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# Arkansas Corn and Grain Sorghum Research Studies 2019

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# Arkansas Corn and Grain Sorghum Research Studies 2019



Victor Ford, Jason Kelley, and Nathan McKinney II, editors



A R K A N S A S A G R I C U L T U R A L E X P E R I M E N T S T A T I O N July 2020 Research Series 669

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Cover: Silking stage corn in plant population trial at the University of Arkansas System Division of Agriculture, Lon Mann Cotton Research Station at Marianna.

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# ARKANSAS CORN AND GRAIN SORGHUM RESEARCH STUDIES - 2019 -

Victor Ford, Jason Kelley, and Nathan McKinney II, Editors

University of Arkansas System Division of Agriculture, Little Rock and Fayetteville, Arkansas

Arkansas Agricultural Experiment Station University of Arkansas System Division of Agriculture Fayetteville, Arkansas 72704

### **INTRODUCTION**

The 2020 Arkansas Corn and Grain Sorghum Research Studies Series is the inaugural edition of this annual report and includes research results on all topics pertaining to corn and grain sorghum production including disease management, environmental/ sustainability, irrigation, post-harvest drying, soil fertility, weed control, and research verification program results.

Our objective is capturing and broadly distributing the results of research projects funded by the Arkansas Corn and Grain Sorghum Board. The intended audience includes producers and their advisors, current investigators, and future researchers. The Series will also serve as a citable archive of research results.

Research reports contained in this publication are 2–3 year summaries. The reports inform and guide our long-term recommendations, but should not be taken solely as our recommended practices. Some reports in this publication will appear in other University of Arkansas System Division of Agriculture's Arkansas Agricultural Experiment Station publications. This duplication is the result of the overlap between disciplines and our effort to broadly inform Arkansas corn and grain sorghum producers of the research being conducted with funds from the Corn and Grain Sorghum Check-off Program. This publication may also incorporate research partially funded by industry, federal, and state agencies.

The use of products and trade names in any of the research reports does not constitute a guarantee or warranty of the products named and does not signify that these products are approved to the exclusion of comparable products. All authors are either current or former faculty, staff, or students of the University of Arkansas System Division of Agriculture, or scientists with the United States Department of Agriculture, Agriculture Research Service.

We extend thanks to the staff at the state and county extension offices, as well as the research centers and stations; producers and cooperators; and industry personnel who assisted with the planning and execution of the programs. A special thanks to Dr. Victor Ford for his time, effort, and support of the Series. This publication is available as a research series online at: https://aaes.uark.edu/communications/publications/

> Victor Ford, Jason Kelley, and Nathan McKinney II, Editors University of Arkansas System Division of Agriculture, Little Rock and Fayetteville, Arkansas

### **ACKNOWLEDGMENTS**

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#### The Arkansas Corn and Grain Sorghum Promotion Board Members 2019–2020

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### 2017–2019 Corn and Grain Sorghum Research Verification Program

J.P. Kelley,<sup>1</sup> C. Capps,<sup>2</sup> B.J. Watkins,<sup>3</sup> and C.R. Stark Jr<sup>4</sup>

### Abstract

During 2017–2019, the Corn and Grain Sorghum Research Verification Program (CGSRVP) was conducted on 20 irrigated corn fields, 1 non-irrigated corn field, 1 irrigated grain sorghum field, and 2 non-irrigated grain sorghum fields. Counties participating included: Arkansas, Chicot, Clay, Conway, Cross, Desha, Faulkner, Jackson, Jefferson, Lawrence, Logan, Mississippi, Monroe, Perry, Pope, Prairie, St. Francis, White, and Yell. Average yields were 208.96 bu./ac for irrigated corn, 144.0 bu./ac for non-irrigated corn, 130.3 bu./ac for irrigated grain sorghum, and 65.2 bu./ ac for non-irrigated grain sorghum. State average corn and grain sorghum yields (irrigated and non-irrigated) from 2017–2019 were 179.7 and 77.5 bu./ac for corn and grain sorghum respectively (USDA-NASS, 2017–2019). Economic returns to total costs/acre were greatest from irrigated corn and averaged \$243.72, \$54.54, and \$131.92 in 2017, 2018, and 2019 respectively when no land charges were applied. Returns to totals costs/acre were all negative for the limited fields of grain sorghum. Seed cost and fertilizer/nutrients accounted for 25% and 28% of total expenses for irrigated corn fields.

### Introduction

The Arkansas Corn and Grain Sorghum Research Verification Program (CGSRVP) represents a public demonstration of research-based Extension recommendations on actual working farms at a field-scale farming environment. The programs stress intensive management with timely inputs and integrated pest management to maximize yields and net returns. The overall goal is to verify that crop management using the University of Arkansas System Division of Agriculture recommendations can result in high yielding and profitable corn and grain sorghum with current technology. The objectives of the programs are to: 1) educate producers on the benefits of utilizing University of Arkansas System Division of Agriculture recommendations for improved yields and/or net returns; 2) conduct on-farm field trials to verify research-based recommendations; 3) aid researchers in identifying areas of production that require further study; 4) improve or refine existing recommendations which contribute to more profitable production; 5) incorporate data into Extension educational programs at the county and state level; and 6) provide in-field training to county agents and producers on current production recommendations.

The CGSRVP started in 2000 after the initiation of a statewide check-off program for corn and grain sorghum, which is distributed by the Arkansas Corn and Grain Sorghum Promotion Board. Since the inception of the program, there have been 149 corn or grain sorghum fields enrolled in the program in 35 counties.

### Procedures

In the fall of each year, the CGSRVP program coordinator sends out requests to county extension agents for program enrollment. County extension agents find cooperators who want to be part of the program and agree to pay production expenses, provide crop expense information for economic analysis, and implement recommended production practices in a timely manner throughout the growing season. During the winter months, the program coordinator and county extension agent meet with the producer to discuss field expectations, review soil fertility, weed control, irrigation, insect control, hybrid recommendations, and provide details of the program. As the planting season begins, the program coordinator along with the county agent and cooperator scout each field weekly and discuss management decisions that are needed that week and the upcoming week. The program coordinator provides the county extension agent and producer with an electronic crop scouting report that outlines recommendations for the week and future expectations.

An on-site weather station provides in-field rainfall data as well as high and low temperature data which is used to calculate accumulated growing degree days for each week. When applicable, irrigation well flow meters are installed prior to initiation of irrigation to document the amount of irrigation water used during the year. Soil moisture sensors are installed in representative areas of the field early in the growing season to provide soil moisture information and are used as a tool to determine initiation, frequency, and termination of irrigation.

### **Results and Discussions**

Overall corn yields during the 3-year period from 2017–2019 ranged from 142.0 bu./ac in Monroe County (2019) to a high of 255.5 bu./ac in Jefferson county (2019) (Table 1). The overall average yield of all corn fields, including

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one non-irrigated field, was 204.7 bu./ac. State average corn yields during this period averaged 179.7 bu./ac (USDA-NASS, 2017–2019). All corn fields were planted within recommended planting date ranges, except for Monroe and White County in 2019 when the planting was delayed due to wet weather. The average planting date for all fields was 13 April with an average harvest date of 17 September. Plant populations averaged 32,736 plants/acre which would be at a recommended level for most fields and hybrids.

Grain sorghum yields ranged considerably from 58.0 bu./ ac in a non-irrigated field in White County (2018) to a high of 130.3 bu./ac in an irrigated field in Cross County (2018) (Table 2). State average grain sorghum yields from 2017–2018 averaged 77.5 bu./ac (USDA-NASS 2017–2019). The three grain sorghum fields enrolled in the program from 2017–2018 were fewer than in past years, but reflect the low acreage of grain sorghum in Arkansas during those years.

Fertilizer applied to fields closely followed current University of Arkansas System Division of Agriculture Cooperative Extension Service (CES) recommendations and were based on soil analysis and yield goals (Tables 3 and 4). Preplant fertilizer applied to corn fields averaged 55-61-78-5-5 lb/ac of nitrogenphosphorus-potassium-sulfur-zinc, where nitrogen applied preplant or at planting totaled approximately 25% of the total nitrogen applied during the season. Sidedress nitrogen applied at the V4-V8 corn growth stage averaged 113 lb of nitrogen/ acre using a nitrogen source of urea, ammonium sulfate, ureaammonium nitrate or a combination of those sources. A pretassel application of nitrogen, typically 100 lb of urea/acre, was made between the V12 and R1 growth stage and is a common and recommended nitrogen management practice in Arkansas. Total nitrogen applied to corn fields was 227 lb nitrogen/acre when averaged across all fields. Applied nitrogen fertilizer resulted in an average yield of 205.8 bu./ac which led to 1 bushel of corn grain for every 1.1 lb of nitrogen fertilizer applied.

Preplant fertilizer applied for grain sorghum averaged 49-40-60-12-5 lb/acre of nitrogen-phosphorus-potassium-sulfurzinc. Total nitrogen for the two non-irrigated fields averaged 115 nitrogen per acre, which resulted in 1.76 lb of nitrogen needed for 1 bushel of grain. The irrigated field with a yield of 130.3 bu./ac and 161 lb of nitrogen fertilizer applied resulted in 1 bushel of grain for every 1.23 lb of nitrogen fertilizer applied.

Pest management practices followed current CES recommendations. None of the corn fields met thresholds requiring an insecticide or foliar fungicide application at any time during the season. Herbicides applied to corn fields varied, but most commonly consisted of a combination of glyphosate, metolachlor, atrazine, and mesotrione that was applied in a one- or two-pass program. The corn field in White County in 2019 was planted to a conventional hybrid and no glyphosate was used. Insects were closely scouted for in grain sorghum and all fields had to be sprayed with Chlorantraniliprole (Prevathon) after heading for control of corn earworms and sorghum webworms; however, sugarcane aphids did not need to be sprayed. The White County grain sorghum field in 2017 was planted to a sugarcane aphidtolerant hybrid, a recommended practice for the management of sugarcane aphid in grain sorghum.

