herbicide rate. Principal component analysis (PCA) and linear discriminant analysis (LDA) were used to model sample FTIR spectra. LDA models for soybean samples taken 0, 3, 7, 14, and 28 DAT identified auxin formulation with 89, 92, 84, 91, and 93% accuracy, respectively. IR spectrum peaks at 1687-1560 cm^{-1} and 1633-1556/1395-1350 cm^{-1} were critical for sample identification in soybeans and cotton, respectively. Future efforts will seek to develop alternative methods for sample preparation and models for more herbicide formulations and multiple rates and tissue types.

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.**

ACTIVITY/PROGRESS

From the preliminary analysis of this procedure in 2016, the first three principal components accounted for 86% of the explained variance of soybean samples collected 28 days after treatment (DAT), while the inclusion of four additional principal components explained an additional 13% of total variance, for a total of 96% of total variance accounted for.

Utilization of a 3-dimensional PCA graph of the initial three principal components illustrates distinct clustering of the five formulations used in the experiment (Blank, Roundup, Banvel, Clarity, Engenia) (Figure 1). Similarly, PCA analysis of soybean samples taken immediately after application, three days after application (DAA), seven DAA, and 14 DAA explained 96, 91, 92, and 92 percent of variance, respectively. Additionally, IR spectra peaks between 1687 and 1560 cm^{-1} were most important to differentiating herbicide formulations, most likely due to the occurrence of peaks corresponding to the aromatic ring of dicamba and the primary or secondary amine from dicamba salt formulations in this range.

We constructed a classification model based on PCA and LDA for both soybeans and cotton at all sampling timings. In dicamba-treated soybean samples, model accuracy was 89, 92, 84, 91, and 93 percent for samples taken at 0, 3, 7, 14, and 28 DAA, respectively (Figure 2). Similarly, model accuracy in 2,4-D-treated cotton samples was 90, 87, 90, 84, and 89 percent at the same sampling timings, respectively. Clearly, the use of FTIR coupled with PCA and LDA analyses demonstrates great promise in the identification of discrete herbicide active-ingredient formulations in damaged plant tissue.

Field response of concentrations of various auxin herbicide formulations has been consistent with existing literature and includes stunting, leaf cupping, chlorosis, and fusion, and stem and petiole epinasty (Figures 6, 7, and 8).

Currently, analysis of spectra from samples treated with the three different preparation methods is being conducted. Thus far little clustering has been found on PCA score plots by tissue type, indicating that tissue type does not play a significant role in determination of PCA/LDA discrimination (Figure 5). The oven drying preparation method has resulted in high clustering of diglycolamine salt damaged samples, but relatively poor clustering of DMA and BAPMA salt formulations (Figures 3 and 4). As a result, a greenhouse study of 50 soybean plants and 50 cotton plants has been initiated to gather further samples for refinement of the oven drying sample preparation method and ultimate decision of which preparation method to utilize.

Status and Future Expectations

Spring + Summer 2018

- Analysis of samples taken from greenhouse trial via oven drying
- Decision on which sample preparation method to use for remaining samples
- Analyzing 940 samples from 2017 with experimentally-determined sample preparation method
- Run, harvest, analyze 840 samples from summer 2018 season
- Analyze field data from 2017 and summer 2018 (visible injury, height, etc.)

Fall 2018

- Finish construction of model
- Prepare first draft of manuscripts
- One per crop or herbicide for total of two manuscripts

