MISSISSIPPI SOYBEAN PROMOTION BOARD
PROJECT NO. 18-2018
2018 ANNUAL REPORT

TITLE: 2,4 D and Dicamba Resistant Soybeans: Stewardship and Testing (Proj. No. 18-2018, BROWN-MSU)

PI: 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

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

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.

Research was conducted in 2017 and 2018 in Starkville, MS to develop a chemometrics and spectroscopy method to create a classification model capable of identifying specific dicamba formulations present in damaged crop tissue. Dicamba diglycolamine (DGA), dimethylamine (DMA), N,N-Bis-(3-Aminopropyl) methylamine (BAPMA), and diglycolamine with potassium acetate (DGAKAC) were applied to susceptible cotton and soybeans at 35, 17.5, 8.75, 4.375, 2.1875, and 1.09375 g dicamba ae/ha, and samples were analyzed with infrared spectroscopy, then were further analyzed using principal component analysis (PCA) and linear discriminant analysis (LDA). Joint PCA-LDA models were only capable of classifying dicamba formulation with 39.82% accuracy, whereas LDA alone was 80 to 85% accurate. This research suggests that with further refining, chemometric analysis of spectral data from damaged crop tissue may be an economical, efficient, and promising application to support management of crop injury cases following OTM of dicamba.

ACTIVITY/PROGRESS

Fourier-Transform Infrared Spectroscopy, or FT-IR, allows for more rapid analysis of samples compared with other spectroscopy methods. The instrument used in this analysis is Thermo Nicolet 6700 FT-IR Spectrometer (Figure 1). The main proponent in using FT-IR compared to other spectrometry methods is that FT-IR is more

rapid and inexpensive. Other spectroscopy methods usually cleave, or remove the salt group of the samples.

An experimental unit containing two rows of soybeans and two rows of cotton were analyzed for this experiment. Dicamba formulations were dicamba N,N-Bis- Methylamine Salt, dicamba diglycolamine salt, dicamba dimethylamine salt, and dicamba diglycolamine salt with potassium acetate. Eight hours following application, three subsamples of three different tissues were collected. The samples were then heated in an oven for two hours and then stored in a freezer at -80°C. The samples were homologized and the IF-TR spectra's were generated for each sample. The spectra's were then analyzed by principal component analysis (PCA) and linear discriminant analysis (LDA) via Unscrambler X 10.3 software.

FIGURES

Figure 1. Thermo Nicolet 6700 FT-IR Spectrometer

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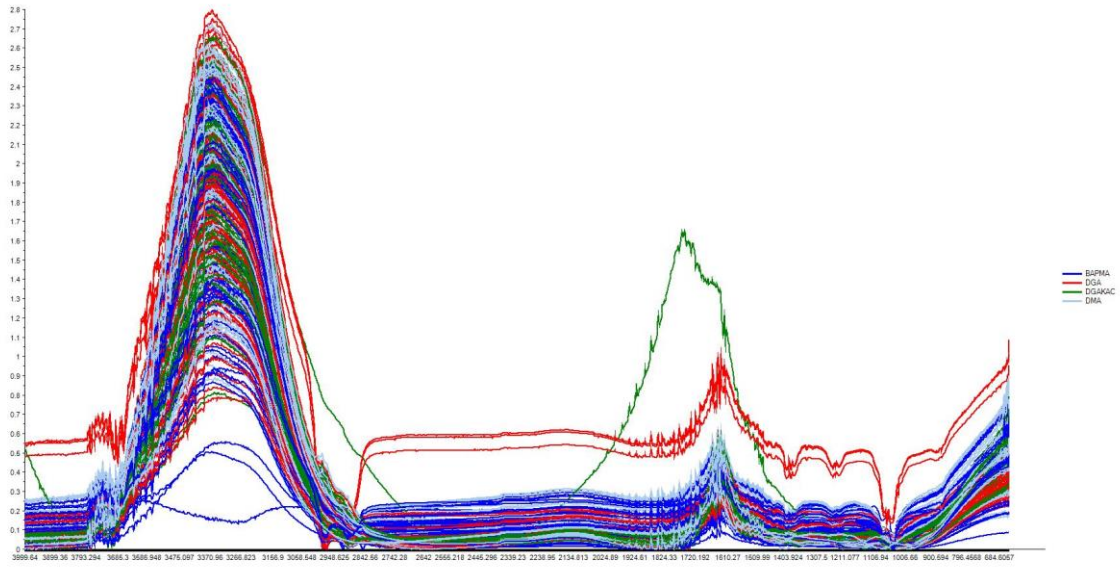

Figure 2. Raw spectra data from soybean samples ranging from 4,000 to 650 cm^{-1}. The samples were treated with different formulations and concentrations of Dicamba.