Irrigation is an important management practice for Arkansas corn. Of the corn verification fields from 2017-2019, 20 out of 21 fields were irrigated and 19 out of 20 were furrow irrigated with only 1 being pivot irrigated. Statewide approximately 90-95% of the corn grown in the state is irrigated (USDA-FSA, 2017–2019). Irrigation initiation, frequency, and termination were scheduled with the help of the Arkansas Irrigation Scheduler program and the use of soil moisture sensors to determine soil moisture content. During 2017–2019, overall irrigation requirements for corn were generally less than in previous years and on average each field was irrigated 4.5 times (Table 5). Each furrow irrigation was estimated to provide 2 acre-inches of irrigation water and each pivot irrigation was estimated to provide 1 acre-inch of water. Average rainfall on corn fields in 2018 and 2019 from planting to maturity was 17.22 inches demonstrating that total rainfall may be adequate for corn production, but the poor distribution of rainfall during the growing season is the reason such a high percentage of Arkansas corn is irrigated. One grain sorghum field was furrow irrigated and it was irrigated 4 times during the season (Table 6).

On-site weather stations provided high and low temperature data for accurate measurement Growing Degree Days (GDD). The formula used to determine GDDs for corn is as follows:

GDDs =	
(Daily Maximum Air Temperature + Daily Minimum Temperature)	50
2	- 50

with a maximum air temperature set at 86 °F and minimum temperature for growth set at 50 °F. During weekly field visits, corn growth stages were recorded and compared to accumulated GDDs. Table 7 shows the 2018–2019 average GDDs accumulated by each growth stage listed. These values align closely with reported GDDs needed to reach maturity for full-season hybrids (110–120 day) that are typically grow in Arkansas. Use of GDDs can accurately predict corn growth stages and is a tool for management decisions such as irrigation termination.

### **Economic Analysis**

Records of field operations on each field that were compiled by the CGSRVP coordinator, county extension agent, and producer serve as the basis for estimating costs and economic returns that are discussed in this section. Production data from the 20 irrigated corn fields, 1 non-irrigated corn field, and 3 grain sorghum fields were applied to determine costs and returns above operating costs, as well as total specified costs. Operating costs and total costs per bushel indicate the commodity price needed to meet each cost type.

Production expenses are expenditures that would generally require annual cash outlays and would be included on an annual operating loan application. Actual quantities of all production inputs as reported by the cooperators are used in this analysis. Input prices are determined by data from the 2017–2019 Crop Enterprise Budgets published by the Cooperative Extension Service and information provided by the producer cooperators. Fuel and repair costs for machinery are calculated using a budget calculator based on parameters and standards established by the American Society of Agricultural and Biological Engineers. Machinery repair costs should be regarded as estimated values for full-service repairs, and actual cash outlays could differ as producers utilize employee labor or provide unpaid labor for equipment maintenance.

Operating expenses include production expenses, as well as interest paid on operating capital and all post-harvest expenses. Post-harvest expenses include, as applicable for each crop, hauling, drying, check-off fees, and other expenses typically incurred after harvest. Post-harvest expenses increase or decrease with yield.

Ownership costs of machinery are determined by a capital recovery method which determines the amount of money that should be set aside each year to replace the value of equipment used in production. Machinery costs are estimated by applying engineering formulas to represent the prices of new equipment. This measure differs from typical depreciation methods, as well as actual annual cash expenses for machinery, but establishes a benchmark that estimates farm profitability.

Operating costs, total costs, costs per bushel, and returns are presented in Table 8 for corn and Table 9 for grain sorghum. Costs in this report do not include land costs, management, or other expenses and fees not associated with production. Corn grain prices used for economic calculations were \$3.75, \$3.35, and \$3.75/bu. in 2017, 2018, and 2019 respectively and were the three week average for the most active weeks of the harvest period each year. Grain sorghum grain prices used for calculations were \$3.35 and \$3.00/bu. in 2017 and 2018 and were also the three-week average price for the most active weeks of the harvest period each year. The average corn yield from the irrigated corn verification fields was 208.96 bu./ac, 144.0 bu./ac for non-irrigated corn, 130.30 bu./ac for irrigated grain sorghum, and 65.15 bu./ac non-irrigated grain sorghum.

The average production expenses from 2017–2019 for irrigated corn fields harvested for grain were \$417.16/ac and ranged from \$375.18 in 2017 to \$445.95 in 2019. On average, fertilizers and nutrients were the largest expense category at \$149.16/ac, or 28% of production expenses for irrigated corn fields. Seed costs averaged \$131.55/ac which was 25% of production expenses on irrigated corn fields.

With an average corn yield of 208.96 bu./acre for all irrigated fields, average operating costs were \$523.94/ac from 2017–2019. Operating costs have steadily increased during the 3-year period from a low of \$490.69 in 2017 to a high of \$551.83 in 2019. This increase is largely contributed to an increase in input costs such as seed, chemical, and fertilizers. Returns to operating costs for all irrigated corn fields from 2017–2019 averaged \$233.10/acre with a low of \$138.30/acre in 2018 to a high of \$332.63/acre in 2017. Average fixed costs over the 3-year period for irrigated fields was \$89.71. Returns to total cost for irrigated fields averaged \$143.39/ac with a low of \$54.54 and a high of \$243.72/ac in 2017. Total specified costs for all irrigated corn fields during 2017–2019 averaged \$2.98/bu. while the one non-irrigated field was \$3.41/bu.

The grain sorghum fields had an average operating cost of \$259.83/ac in 2017–2018. Fertilizers and nutrients were 34%

of production expenses with an average expense of \$88.18/ac. Seed cost averaged \$19.18/ac and was 7% of production expenses. Operating expenses averaged \$259.83 which is \$3.20/ bu. as determined by the average yields among fields. Returns to operating costs averaged \$9.21/ac. Fixed costs averaged \$77.40/ac. This leads to average total costs of \$337.23/ac, or \$4.18/bu. Returns to total specified costs averaged -\$68.20/ac during 2017–2018.

### **Practical Applications**

The corn and grain sorghum research verification program continues to serve as a field-scale demonstration of all CES recommendations for growing corn and grain sorghum in Arkansas. It serves as a method to evaluate recommendations and make adjustments or define areas that may need more research in the future. The program results are assembled into a database to allow long-term monitoring of agronomic and economic trends of Arkansas corn and grain sorghum production. The program also aids in educating new county agents and producers who are less familiar with current production recommendations.

Areas of ongoing research that are being evaluated in the corn and grain sorghum research verification program fields include use of foliar tissue testing during the season to evaluate whether current fertilizer recommendations for corn provide adequate levels of nutrients in the plants; in particular, tissue samples are taken during the V10-tassel stage to determine whether nitrogen levels in the plant are adequate and if a pretassel nitrogen application is needed. End of season corn stalk nitrate samples are also collected to determine if nitrogen was adequate during the season and to evaluate overall nitrogen efficiency. Soil moisture sensors are being used in all corn fields to track soil moisture levels and will help serve as a testing program for using for soil moisture sensors for irrigation initiation, timing throughout the season, and termination. The verification fields also serve as a pest management monitoring program for foliar diseases in corn such as southern rust and sugarcane aphids in grain sorghum to alert growers of potential developing pest problems.

The verification program highlighted that corn can be a profitable crop, especially when yields of 200 bu./ac are produced with careful management following current recommendations and keeping inputs costs relatively low. However the program also highlighted that grain sorghum yields and/or overall economic returns need to increase before acres of grain sorghum can increase. The relatively low yields of non-irrigated grain sorghum were not profitable, but a high yielding irrigated field also did not produce a profit. More work is needed to evaluate ways to make grain sorghum more profitable during times of low grain prices.

### Acknowledgments

The authors appreciate the support provided by Arkansas corn and grain sorghum producers through check-off funds administered by the Arkansas Corn and Grain Sorghum Promotion Board. In addition we appreciate the cooperation of participating producers and County Extension agents who are enrolled in the program. Support was also provided by the University of Arkansas System Division of Agriculture.

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Table 1. 2017–2019 Corn Research	Verification Program locations,	, hybrid planted, field size, row spacing,
previous crop,	plants per acre, plant date, harv	vest date, and yield.

		Field	Row	Previous	Plants	Plant	Harvest	
County/Year	Hybrid	Size	Space	Crop	per acre	Date	Date	Yield
		(acres)	(inches)					(bu./ac)
Jackson/17	Pioneer 2089VYHR	40	30	Soybean	34,000	3/31	9/12	223.9
Prairie/17	Armor 1500PRO2	40	30	Soybean	32,500	4/1	9/15	201.0
River Valley/17	Terral REV23BHR55	47	30	Soybean	31,000	4/10	8/29	214.3
St Francis/17	Armor 1717PRO2	53	38	Soybean	35,000	3/24	8/23	239.0
Arkansas/18	AgriGold 6499VT2	35	30	Corn	31,400	4/20	10/30	178.1
Chicot/18	Ag Venture AV8614	48	38	Soybean	28,400	4/21	9/14	144.0
Clay/18	Pioneer 1870YHR	50	30	Soybean	29,000	4/11	9/14	217.5
Desha/18	Pioneer 1870YHR	45	38	Soybean	30,000	3/21	9/15	198.0
Jackson/18	Pioneer 1870YHR	40	30	Corn	32,100	4/10	9/19	240.1
Jefferson/18	DeKalb 67-72VT2P	80	38	Soybean	32,000	4/19	10/4	174.0
Prairie/18	Dyna-Gro D57VC51	40	30	Soybean	30,500	3/21	9/19	188.0
Arkansas/19	AgriGold 6499VT2P	44	30	Soybean	33,333	4/24	10/1	220.1
Chicot/19	DeKalb 67-44VT2P	42	38	Soybean	32,800	4/23	9/18	208.0
Clay/19	DeKalb 70-27VT2P	34	30	Soybean	33,000	4/1	9/8	232.2
Desha/19	Mission A1687VT2P	57	38	Soybean	32,500	4/24	9/5	207.7
Jefferson/19	Dyna-Gro 57VC51	146	30	Soybean	32,800	4/23	9/12	255.5
Lawrence/19	Pioneer P1870AM	15	30	Soybean	35,800	4/6	9/10	220.3
Mississippi/19	Progeny 6116VT2P	30	30	Soybean	33,000	4/29	9/30	187.3
Monroe/19	Progeny 5115VT2P	71	30	Soybean	39,000	5/18	9/28	142.0
Prairie/19	Dyna-Gro D57VC51	110	30	Soybean	33,000	3/27	9/6	195.4
White/19	Dyna-Gro D57CC51	65	30	Soybean	36,333	5/18	9/30	212.2
Mean		54			32,736	4/13	9/17	204.7

Table 2. 2017–2018 Grain Sorghum Research Verification Program locations, hybrid planted, field size, row spacing, previous crop, plants per acre, plant date, harvest date, and yield.