FIGURES

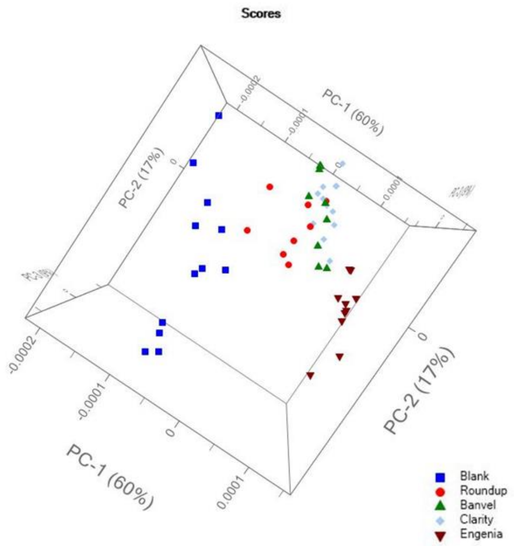

Figure 1. Principal Component Analysis of Soybean Tissue 28 DAT

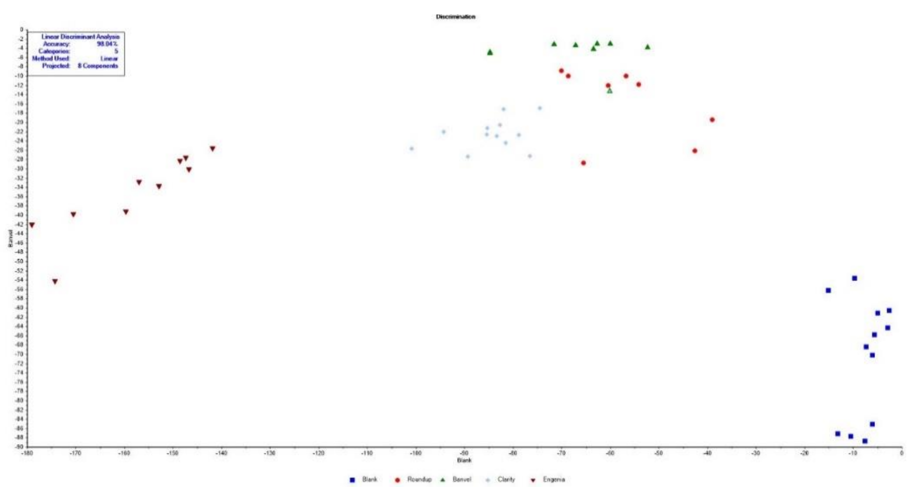

Figure 2. LDA of soybean tissues 28 DAA of dicamba.

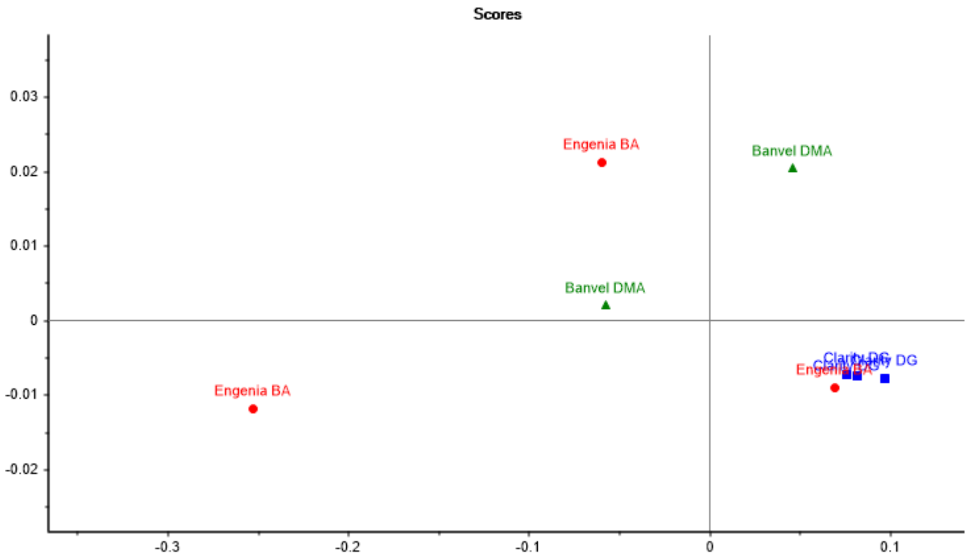

Figure 3. PCA score plot grouped by herbicide formulation damaged by a 1/32X rate of dicamba after oven drying and grinding.

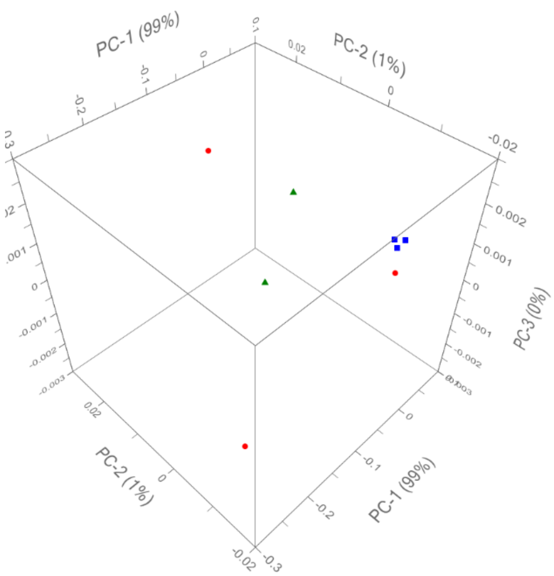

Figure 4. Three-dimensional PCA score plot grouped by herbicide formulation damaged by a 1/32X rate of dicamba after oven drying and grinding.