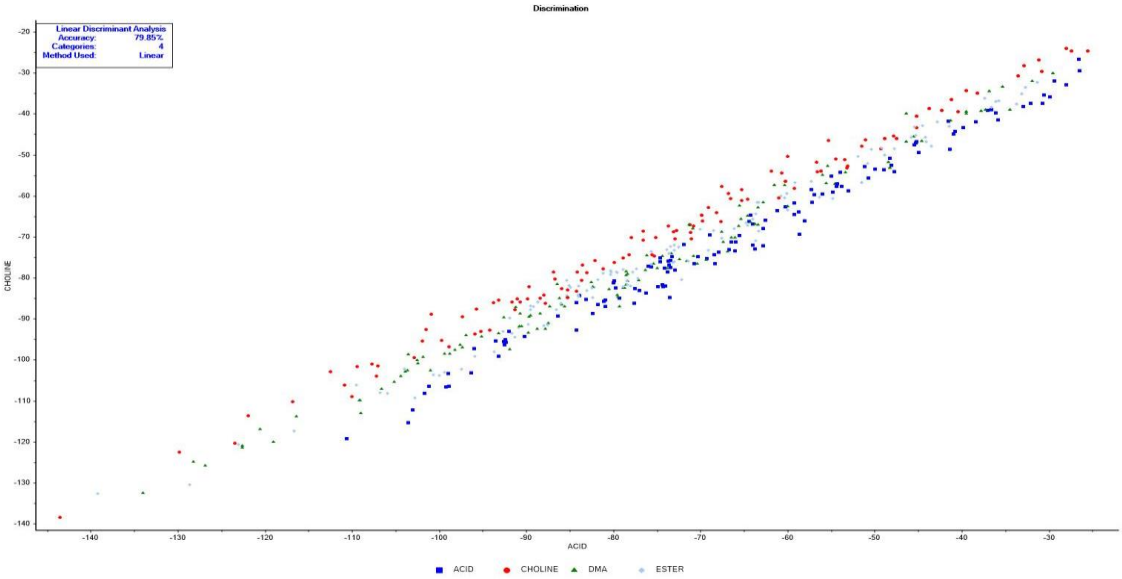

Figure 3. Linear Discriminant Analysis plot without Principle Component Analysis from soybeans treated varying formulations of Dicamba with varying functional groups resulting in a 80% identification accuracy.

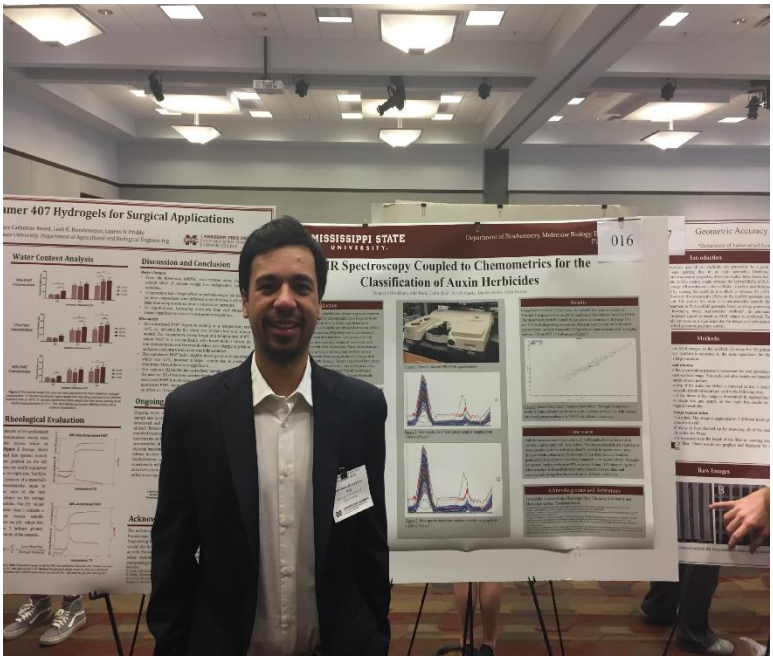

Figure 4. Undergraduate Student Ben Blackburn presents research data at the Spring 2019, Undergraduate Research Symposium.

Using Unscrambler X 10.5 software, the spectral data were analyzed via Principle Component Analysis (PCA) and Linear Discriminant Analysis (LDA). The model using both PCA and LDA produced an accuracy of 48% in distinguishing between the different formulations for soybeans. Analysis using only LDA produced more accurate results ranging from 80% (figure 3).

With the increased use of herbicides such as Dicamba, the likelihood of an increase of plant injury will likely follow. The data produced in this experiment show promising strides in helping identify specific formulations causing damage to both cotton and soybean crops. Linear Discriminant Analysis produced the highest percent accuracy compared to being paired with Principle Component Analysis with up to 90% accuracy. Using LDA alone along with other analytical tools could provide future research opportunities and improvements in regard to the classification of Auxin herbicides.