					Plants	-	Harvest	
		Field	Row	Previous	per	Plant	Date	
County/Year	Hybrid	Size	Space	Crop	acre	Date		Yield
		(acres)	(inches)					(bu./ac)
White/17	Sorghum Partner 7715	21	30	Soybean	123,000	4/25	9/10	72.3
Arkansas/18	Dekalb 53-53	24	30	Soybean	64,875	5/1	9/24	58.0
Cross/18	Dekalb 53-53	83	30	Soybean	94,000	5/3	9/21	130.3
Mean		43			93,958	4/29	9/18	86.9

	Preplant							
County/Year	Fertilizer	Sidedress	Pretassel <sup>a</sup>	Total Fertilizer	Soil Type			
Applied Fertilizer Ib/ac of N-P-K-S-Zn								
Jackson/17	46-90-90-0-10	150-0-0-0-0	46-0-0-0-0	242-90-90-0-10	Calhoun Silt Loam			
Prairie/17	60-60-90-0-0	113-0-0-24-0	46-0-0-0-0	219-60-90-24-0	Immanuel Silt Loam			
River Valley/17	67-0-60-24-0	120-0-0-0-0	46-0-0-0-0	233-0-60-24-0	Dardanelle Silt Loam			
St Francis/17	67-110-80-34-20	115-0-0-0-0	46-0-0-0-0	228-110-80-34-20	Bowdre Silty Clay Loam			
Arkansas/18	50-60-90-10-10	138-0-0-0-0	46-0-0-0-0	234-60-90-10-10	Calloway Silt Loam			
Chicot/18	60-60-60-0-5	113-0-0-24-0	46-0-0-0-0	219-60-60-24-5	Rilla Silt Loam			
Clay/18	40-0-80-24-0	134-0-0-0-0	46-0-0-0-0	220-0-50-24-0	Falaya Silt Loam			
Desha/18	92-25-0-12-10	92-0-0-0-0	46-0-0-0-0	230-25-0-12-10	Tutwiler Silt Loam			
Jackson/18	60-90-90-0-10	115-0-0-12-0	46-0-0-0-0	221-90-90-12-10	Calhoun Silt Loam			
Jefferson/18	60-60-60-0-10	115-0-0-0-0	46-0-0-0-0	221-60-60-0-10	Rilla Silt Loam			
Prairie/18	56-104-76-0-0	115-0-0-0-0	46-0-0-0-0	217-104-76-0-0	Immanuel Silt Loam			
Arkansas/19	50-110-115-0-5	124-0-0-14-0	46-0-0-0-0	220-110-115-14-5	Ethel Silt Loam			
Chicot/19	46-60-90-0-5	130-0-0-24-0	46-0-0-0-0	222-60-90-24-5	McGehee Silt Loam			
Clay/19	51-80-80-0-0	124-0-0-24-0	46-0-0-0-0	221-80-80-24-0	Falaya Silt Loam			
Desha/19	69-90-147-0-0	113-0-0-24-0	46-0-0-0-0	228-90-147-24-0	Herbert Silt Loam			
Jefferson/19	2Ton Litter +46-	130-0-0-24-0	46-0-0-0-0	222-40-60-24-0	Rilla Silt Loam			
	40-60-0-0							
Lawrence/19	46-70-60-0-10	130-0-0-24-0	46-0-0-0-0	222-70-60-24-10	Beulah Sandy Loam			
Mississippi/19	78-79-0-0-10	130-0-0-0-0	46-0-0-0-0	254-79-0-0-10	Earle Clay			
Monroe/19	46-0-81-0-0	129-0-0-24-0	46-0-0-0-0	221-0-81-24-0	Bosket Fine Sandy Loam			
Prairie/19	46-100-108-0-10	128-0-0-24-0	46-0-0-0-0	220-100-108-0-10	Immanuel Silt Loam			
White/19	23-0-120-0-0	112-0-0-20-0	106-0-0-19-0	241-0-120-39-0	Calhoun Silt Loam			
Mean	55-61-78-5-5	113-0-0-12-0	49-0-0-1-0	227-61-78-17-5				

Table 3. 2017–2019 Corn Research Verification Program locations, preplant, sidedress, pre-tassel and total
fertilizer applied, and soil type.

<sup>a</sup> Applied between V12 to R1(silking) corn growth stages.

Table 4. 2017–2018 Grain Sorghum Research Verification Program locations, preplant, sidedress,
late-season and total fertilizer applied, and soil type.

	late-season and total leftilizer applied, and soil type.						
	Preplant		Late-				
County/Year	Fertilizer	Sidedress	Season	Total Fertilizer	Soil Type		
	App	olied Fertilizer It	o/ac of N-P-K-	S-Zn			
White/17	50-0-60-0-0	60-0-0-0-0	0-0-0-0-0	110-0-60-0-0	Calhoun Silt Loam		
Arkansas/18	50-60-60-24-5	69-0-0-0	0-0-0-0-0	119-60-60-24-5	Calloway Silt Loam		
Cross/18	46-60-60-12-10	115-0-0-0-0	0-0-0-0-0	161-60-60-12-10	Collins Silt Loam		
Mean	49-40-60-12-5	81-0-0-0-0	0-0-0-0-0	130-40-60-12-5			

County/Year	Irrigation Type	Irrigation Frequency <sup>a</sup>	Rainfall from planting to maturity
•	5 //		(inches)
Jackson/17	Furrow	4	NAb
Prairie/17	Furrow	3	NA
River Valley/17	Pivot	4	NA
St Francis/17	Furrow	2	NA
Arkansas/18	Furrow	4	8.85
Chicot/18	Non-Irrigated	0	10.72
Clay/18	Furrow	6	12.84
Desha/18	Furrow	6	16.96
Jackson/18	Furrow	7	13.09
Jefferson/18	Furrow	6	11.33
Prairie/18	Furrow	6	17.33
Arkansas/19	Furrow	3	18.99
Chicot/19	Furrow	5	23.40
Clay/19	Furrow	2.5	25.30
Desha/19	Furrow	6	17.02
Jefferson/19	Furrow	5	22.45
Lawrence/19	Furrow	5	23.59
Mississippi/19	Furrow	4	16.90
Monroe/19	Furrow	6	15.71
Prairie/19	Furrow	5	22.50
White/19	Furrow	5	15.86
Mean		4.5	17.22

 Table 5. 2017–2019 Corn Research Verification Program locations, irrigation type, number of irrigations, and rainfall from planting to maturity.

<sup>a</sup> Each furrow irrigation supplied approximately 2 acre-inches of irrigation water and each pivot irrigation applied approximately 1 acre-inch.

<sup>b</sup> Rainfall from planting to maturity is not available.

Table 6. 2017–2018 Grain Sorghum Research Verification Program locations, irrigation type, number of
irrigations, and rainfall from planting to maturity.

County/Year Irrigation Type		Irrigation Frequency <sup>a</sup>	Rainfall from planting to maturity
			(inches)
White/17	Non-irrigated	0	
Arkansas/18	Non-irrigated	0	8.03
Cross/18	Furrow	4	8.23
Mean			8.13

<sup>a</sup> Each furrow irrigation supplied approximately 2 acre-inches of irrigation water.

Table 7. Corn growth stage and corresponding average accumulated growing degree days
determined by weekly field visits in all corn fields in 2018 and 2019.

Corn Growth Stage	Accumulated Growing Degree Days From Planting
VE - Emergence	157
V2	292
V4	449
V6	588
V8	789
V10	958
V12	1081
V14	1218
V16	1343
R1 – Silking	1537
R2 – Blister	1692
R3 – Milk	1858
R4 – Dough	2032
R5 – Dent	2203
R6 - Physiological Maturity (Black Layer)	2831

	Irrigated					Non-Irrigated	
				Simple	% of		% of
Receipts	2019	2018	2017	Average	Budget	2018	Budget
Yield (bu./ac)	208.05	199.28	219.55	208.96		144.0	
Price (\$/bu.)	3.75	3.35	3.75	3.62		3.35	
Total Crop Revenue \$	780.19	667.60	823.31	757.03		482.40	
Seed	133.33	138.81	122.50	131.55	25.11%	138.13	32.14%
Fertilizers & Nutrients	165.04	141.40	141.04	149.16	28.47%	135.84	31.61%
Herbicides	46.64	42.74	27.61	38.99	7.44%	29.28	6.81%
Insecticides	0.00	0.00	0.00	0.00	0.00%	0.00	0.00%
Fungicides	0.00	0.00	0.00	0.00	0.00%	0.00	0.00%
Other Chemicals	0.49	0.00	0.00	0.16	0.03%	0.00	0.00%
Custom Applications	16.55	22.17	15.75	18.16	3.47%	14.00	3.26%
Diesel Fuel, Field Activities	16.82	14.03	10.13	13.66	2.61%	14.27	3.32%
Irrigation Energy Costs	13.20	21.09	14.29	16.19	3.09%	0.00	0.00%
Other Inputs, Pre-harvest	3.88	3.88	3.88	3.88	0.74%	0.00	0.00%
INPUT COSTS	395.94	383.05	328.02	369.00		328.31	
Fees	6.00	6.00	6.00	6.00	1.15%	6.00	1.40%
Crop Insurance	13.00	13.00	13.00	13.00	2.48%	0.00	0.00%
Repairs & Maintenance	20.57	18.43	16.75	18.58	3.55%	14.27	3.32%
Labor, Field Activities	10.43	9.16	7.95	9.18	1.75%	8.69	2.02%
PRODUCTION EXPENSES	445.95	430.36	375.18	417.16		357.27	
Interest	12.26	9.25	8.06	9.86	1.88%	7.68	1.79%
Post-harvest Expenses	93.63	89.64	98.80	94.02	17.95%	64.80	15.08%
Custom Harvest	0.00	0.00	0.00	0.00	0.00%	0.00	0.00%
Total Operating Expenses	551.83	529.29	490.69	523.94	100.00%	429.75	100.00%
Returns to Operating Expenses	228.37	138.30	332.63	233.10		52.65	
Capital Recovery & Fixed Costs	96.45	83.76	88.91	89.71		61.21	
Total Specified Expenses	648.28	613.06	579.60	613.65		490.96	
Returns to Specified Expenses	131.92	54.54	243.72	143.39		-8.56	
Operating Expenses/bu.	2.70	2.69	2.23	2.54		2.98	
Total Specified Expenses/bu.	3.18	3.12	2.64	2.98		3.41	