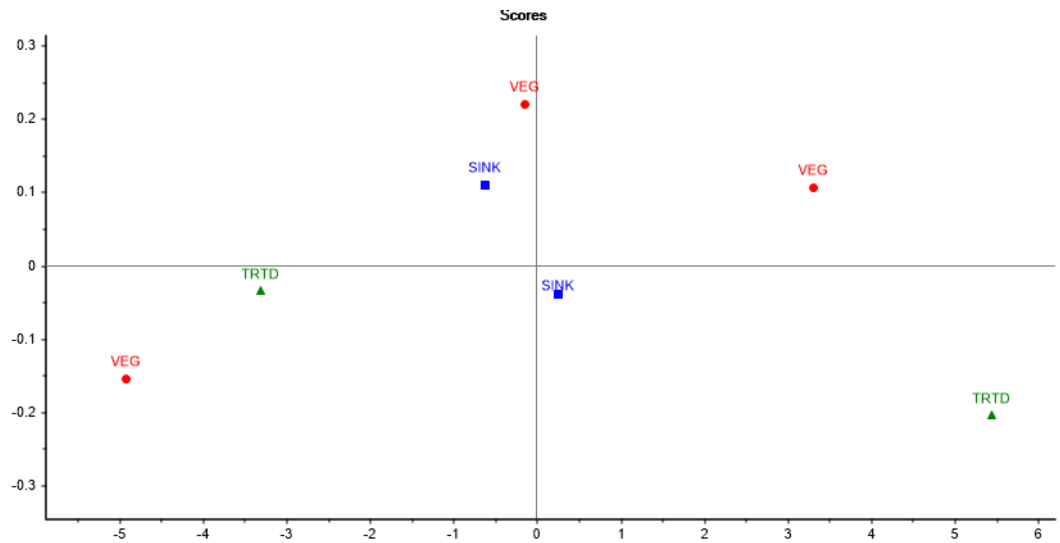

Figure 5. PCA score plot grouped by various soybean tissue types damaged by a 1/32X rate of dicamba formulated as a diglycolamine salt.

Figure 6. NTC plot


Figure 7. Plot treated with a 1/256X concentration of 2,4-D acid


Figure 8. Plot treated with a 1/128X concentration of dicamba as diglycolamine salt.

Using these statistical techniques, the various herbicides were successfully differentiated up to 28 DAA. Promising models have been developed that can determine which type of 2,4-D and dicamba were applied to soybean plants up to 28 days after application. In the future, more samples will be analyzed in order to increase the precision of the model, and more unknowns will need to be run to increase the robustness of the 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.

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

Theses/Dissertations Completed by Graduate Students:
Cedric Reid, Ph.D. Biochemistry (Degree Conferred, August 2017) Dissertation Title: Monitoring *Aspergillus flavus* and aflatoxin accumulation in inoculated miaze hybrids (Chapter VI: Identifying Auzin Herbicide Formulations in Soybeans Using FT-IR)

Published Abstracts and Presentations:

Wilkerson, T., Tomaso-Peterson, M., Golden, B. R., Lu, S., Brown Johnson, A. E., Allen, T. W., (February 2017). "Assessing applications of secondary nutrients as a management strategy for charcoal rot of soybean." Southern Division-American Phytopathological Society, SD-APS, College Station, TX.

Brown, A. E., Sparks, D. L., Reid, C. X., (July 2017). "Method Development for New 2,4 D and Dicamba Formulations." MAIC, Perdido Beach, AL.

Brown Johnson, A. E., Sparks, D. L., Reid, C., Meredith, A. N. , Reynolds, D. B., (August 23, 2017). "FT-IR Testing Method and Stewardship for 2,4-D and Dicamba Resistant Crops." 254th ACS National Meeting and Exposition, American Chemical Society, Washington, D.C.
Z.A. Carpenter, D. Reynolds, A.B. Johnson, A. Meredith, M. Green, (January 2018) "Comparison of Various Tank Cleaners for Removal of Dicamba from Contaminated Sprayers." Southern Weed Science Society Meeting, Atlanta, GA.

A. Brown (January 2018) "Analytical Method Development to Support Ag Research Programs." Southern Weed Science Society Meeting, Atlanta, GA.

B. Sperry, D. Reynolds, J. Bond, J. Ferguson, G. Kruger, A. Brown-Johnson, (January 2018) "Effect of Nozzle, Carrier Volume, and Cover Crop Residue on Residual Herbicide Efficacy." Southern Weed Science Society Meeting, Atlanta, GA.

Z.A. Carpenter, D. Reynolds, A.B. Johnson, A. Meredith, M. Green, (January 2018) "Determining the Effects of Increased Rinse Volumes on Dicamba Removal from Contaminated Sprayers." Southern Weed Science Society Meeting, Atlanta, GA.