IMPACTS

The expected outcome of this study will be the development FT-IR methodologies for identifying low volatility 2,4 - 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 the 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:
John Tyler Buol, Ph.D. Biochemistry (Degree Conferred, May 2019) Dissertation Title: Stewarding 2,4-D and Dicamba based weed control technologies in cotton and soybean production systems.

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Published Abstracts and Presentations:

Buol, J. (Author & Presenter), Reynolds, D. B. (Author), Brown Johnson, A. E. (Author), Reid, C. (Author), Southern Weed Science Society, "Developing a Traditional Fourier-Transform Infrared Spectroscopy Method for Detection and High-Fidelity of Various Auxin Herbicide Formulations in Damaged Soybean and Cotton Tissue," SWSS, Atlanta, GA. (January 23, 2018).

Sperry, B. (Author & Presenter), Reynolds, D. B. (Author), Bond, J. A. (Author), Ferguson, J. C. (Author), Kruger, G. (Author), Brown Johnson, A. E. (Author), Southern Weed Science Society, "Effect of Nozzle, Carrier Volume, and Cover Crop Residue on Residual Herbicide Efficacy," SWSS, Atlanta, GA. (January 23, 2018).

Brown Johnson, A. E. (Author & Presenter), Southern Weed Science Society, "Analytical Method Development to Support Ag Research Programs," SWSS, Atlanta, GA. (January 21, 2018).

Toler, J. (Author & Presenter), Gibbons, M. (Author & Presenter), Meredith, A. N. (Author), Wilkerson, T. (Author), Allen, T. (Author), Green, M. (Author), Sparks, D. L. (Author), Brown Johnson, A. E. (Author), Annual Meeting of the Southern Section of AOAC, Int., "Isolation and Analysis of Botryodiplodin in Soybean Plants by Gas Chromatography Coupled to Mass Spectrometry," SSAOAC, Atlanta, GA. (April 18, 2018).

Green, M. (Author & Presenter), Phillips, A. (Author), Toler, J. (Author), Meredith, A. (Author), Carpenter, Z. (Author), Reynolds, D. B. (Author), Brown Johnson, A. E. (Author), Association of Offical Analytical Chemists (AOAC), "Measuring the Reduction of Dicamba Carryover in Pesticide Application Equipment by LC-MS/MS," Southern Section AOAC International, Atlanta, GA. (April 17, 2018).

Blackburn, B. (Author & Presenter), Buol, J. (Author), Reid, C. (Author), Reynolds, D. (Author), Sparks, D. (Author), Brown, A. (Author), Undergraduate Research Symposium, “FTIR Spectroscopy Coupled to Chemometrics for the Classification of Auxin Herbicides,” MSU (April 16, 2019).

Funding Leveraged:

Ferguson, J. C., Reynolds, D. B. (Co-Principal), Brown Johnson, A. E. (Co-Principal), Sponsored Research, "Reducing herbicide antagonism through novel spray application methods and improved understanding of herbicide antagonism chemistry", Mississippi State University - MAFES, Mississippi State University, $49,795.00, Awarded. (start: January 1, 2018, end: December 31, 2018).

Brown, A. E. (Principal), Sparks, D. L. (Co-Principal), Hagood, G. (Co-Principal), Boone, J. Scott (Co-Principal), Meredith, A. N. (Co-Principal), Childers, C. (Co-Principal), Grant, "Maintaining and Enhancing the MSCLs Scope of ISO/IEC 17025 Accreditation", United States Food and Drug Administration (FDA) (DHHS), Federal, $144,999.00, Awarded. (start: September 5, 2018, end: June 30, 2019).

Simpson, C. L. (Co-Principal), Burger, L. M. (Principal), Kobia, C. M. (Co-Principal), Brown Johnson, A. E., Lemons, L. L., Grant, "Engaging Women and Minorities in Agriculture-Related Stem Disciplines Through Mentoring, Leadership, and Experiential Learning", USDA-National Institutue of Food and Agriculture (USDA-NIFA), Federal, $99,989.42. Awarded. (start: October 15, 2018, end: October 31, 2021).

Baird, R. E., Mlsna, D. A., Mlsna, T. E., Brown Johnson, A. E., Gude, V. G., Yu, F., Street, J. T., Sparks, D. L., Fitzkee, N. C., Zhang, D., Emerson, J. P., Grant, "Research Experience for Undergraduates- Food, Energy and Water Security", National Science Foundation, Federal, $274,977.00, Awarded. (start: April 1, 2017, end: March 31, 2020).