# Table 8. Operating costs (\$), total costs, and returns for corn research verification program fields, 2017–2019.

Irrigated Non-Irrigated							
	2018	2018	2017	Simple	% of		
Receipts	Cross Co.	Arkansas Co.	White Co.	Average	Budget		
Yield (bu./ac)	130.30	58.00	72.30	86.87	Duagot		
Price (\$/bu.)	3.00	3.00	3.35	3.12			
Total Crop Revenue \$	390.90	174.00	242.21	269.04			
Seed	18.53	13.80	25.20	19.18	7.38%		
Fertilizers & Nutrients	108.98	105.27	50.30	88.18	33.94%		
Herbicides	33.06	17.25	14.75	21.69	8.35%		
Insecticides	18.50	18.50	16.30	0.00	0.00%		
Fungicides	0.00	0.00	0.00	0.00	0.00%		
Other Chemicals	0.00	0.00	0.00	0.00	0.00%		
Custom Applications	28.00	14.00	21.00	21.00	8.08%		
Diesel Fuel, Field Activities	16.10	13.10	12.77	13.99	5.38%		
Irrigation Energy Costs	12.22	0.00	0.00	4.07	1.57%		
Other Inputs, Pre-harvest	3.88	3.88	0.00	2.59	1.00%		
INPUT COSTS	239.33	181.93	140.32	187.19			
Fees	6.00	6.00	6.00	6.00	2.31%		
Crop Insurance	13.00	13.00	13.00	13.00	5.00%		
Repairs & Maintenance	17.10	14.62	17.44	16.39	6.31%		
Labor, Field Activities	10.88	8.81	10.06	9.92	3.82%		
PRODUCTION EXPENSES	286.31	224.36	186.18	232.28			
Interest	6.16	4.82	3.91	4.96	1.91%		
Post-harvest Expenses	33.88	15.08	18.80	22.59	8.69%		
Custom Harvest	0.00	0.00	0.00	0.00	0.00%		
Total Operating Expenses	326.34	244.26	208.89	259.83	100.00%		
Returns to Operating Expenses	64.56	-70.26	33.32	9.21			
Capital Recovery & Fixed Costs	83.07	72.53	76.60	77.40			
Total Specified Expenses	409.41	316.80	285.49	337.23			
Returns to Specified Expenses	-18.51	-142.80	-43.29	-68.20			
Operating Expenses/bu.	2.50	4.21	2.89	3.20			
Total Specified Expenses/bu.	3.14	5.46	3.95	4.18			

Table 9. Operating costs (\$), total costs, and returns for grain sorghum research verification program fields. 2017–2018.

### Gene Editing: A New Approach to Overcome Mycotoxins and Environmental Stress in Arkansas Corn Production

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### Abstract

Many U.S. corn growers find it impossible to guarantee that their crops will not exceed acceptable levels of mycotoxins. Tools and strategies currently available to manage mycotoxins are not consistently effective, and the impact of this risk on U.S. corn growers ranges from reduced profitability, long-term shifts in production away from corn, and even economic ruin from mycotoxin outbreaks. Aflatoxins, one of the most important classes of mycotoxins in corn, are associated with pre-harvest ear rots caused by A. flavus. Aflatoxins have been linked to acute and chronic disorders in animals and are classified as human carcinogens. Environmental stress, particularly heat and drought, are closely associated with pre-harvest aflatoxin contamination. Currently, aflatoxin mitigation tools are limited and partially effective at best. Thus, novel management tools are needed urgently to reduce the impact of aflatoxins in corn. Gene editing, a recent breakthrough technology for non-transgenic manipulation of plant genomes, has tremendous promise to augment corn's resistance to biotic and abiotic stress. The overall goal of this project is to utilize gene editing to improve the resistance of corn to aflatoxin contamination, in part by augmenting resistance to environmental stress. The specific objectives are to: 1) use gene editing for non-transgenic, precision manipulation of corn genes involved in resistance (or susceptibility) to aflatoxin and environmental stress, and 2) genetically map genes/pathways in corn underlying resistance and/or susceptibility to aflatoxin and environmental stress. To this end, we developed a tissue culture-based delivery system for gene editing in corn, from which non-transgenic plants can be regenerated. We also identified candidate genes for editing and developed protocols to create gene editing constructs. This information has provided a crucial foundation to advance gene editing as a tool for aflatoxin control in corn.

### Introduction

Aflatoxins are among the most carcinogenic naturally occurring compounds known to humankind. In the context of corn (Zea mays L.) pathology, the primary producers of aflatoxins are Aspergillus flavus and A. parasiticus (Bennett and Klich, 2003). Although more than 16 different aflatoxin analogs have been described (Bhatnagar et al., 2003), aflatoxin  $B_1$  is regarded as the most toxic and commonly associated with corn. Aflatoxin  $B_1$  consumption has been linked to a range of adverse health effects, including liver cancer, immunosuppression, and growth retardation (Boonen et al., 2012). The Food and Agriculture Organization (FAO) estimates that 25% of world food crops are affected by aflatoxins, and corn is particularly susceptible (Eskola et al., 2019). Environmental conditions such as drought, extreme temperature, and corn ear injury are favorable for Aspergillus infection and aflatoxin production in corn (Reverberi et al., 2010).

Decades of conventional breeding in public- and privatesector research programs have failed to produce corn hybrids with acceptable resistance to aflatoxin (Brown et al., 2013). Some level of genetic resistance is known to exist, but it is mostly found in tropical corn germplasm, which is not suitable for modern, row-crop agriculture. Linkage drag and other issues have made it nearly impossible to move aflatoxin resistance from tropical lines into commercial germplasm (Warburton et al., 2017). Transgenic approaches offer hope for a quicker solution (Thakare et al., 2017). In previous work, the Bluhm lab created transgenic corn designed to silence fungal genes involved in aflatoxin biosynthesis. In 2016, enough seed was available for replicated experiments, which showed that these lines had up to 50% less mycotoxin accumulation than non-transgenic controls. However, two drawbacks of transgenic resistance are 1) public perception of transgenic material, and 2) the time and cost to get regulatory approval for new transgenes in food crops. Neither issue is insurmountable, but both must be considered to bring new transgenics to market, and ultimately into the hands of growers.

Recently, a new technique known as gene editing has become feasible in crop plants, including corn and sorghum (Jaganathan et al., 2018; Kelliher et al., 2019). In this process, a technology known as CRISPR-Cas9 is used to customize the sequence of one or more plant genes in order to change specific traits (Ran et al., 2013). A few important points set gene editing apart from transgenics. First, gene editing modifies genes already present in the plant genome. It can be used to inactivate genes associated with susceptibility to stress, such as aflatoxin accumulation, up-regulate genes involved in resistance, or change the sequence of a gene in order to change its function. Second, gene editing can be done in corn without transgenic approaches—there is no insertion of foreign DNA into the corn genome. Third, since gene editing can be done

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non-transgenically, it is shaping up to be regulated much differently (and less strictly) than transgenic plants. Recently, a gene edited mushroom that resists bruising has led the way: the USDA exempted the mushroom from its regulatory process (Waltz, 2016).

Philosophically, the plant pathology research community should explore aflatoxin resistance from new perspectives. In humans, a fever is a symptom of an infection or other malady; treating the fever can bring temporary relief, but does not solve the underlying cause. Similarly, aflatoxin contamination can be conceptualized as a symptom of stressed corn, with two of the biggest culprits being heat and drought stress. Considerable research has focused on preventing aflatoxin accumulation as a symptom, without adequately addressing the fundamental, underlying problems of how corn responds to environmental stress. However, a greater focus on addressing genetic resistance to stress will naturally augment resistance to aflatoxin accumulation in corn.

Thus, the research objectives of this project are to: 1) Use gene editing for non-transgenic, precision manipulation of corn genes involved in resistance (or susceptibility) to aflatoxin and environmental stress, and 2) Genetically map genes/pathways in corn underlying resistance and/or susceptibility to aflatoxin and environmental stress. These genes will also be used as targets for gene editing.

### Procedures

### **Objective 1**

Because gene editing is a new technology, the first step of the process was to create an assemblage of tools, skills, and resources required to apply gene editing in corn. This is somewhat analogous to building a new assembly line in a factory in order to create new products consistently and efficiently. Although gene editing has been used widely in model plants (Pandey et al., 2019), there are comparatively fewer reports of successful gene editing in corn (Young et al., 2019). Some of the specific tools required for gene editing in corn have not been extensively tested by the scientific community and therefore require varying levels of optimization. Fundamental tools required for successful gene editing in corn include a robust tissue culture system, the ability to create and regenerate protoplasts, efficient delivery of gene editing constructs into corn protoplasts and/or tissue culture cells, the ability to efficiently regenerate non-transgenic, edited plants, and high-throughput screening for gene editing events.

In parallel to developing the fundamental tools required for gene editing, it is also important to identify candidate genes in corn that regulate stress responses. One of the most promising categories of genes to target is transcription factors—genetic relay switches that regulate the expression of other (often numerous) downstream genes involved in front-line responses to environmental stimuli (like stress) (Meshi and Iwabuchi, 1995). Some families of plant transcription factors are known to be involved in various stress responses (Alves et al., 2013; Joshi et al., 2016), although some of these families are comprised of hundreds of genes. To narrow down which specific genes to target, complementary sources of information were utilized, including published literature regarding the function of specific transcription factors in corn (and homologous genes from related crop plants) and public data sets of genome-wide gene expression analyses in response to environmental stress.

After optimizing the gene editing pipeline and identifying candidate genes, the next step is to perform gene editing. This study uses the CRISPR-Cas9 system with transient (non-transgenic) expression of constructs for gene editing. We screen for bi-allelic (homozygous) editing events, regenerate plants from edited protoplasts and/or tissue culture material, and increase seed for two generations. Initially, edited material is evaluated in greenhouse assays to determine resistance to heat stress, drought stress, and aflatoxin accumulation. Promising lines are then evaluated in field conditions at multiple sites. Drought stress can be induced by withholding irrigation as needed; heat stress is more difficult to induce due to dependence on the weather, but is increased by delaying planting dates so that reproductive development occurs during the hottest part of summer.

### **Objective 2**

A genetic approach is used to identify corn genes involved in stress resistance (and susceptibility) that function specifically in Arkansas production conditions. This component of the project provides an additional source of gene targets for editing as described above. Initially, three inbred corn lines were selected that are highly susceptible to environmental stress and aflatoxin accumulation, and pilot gene expression analyses were performed to identify promising target genes. However, because new, cost-effective techniques have become accessible for association mapping, the gene discovery strategy was modified to phenotype a corn diversity panel for stress responses, particularly at V7-R3 stages of development. Phenotyping data will be used for association mapping; the mapping interval will subsequently determine the strategy to clone the specific gene(s) underlying the trait. A conceptually similar approach has successfully identified genes involved in disease and insect resistance (Stagnati et al., 2020; Rossi et al., 2020; Jiménez-Galindo et al., 2019; Samayoa et al., 2015) and has been applied to specific components of environmental stress (Gao et al., 2019a). Genes identified in this approach will be modified via gene editing, as described above, to improve stress resistance; the exact strategy will depend on the type(s) of genes identified.

### **Results and Discussion**

The foremost requirement to perform gene editing in corn is to implement a robust, reliable tissue culture system. The core of this approach is to grow undifferentiated (and uncontaminated) corn cells in culture in laboratory conditions (Thorpe, 2013). These cells are totipotent, in that any individual cell is capable of re-forming a healthy corn plant. A wide variety of cell culture media and additives, sterilization techniques, and growth conditions were evaluated (Green and Phillips, 1975; Brar et al., 1979; Phillips et al., 1988; Frame et al., 2006; Jiang et al., 2015; Silvarajan et al., 2017). Ultimately, techniques from various sources of published scientific literature and our own adaptations and modifications were blended in order to develop a robust system for corn tissue culture at the University of Arkansas System Division of Agriculture (Fig. 1A). With this approach, we were able to propagate cell cultures from numerous inbred lines of corn. With periodic transfer to fresh growth medium, cultures can be propagated for >12 months. The regeneration of plants from tissue culture cells was highly efficient (Fig. 1B) and produced plants that developed normally through all growth stages (including reproductive development) in greenhouse conditions.

The corn cell lines described above were utilized in liquid suspension cultures to create source material for protoplasting. Corn protoplasts are essentially cells stripped of their cell walls, which makes them more receptive and accessible to receive gene editing constructs. However, protoplasts are notoriously fragile, and regeneration of corn plants from protoplasts can be challenging. Techniques were extracted from several published studies (Cao et al., 2014; Gao et al., 2019b) and the authors' personal experience in order to develop a corn protoplasting protocol. Although protoplasts were viable, the efficiency of regeneration into cells with cell walls and subsequent production of viable plants requires further optimization for large-scale genome editing via protoplasting. Parameters being evaluated with highest priority include age of culture suspensions used as source material (older appears to be better), regeneration conditions, particularly the osmoticum, and potential genotypic background effects (some inbred lines appear to work better than others).

For delivery of gene editing constructs into corn cells and protoplasts, we explored two technologies. The first is a gene gun (Fig. 2), which uses compressed helium at high pressure to physically force DNA into corn tissue culture cells in a process known as biolistics (Baltes et al., 2017). The second is Agrobacterium, which naturally evolved to infuse DNA into plant genomes (Nester, 2015). When modified, Agrobacterium is unable to complete the transfer of DNA into the plant genome, but the gene editing components are still expressed in plant cells, thus leading to non-transgenic editing events. The majority of our efforts to date have focused on biolistic approaches for delivery of gene editing constructs. We have combined information from published protocols (Frame et al., 2000; Lowe et al., 2009; Liu et al., 2019) and the authors' personal experience to optimize construct delivery. Currently, we have an acceptable level of transient expression for gene editing. Although we have also explored pilot experiments utilizing Agrobacterium, a key concern is that governmental regulations are still evolving regarding the definition of genetically modified plant material in light of emerging gene editing technologies. Thus, it is still not fully clear how plants will be labeled when Agrobacterium is utilized in their development-even when foreign DNA is not inserted into the genome of corn cells.

To identify gene editing events in corn tissue culture cells and/or young, regenerated plantlets, a next-generation DNA sequencing approach was developed for high-throughput screening. High-throughput screening is crucial for several important reasons. First, the efficiency of gene editing can vary substantially in populations of cells; when efficiency is low, it is crucial to screen large numbers of cell lines in order to obtain edited material. Second, the key focus of this project is to perform gene editing non-transgenically, and thus the introduction of selectable markers (such as antibiotic resistance) is not feasible. As a result, large numbers of 'escapes' are possible—viable cells in which gene editing did not occur. Third, early and accurate screening of cell populations identifies highly efficient (or inefficient) gene editing events before excessive time is invested in culture maintenance, which allows us to focus on regenerating plants from the most successful editing experiments. In turn, this allows the most efficient use of existing greenhouse and laboratory space and accelerates the creation of edited corn lines.

To create a semi-quantitative assay to assess successful gene editing in corn (cell populations or pooled individual lines), we adapted a protocol recently developed in our research program for target-enrichment sequencing to identify genomic lesions in fungal mutant populations (Sharma, 2018). With this approach, we designed customized DNA-oligo 'capture probes' that corresponded to gene editing targets in corn. We then extract DNA in bulk from edited cell lines and/or plantlets, and use the capture probes to 'fish out' (enrich) DNA sequences corresponding to the gene of interest from the pooled DNA sample. Finally, we sequence this enriched sample of DNA at considerable depth (>1000× coverage) via next-generation DNA sequencing. This provides a semi-quantitative analysis of overall editing efficiency and a profile of the types of editing events created (there is often a degree of variability at the DNA sequence level regarding editing events). Multiple genes can be targeted in the same sequencing strategy, which allows us to multiplex gene editing events (target multiple genes for editing at the same time). Additionally, depending on how sample pools are organized (e.g., the number of individual cell lines/ plantlets per pool), this approach can also be used to quickly identify rare individuals with specific editing events.

Candidate genes for gene editing were identified based on predicted molecular function (transcriptional regulators) and putative involvement in environmental stress responses (drought, heat tolerance, etc.). In plants, abscisic acid (ABA) is a key signaling intermediary for environmental stress, including drought (Cutler et al., 2010). Corn, in particular, utilizes both ABA-dependent and ABA-independent signaling pathways to respond at the transcriptional level to heat and drought (reviewed by Kimotho et al., 2019). To identify candidate transcription factors for gene editing in corn, we focused primarily on three gene families: MYB/MYC (heat and drought responsive, ABA-dependent); WRKY (heat and drought responsive, ABA-dependent); and DREB (heat responsive, ABA-independent). Of the 72 MYB/MYC genes identified in the maize genome (Du et al., 2013), at least 22 were induced after exposure to abiotic stress (Chen et al., 2017). Three of these genes (ZmMYB30, ZmMYB36, and ZmMYB95) were selected as finalists for gene editing, with the specific strategy of increasing expression levels through promoter modifications. Among WRKY transcription factors, which comprise the largest superfamily of plant transcription factors (Tripathi et al., 2014), a subset of genes was identified that are either induced or suppressed during heat and drought stress, including *ZmWRKY17*, *ZmWRKY33*, *ZmWRKY40*, *ZmWRKY44*, *ZmWRKY58*, and *ZmWRKY106*. The differential expression of WRKY transcription factors in response to stress is intriguing, as inactivation via gene editing may convey increased stress tolerance. Of the DREB transcription factors, *ZmDREB1A*, *ZmDREB2A*, *ZmDREB2.7*, *ZmDREB3*, and *ZmDREB4* were identified as candidate genes for editing. The DREB proteins (an acronym derived from dehydration responsive element binding) have been broadly associated with stress responses in corn, particularly heat and drought stress (Zhuang et al., 2010).

Expression profiles of transcription factors from these families were cross-referenced in other data sets, including responsiveness to infection by A. flavus (Jiang et al., 2011; Kelley et al., 2012; Dhakal et al., 2017; Shu et al., 2017). Somewhat surprisingly, published studies examining the transcriptional response of corn to A. flavus infection identified very few transcription factors as candidate genes involved in resistance or susceptibility. This is likely because the depth of sequencing in these studies was insufficient to identify regulatory genes, the timepoints selected for analysis were substantially later than initial regulatory events triggering metabolic responses, regulatory responses were masked due to the complexity of the plant-fungal interaction, a majority of defense components were constitutively expressed as a baseline, and anticipatory defense response, and/or corn's responses to environmental stress supersede specific transcriptional responses to fungal infection.

To identify corn genes involved in stress tolerance, we are evaluating multiple-parent advanced-generation inter-cross ('MAGIC') lines of maize (Holland, 2015). These lines facilitate mapping of genes associated with environmental stress responses more quickly and with greater confidence compared to other genetic resources and approaches (Dell'Acqua et al., 2015). Inbred lines are planted, in randomized replication, in field conditions and phenotyped pertaining to heat and drought stress. Phenotyping data are superimposed on existing genetic data for each line, which facilitates association mapping. In some (fortunate) cases, this approach could identify specific candidate transcription factor genes. In most cases, however, we anticipate identifying specific regions of the corn genome associated with environmental stress responses in Arkansas. We can then use this information to corroborate the genomic location of known transcription factors, which will provide additional lines of evidence for selected targets and potentially elevate the priority of some candidates over others for gene editing

### **Practical Applications**

Environmental conditions in Arkansas can be stressful for corn, which requires additional inputs for management and introduces risk for growers. When prices are low, the cost of additional inputs is even more problematic. Aflatoxin remains one of the most unpredictable, difficult to manage potential problems for Arkansas corn producers. We believe that the long-term outlook for profitable corn production in Arkansas and other Southeastern states depends to a considerable extent on stress and aflatoxin management. Through gene editing, our ultimate aim is to develop new genetic material that will ultimately lead to corn hybrids specifically customized for Arkansas production conditions. We anticipate that creating gene-edited, stress-resistant material will require three to six years of research, development, and field testing. Once gene edited material has been thoroughly assessed, the modified genes can be introgressed into advanced breeding lines and/or the parents of commercial hybrids (or edited directly in such lines) within another two to three years.

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Fig. 1. Key components of the corn tissue culture system. (A) Corn tissue culture cells. (B) Juvenile plants regenerated from undifferentiated corn tissue culture cells.



Fig. 2. Biolistic particle bombardment of corn tissue culture cells using a gene gun.

# Detection, Spread and Economic Impact of Southern Rust in Arkansas Corn Fields Using Remote Sensing and Spatial Analysis Technologies

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### Abstract

Southern rust in corn is caused by the fungus Puccinia polysora (Underwood) and is the most economically important foliar disease in corn production in Arkansas. The disease does not overwinter in the state, but rather in warmer climates to the south. Southern rust can cause severe yield loss if not managed correctly. During the 2017, 2018, and 2019 growing seasons, 10 corn fields were scouted, and the amounts of southern rust determined on a spatial grid. After scouting and disease confirmation, fields were marked with GPS point locations in a grid pattern across the entire field. After each field was marked, ratings of disease severity (percent leaf with southern rust) were taken below the ear leaf, at the ear leaf, and above the ear leaf at each point at least two times until physiological maturity. Data analyses showed that southern rust did not occur randomly, which is a common thought about foliar diseases. However, the disease spread in a uniform or dispersed fashion across each field but multiplied differently in localized clusters throughout the field, a distribution that indicates the disease is likely dependent on some clustered environmental phenomenon that favors its development in certain areas of fields over others. When soil samples were collected after harvest at each GPS point and nutrient concentrations determined, there was a significant positive correlation between relative levels of phosphorus and southern rust severity in 6 of 10 fields (P = 0.10). Imagery was collected and a normalized difference vegetative index (NDVI) ratio was calculated just prior to tasseling for 1 field in 2017 and for 4 fields in 2018. There was a significant positive correlation with relatively higher NDVI and relatively higher southern rust severity many weeks later in the season (P = 0.10) in all of them.

### Introduction

Southern rust (SR), *Puccinia polysora* (Underwood), is a troublesome disease that can cause widespread damage and yield loss without proper management. The fungus can be identified by small orange pustules clustered together on the upper surface of the corn leaf. The SR pustules tend to be found first in the lower leaves of the corn plant and when the weather is favorable, spores advance up the plant and spread. The pathogen does not overwinter in Arkansas as the majority of inoculum, called urediniospores, are blown in annually from the south (Vincelli, 2010).

Southern rust is typically confirmed each year in the southernmost parts of the United States, Texas and Florida. Most SR incidence occurs in the lower Mississippi Valley and Texas, but the disease has been confirmed as far north as Massachusetts (Melching, 1975). Favorable weather conditions, approximately six hours of dew, and temperatures ranging from 77–82 °F are required for infection and disease development (Rodriguez et al., 1980). After the disease is established and favorable weather conditions are present, the urediniospores are dispersed through the field via wind and rain. These spores serve as both primary and secondary inoculum. Southern rust pustules can be found on husks and stalks in severe cases. Since

the pathogen does not overwinter in Arkansas, tillage practices do not influence local disease incidence.

Corn hybrids vary in susceptibility to the pathogen, but most hybrids planted are susceptible to southern rust. A single resistance gene, *Rpp9*, from a South African maize hybrid, was first identified in 1965 (Ullstrup, 1965). The *Rpp9* gene was bred into many hybrids but these are not commonly marketed due to yield limitations. Today, there are 11 genes controlling specific resistance to *P. polysora*, which are designated *Rpp1* to *Rpp11*. However, these resistant genes have been associated with yield drag, which is a negative effect on grain yield. Foliar fungicides can be applied to corn if levels of SR are high. However, growers typically do not hire a corn scout, so detection is problematic in many cases. This often results in prophylactic applications of foliar fungicide as opposed to disease scouting and a more discovery-based approach.

Currently, there are no good options for management of SR other than scouting and spraying. Without a reliable method of scouting and a data driven economic threshold, treatment of the disease is most problematic for growers in Arkansas and the mid-South. Also, SR is often misidentified earlier in the season as a similar looking disease called common rust (Fig. 1). This has caused unnecessary fungicide applications that do not add value to the crop.

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A pilot study suggested that SR builds preferentially in fields and is positively correlated with improved plant health. If the disease is related to a measurable source of variability, this could be used to inform scouting plans. The objectives of this work were to determine how SR moves throughout the field once established and how the disease builds relative to other factors within fields. These findings should allow for the development of a more reliable economic threshold and a predictive scouting procedure.

### **Procedures**

Southern rust was scouted until at least three fields were found with the disease in 2017, 2018, and 2019. In 2018 and 2019, fields in the University of Arkansas System Division of Agriculture's Arkansas Corn Research Verification Program (CRVP) were used. The distributions of SR were tracked from first detection to physiological maturity. Once SR was confirmed, the field was spatially marked with SMS Mobile software (Ag Leader Technology, Ames, Iowa) running on a Yuma 2 GPS system (Trimble Inc., Sunnyvale, California). Points were marked along rows in a distribution representative of field scale, at least 16 ft apart. The number of points per row and field were dependent on the size and orientation of the field relative to row and planting direction. Each field was rated at the first detection of SR and every two weeks following until physiological maturity. At every GPS point, a linear 10-ft area of row was rated as a total percentage of SR below the ear leaf (BEL), at the ear leaf (EL), and above the ear leaf (AEL). After harvest, soil samples were collected from every GPS-marked point at each field. Soil was collected and placed in 1-gal plastic bags labeled according to each field and GPS point, and loaded into ice chests. Soil was sent to the University of Arkansas System Division of Agriculture's Soil Testing and Research Laboratory in Marianna, Arkansas.

The marked GPS points were exported as a shape file (.shp) from the Yuma 2. All disease rating data were recorded in an Excel spreadsheet (Microsoft Corporation, Redmond, Washington) by GPS position, and then copied into a database file (.dbf) that accompanied the .shp file for each field. The .shp file was imported into ArcMap (ESRI, Redlands, California) and projected to the coordinate system WGS1984 UTM15N where precise distances were measured. Data from the SR ratings at each plant part were visualized and analyzed spatially in ArcMap and GeoDa (Center for Spatial Data Science, University of Chicago, Chicago, Illinois) software. In ArcMap, data were spatially interpolated at each rating date and plant part for visualization. In ArcMap, a binary Moran's I was used to determine the distribution of points positive for SR at each rating date and plant part. Moran's I is a measure of spatial autocorrelation to determine how variables agree with themselves across space to determine if their distributions are clustered (patchy), random (no pattern), or dispersed (like a checkerboard or grid) (Moran, 1950). For the binary Moran, field points were queried and points positive were analyzed. In GeoDa, a quantitative Moran's I was computed to show distribution of SR percentage at each rating date and plant part.

Due to institutional limitations for aerial imagery collection, Mavrx LLC (San Francisco, California) was hired to collect aerial

imagery from various designated research fields and returned normalized difference vegetation index (NDVI) images of the fields at different dates through the 2017 and 2018 growing seasons. At each field, outliers were removed and the NDVI image was spatially joined to the .shp file that contained SR data. A 10-ft, 20-ft, and 30ft radius was analyzed from the NDVI image around each point at the second (final) rating for EL and AEL. Only the final rating was analyzed in comparison to the NDVI images due to the adequate amount of SR compared to a lesser amount from all fields' first ratings. These analyses were completed to determine correlation between the NDVI and SR severity levels. In 2017, only one field was flown by Mavrx prior to tassel. In 2018, all 7 corn fields used in the CRVP were flown by Mavrx LLC (San Francisco, California) in early June 2018, where NDVI images were obtained. These 7 corn fields were scouted weekly and 3 were found to be infested with SR early enough in the growing season to collect data and study. In 2019, all CRVP fields were flown using an unmanned aerial system (UAS), DJI Phantom 4 Pro (DJI, Inc., Los Angeles, California) carrying a sensor capable of collecting near-infrared imagery to calculate a NDVI (Sentera Inc., Minneapolis, Minnesota). However, due to equipment malfunction, fields could not be flown prior to tasseling.

Upon detection of SR in a field during the 2018 and 2019 growing season, aerial imagery was collected from each field using the Phantom 4 UAS at the time of rating for every rating.

### **Results and Discussion**

Across 3 years, 10 fields were found to be positive for SR (Table 1). In the 2017 (Table 2), 2018 (Table 3), and 2019 (Table 4) growing seasons, results from the spatial study showed that the distribution of points positive for SR began in a random or significantly dispersed distribution most of the time and progressed to entirely dispersed as SR was found at most data points in the field by maturity (P = 0.05). The most important finding from this work is that as the season progressed, the quantitative distribution of SR became clustered meaning that the epidemic did not spread in equal severity field-wide but built preferentially in areas (Fig. 2). In 6 of 10 fields, there was a positive and significant spatial relationship between SR severity at the ear leaf or above the ear leaf and soil phosphorus concentrations from post-harvest sampling (Table 5). This relationship was not as reliable in 2018 and 2019 as the overall severity of SR was less in the fields sampled (an example of the relationship to soil phosphorus is shown in Fig. 3). In 1 field in 2017 (Fig. 4), and 4 fields in 2018, pre-tassel normalized difference vegetation indices indicated a positive spatial relationship with SR severity as well (Table 6). Imagery from dates after tasseling was inconsistent and is not presented in this publication.

### **Practical Applications**

The evidence from this work suggests that SR spreads throughout fields but builds preferentially according to more favorable microenvironments within the corn canopy. The totality of the conditions favorable for more prolific reproduction are not fully understood at the completion of this study. Pretassel imagery could help differentiate areas in fields where the corn crop is relatively more healthy and the corn leaf canopy more dense. In theory, the more dense canopy should add some level of protection for the fungus that is sensitive to ultraviolet radiation and dessication. The canopy could also provide a microclimate that allows free water on the leaf surface to exist longer increasing the time that fungal spore germination and infection could occur. These areas of field relative variability could be used to help locate the initial onset of fungal reproduction and disease caused by SR. It will be important to explore these relationships in the future and continue this work to help develop a more predictive scouting model, or possibly a tool, for SR detection and aid in more informed management decisions (such as timing of fungicide applications). The current economic threshold is 5% SR on the ear leaf prior to R3. Fungicide applications beyond this date do not consistently add value to the corn crop nor does late-season development of SR decrease yield significantly.

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# Table 1. Fields rated for southern rust in Arkansas from 2017–2019. Table shows location and size of fields. The dates rated, plant parts rated, and growth stage at each rating is shown.

Year	Field	County	Coordinates	Acres	Dates Rated <sup>a</sup>	Growth Stage
2017	Pickens_17	Desha	33°50'3.91"N, 91°29'19.38"W	61	12 July	Dent (R5)
					26 July	Diack Layer (NO)
	Plumerville_17	Conway	35°7'52.61"N, 92°39'16.10"W	59	18 July	Hard Dough (R4) Dent (R5.5)
	Grady 1	Lincoln	24° 2'42 66"N 01°40'18 05"W		2 August	2011 (1010)
	Grady_1	LINCOIN	34 2 43.00 N, 91 40 18.95 W	81	20 July	Black Layer (R6)
	Grady_2	Lincoln	34°7'16.45"N, 91°40'38.06"W	54	28 June 15 July 26 July	Milk (R3) Hard Dough (R4) Dent (R5)
2018	Jackson_18	Jackson	35°33'44.32"N, 91°3'47.66"W	29	26 July 6 August	Dent (R5) Black layer (R6)
	Prairie_18	Prairie	34°58'58.18"N, 91°35'9.64"W	32	26 July 6 August	Dent (R5) Black layer (R6)
	Clay_18	Clay	36° 16'38.38"N, 90°27'36.44"W	52	26 July 6 August	Dent (R5) Black layer (R6)
2019	Dumas_19	Desha	33°51'57.69"N, 91°28'3.39"W	32	19 August 3 September	Black layer (R6) Post black layer
	White_19	White	35°8'49.92"N, 91°38'31.89"W	65	12 August 29 August	Dent (R5) Black layer (R6)
	Pickens_19	Desha	35°8'49.92"N, 91°38'31.89"W	34	30 July 8 August	Dent (R5) Black layer (R6)

<sup>a</sup> GPS points within corn fields were marked and the amount of southern rust determined within a 10-ft section of row below the ear leaf, at the ear leaf, and above the ear leaf for each individual point location on the date indicated.

Cluste	Clustered and dispersed distributions are considered statistically significant ( $P = 0.10$ ).							
	# points		Binary	Moran's I <sup>b</sup>	Quantitat	ive Moran's I <sup>c</sup>		
Field	positive for SR	Plant Part <sup>a</sup>	P-value	<b>Distribution</b> <sup>d</sup>	P-value	Distribution		
Grady_1_17	73	EL 1	0.001	dispersed	0.003	clustered		
	73	AEL 1	0.001	dispersed	0.001	clustered		
Pickens_17	4	BEL 1	<0.001	dispersed	0.275	random		
	4	EL 1	<0.001	dispersed	0.229	random		
	0	AEL 1	No Value	NC	No Value	NA		
	100	EL 2	0.001	dispersed	0.001	clustered		
	100	AEL 2	0.001	dispersed	0.001	clustered		
Grady_2_17	11	BEL1	0.112	random	0.269	random		
	1	EL 1	No Value	NC	0.227	random		
	0	AEL 1	No Value	NC	No Value	NA		
	100	BEL 2	0.001	dispersed	0.001	clustered		
	100	EL 2	0.001	dispersed	0.001	clustered		
	100	AEL 2	0.001	dispersed	0.098	clustered		
	100	EL 3	0.001	dispersed	0.001	clustered		
	100	AEL 3	0.001	dispersed	0.001	clustered		
Plumerville_17	78	BEL1	0.001	dispersed	0.002	clustered		
	42	EL 1	0.001	dispersed	0.155	random		
	21	AEL 1	0.003	dispersed	0.355	random		
	80	BEL 2	0.001	dispersed	0.001	clustered		
	80	EL 2	0.001	dispersed	0.001	clustered		
	80	AEL 2	0.001	dispersed	0.002	clustered		

Table 2. Southern rust (SR) distributions from fields in 2017.	
Clustered and dispersed distributions are considered statistically significant (P	= 0.1

<sup>a</sup> SR severity ratings at the ear leaf (EL), above the ear leaf (AEL), or below the ear leaf (BEL)

at the first (1) or second (2) rating date.

<sup>b</sup> Moran's, I calculated from the points positive for SR (either 1 for positive or 0 for negative). <sup>c</sup> Moran's I calculated from the amount of SR found at a given point and plant part.

<sup>d</sup> Distributions are either clustered (grouped/clumped), random, or dispersed (checkerboard-like/ evenly distributed).

	# points		Binary	Moran's I⁵	Quantitativ	e Moran's lº
Field	positive for SR	Plant Parta	P-value	<b>Distribution</b> <sup>d</sup>	P-value	Distribution
Jackson_18	12	EL 1	0.001	dispersed	0.42	random
	0	AEL 1	No Value	NA	No Value	NA
	77	EL 2	0.001	dispersed	0.001	clustered
	17	AEL 2	0.005	dispersed	0.01	clustered
Prairie_18	24	BEL 1	0.001	dispersed	0.001	clustered
	10	EL1	0.001	dispersed	0.01	clustered
	2	AEL 1	<0.001	dispersed	0.26	random
	100	EL 2	0.003	dispersed	0.001	clustered
	99	AEL 2	0.005	dispersed	0.002	clustered
Clay_18	12	BEL1	0.001	dispersed	0.18	random
	4	EL 1	<0.001	dispersed	0.05	clustered
	0	AEL 1	No Value	NA	No Value	NA
	44	EL 2	0.001	dispersed	0.001	clustered
	37	AEL 2	0.001	dispersed	0.002	clustered

Table 3. Southern rust (SR) distributions from fields in 2018. Clustered and dispersed distributions are considered statistically significant (P = 0.10).

<sup>a</sup> SR severity ratings at the ear leaf (EL), above the ear leaf (AEL), or below the ear leaf (BEL) at the first (1) or second (2) rating date.

<sup>b</sup> Moran's I calculated from the points positive for SR (either 1 for positive or 0 for negative).

<sup>c</sup> Moran's I calculated from the amount of SR found at a given point and plant part.

<sup>d</sup> Distributions are either clustered (grouped/clumped), random, or dispersed (checkerboard-like/evenly distributed).

Clu	stered and disper	sed distributio	ns are consid	ered statistically	significant (P	= 0.10).
	# points		Binary	Moran's I <sup>b</sup>	Quantitati	ive Moran's I <sup>c</sup>
Field	positive for SR	Plant Parta	<i>P</i> -value	<b>Distribution</b> <sup>d</sup>	P-value	Distribution
Pickens_19	35	EL 1	0.21	random	0.001	clustered
	36	AEL 1	0.31	random	0.001	clustered
	33	BEL1	0.34	random	0.001	clustered
	91	EL 2	<0.001	dispersed	0.49	random
	93	AEL 2	<0.001	dispersed	0.09	clustered
	91	BEL2	<0.001	dispersed	0.21	random
	99	AEL3	<0.001	dispersed	0.06	clustered
White_19	0	EL 1	No value	NA	No value	NA
	0	AEL 1	No value	NA	No value	NA
	0	BEL1	No value	NA	No value	NA
	85	EL 2	<0.001	dispersed	0.001	clustered
	84	AEL 2	<0.001	dispersed	0.18	random
	49	BEL 2	0.31	random	0.02	clustered
Dumas_19	28	EL 1	0.36	random	0.04	clustered
	26	AEL 1	0.51	random	0.03	clustered
	15	BEL1	0.01	clustered	0.02	clustered
	83	EL 2	<0.001	dispersed	0.001	clustered
	99	AEL 2	<0.001	dispersed	0.001	clustered
	0	BEL 2	No value	NA	No value	NA

Table 4. Southern rust (SR) distributions from fields in 2019.	
lustered and dispersed distributions are considered statistically significant (	P = 0.1

<sup>a</sup> SR severity ratings at the ear leaf (EL), above the ear leaf (AEL), or below the ear leaf (BEL) at the first (1) or second (2) rating date.

<sup>b</sup> Moran's I calculated from the points positive for SR (either 1 for positive or 0 for negative).

<sup>c</sup> Moran's I calculated from the amount of SR found at a given point and plant part.

<sup>d</sup> Distributions are either clustered (grouped/clumped), random, or dispersed (checkerboard-like/evenly distributed).

			Independent	-	
Year	Field	# of points <sup>a</sup>	variable	<b>Relationship</b> <sup>b</sup>	<b>Correlation</b> <sup>c</sup>
2017	Pickens_17	99	Р	+	0.02
	Grady_1_17	71	Р	+	<0.0001
	Grady_2_17	94	Р	+	0.06
	Plumerville_17d	21	Р	+	0.04
2018	Prairie_18	94	Р	+	0.07
	Jackson_18	73	Р		NS
	Clay_18	108	Р		NS
2019	Dumas_19 <sup>d</sup>	26	Р	+	0.07
	White_19	99	Р		NS
	Pickens_19	99	Р		NS

 Table 5. Southern rust severity at the ear leaf and relationship to soil phosphorus sampled post harvest using spatially collected data from corn fields across three years.

<sup>a</sup> GPS marked points where southern rust was found.

<sup>b</sup> (+) indicates a positive relationship between southern rust severity and soil phosphorus while (-) indicates an inverse relationship.

• A *P*-value of 0.10 or less indicates a significant relationship between southern rust severity and soil phosphorus using spatial regression analysis.

<sup>d</sup> Ratings from above the ear leaf plant part.

				Radius around	
Field	Plant part	Model <sup>a</sup>	# of points <sup>b</sup>	point (m) <sup>c</sup>	P-value
Prairie_18	EL2	LAG	98	3	0.002
	EL2	LAG	98	6	0.006
	EL2	LAG	98	9	0.001
	AEL2	LAG	94	3	0.002
	AEL2	LAG	94	6	0.100
	AEL2	LAG	94	9	0.003
Jackson_18	EL2	LAG	76	3	0.090
	EL2	LAG	76	6	0.080
	EL2	LAG	76	9	0.060
	AEL2	LAG	76	3	0.060
	AEL2	LAG	76	6	NS
	AEL2	LAG	76	9	0.040
Clay_18	EL2	LAG	108	3	0.007
	EL2	LAG	108	6	0.010
	EL2	LAG	108	9	0.020
	AEL2	LAG	108	3	0.030
	AEL2	LAG	108	6	0.020
	AEL2	LAG	108	9	NS

Table 6. The table represents correlations between early normalized difference vegetative index (NDVI)
images taken on 2 June 2018 and southern rust severity at different corn fields and on different plant part
ratings, ear leaf (EL) and above ear leaf (AEL), in the 2018 growing season.

<sup>a</sup> Spatial lag model used after running ordinary least squares regression and diagnostics indicated spatially dependencies of variables.

<sup>b</sup> GPS marked points where data collection occurred.

<sup>c</sup> Radius of sampled pixel data around each point for analysis.



Fig. 1. Southern rust (left) compared to common rust (right) on infected corn leaves. Southern rust spores are identified by small orange to tan pustules clustered together on the upper surface of the corn leaf. Common rust tends to be present on both sides of the leaf and appear dark red to brown. Southern rust can cause severe damage to corn fields in Arkansas when conditions favor disease development. Common rust does not impact yields in the state.



Fig. 2. Southern rust (SR) distribution and severity in the Pickens field at the first rating below the ear leaf (2A) on 12 July 2017 at R5 (Dent) and at the second rating (2B and 2C) on 26 July 2017 at the ear leaf and above the ear leaf, respectively. Blue dots indicate the spatially marked GPS points infested with SR. The interpolated color map indicates the estimate of severity using ordinary kriging. All three distributions were significantly dispersed (P < 0.0001) as calculated using a binary Moran's I of points positive for SR. Calculated using a quantitative Moran's I, SR quantities were random upon establishment below and at the ear leaf and became clustered as disease severity progressed at the ear leaf and above the ear leaf (P = 0.001).



Fig. 3. Prairie County corn field in 2018. The correlation between southern rust (SR) severity (3A) and (3B) phosphorus (P) levels (*P* = 0.05) is positive across relative spatial changes. The interpolated color map indicates estimated severity using ordinary kriging. Blue dots indicate points rated for southern rust and points soil fertility samples were collected.



Fig. 4. Normalized difference vegetative index (NDVI) image from 11 Jun 2017 (4A). NDVI images show relative crop performance changes throughout the field with green indicating healthier plants and red indicating less healthy. The second image (4B) shows southern rust (SR) severity at ear leaf on 14 Jul 2017. The interpolated color map indicates estimated severity using ordinary kriging. Southern rust severity levels and NDVI data were significantly correlated (*P* = 0.05).
## Preemergence and Postemergence Corn Tolerance to Photosystem II-Inhibiting Herbicides

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#### Abstract

Weed control in corn has traditionally relied on atrazine as a foundational tool to control problematic weeds such as Palmer amaranth and barnyardgrass. However, recent discovery of atrazine in aquifers and other water sources may pose potential restrictions on its use. Therefore, research was initiated in 2017 to explore potential atrazine replacements. Field experiments were conducted in 2017 and 2018 in Fayetteville, Arkansas, to test the tolerance of corn to preemergence and postemergence applications of different photosystem II (PSII) inhibitors alone or in combination with mesotrione or S-metolachlor. All experiments were designed as a two-factor factorial, randomized complete block with the two factors being 1) PSII herbicide and 2) the herbicide added to create the mixture. The PSII herbicides were prometryn, ametryn, simazine, fluometuron, metribuzin, linuron, diuron, atrazine, and propazine. The second factor consisted of either no additional herbicide, S-metolachlor, or mesotrione. Treatments were applied immediately following planting in the preemergence experiments and at 30-cm corn for the postemergence experiments. For the preemergence study, low levels of crop injury (<15%) were observed at 14 and 28 days after application (DAA) and corn height was influenced by the PSII herbicide applied; however, crop density and yield did not differ from the nontreated plots. For the postemergence study, crop injury, height relative to the nontreated, and yield relative to the nontreated were all impacted by PSII herbicide and herbicide added. Diuron-, linuron-, metribuzin-, and simazine-containing treatments applied preemergence and metribuzin- and simazine-containing treatments applied postemergence should be further investigated as to their utility to replace atrazine.

#### Introduction

Weed control is a necessity for corn producers, as poor weed control can negatively impact yields. Weeds compete with corn for soil nutrients, water, and light. Smith and Scott (2017) demonstrated that just one Palmer amaranth [Amaranthus palmeri (S.) Wats.] per 4 ft of row that goes uncontrolled in corn for 4 weeks after emergence can potentially reduce yields by 4%. Eliminating this competition encourages corn to produce grain at its fullest potential. Weeds can also impede harvest. Bensch et al. (2003) showed that Palmer amaranth can grow up to 6 feet tall in less than 40 days. This means that late-season infestations of weeds could result in less than optimal harvest conditions. So, whether it is early in the growing season or late in the growing season, weed control is vital. Troublesome weeds in corn in the southern U.S. include morningglories (Ipomoea ssp.), Texas millet [Panicum texana (Buckley) R. Webster], broadleaf signalgrass [Urochloa platyphylla (Munro ex C. Wright) R. Webster], johnsongrass [Sorghum halepense (L.) Pers.], sicklepod [Senna obtusifolia (L.) H.S. Irwin and Barneby], nutsedges (Cyperus ssp.), and Palmer amaranth (Webster and Nichols, 2012).

In 2016, over 55 million pounds of atrazine were applied in the U.S. (USDA-NASS, 2018). Atrazine, a photosystem II (PSII)-inhibiting herbicide, has been the foundation for weed control in corn for over 70 years. The PSII-inhibiting herbicides make up Weed Science Society of America (WSSA) groups 5, 6, and 7, with the largest group of PSII-inhibiting herbicides coming from group 5. The PSII-inhibiting herbicides create oxidative stress to the D1 protein by halting electron flow within the photosynthetic electron transport chain (Abendroth et al., 2006).

Atrazine alone and in combination with other herbicides provides corn growers with an unmatched tool for weed control. However, there are some potential issues with this tool. Survey results from Barbash et al. (2006) indicated that atrazine is routinely found in drinking water aquifers and shallow groundwater under agricultural areas. Contamination of groundwater may pose health concerns for the general public given the effects that endocrine disruptors can have on human cells (Lasserre et al., 2009). One way to decrease the prevalence of atrazine in groundwater is by reducing the amount used in agriculture, specifically corn. Hence, research was initiated to test the tolerance of corn to several other PSII-inhibiting herbicides alone and in combination with mesotrione and *S*-metolachlor that could potentially replace atrazine.

#### Procedures

#### **Common Trial Methodology**

Field experiments were conducted in 2017 and 2018 to test the tolerance of corn to preemergence (PRE)- and postemergence (POST)-applied PSII-inhibiting herbicides. All ex-

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periments were planted to Pioneer hybrid 1197YHR at 32,000 seeds per acre into conventionally tilled and raised beds. Plot sizes were 12 ft by 20 ft long and rows were spaced 36 in. apart. Plots were maintained weed-free with POST applications of glufosinate and glyphosate on an as-needed basis. All trials were furrow irrigated and otherwise managed according to the Arkansas Corn Production Handbook (Espinoza and Ross, 2018).

#### **Experimental Sites**

All field experiments were conducted on a Convent silt loam at the University of Arkansas System Division of Agriculture's Milo J. Shult Agricultural Research and Extension Center (SAREC) in Fayetteville, in 2017 and 2018. The soil at Fayetteville consisted of 34% sand, 53% silt, and 13% clay, with an organic matter content of 1.5% and a pH of 6.8.

## PRE-Tolerance Study Setup and Data Collection

All experiments were designed as a two-factor factorial, randomized complete block with the two factors being 1) PSII herbicide and 2) the herbicide added to create the mixture. The PSII herbicides included ametryn, atrazine, diuron, fluometuron, linuron, metribuzin, prometryn, propazine, and simazine. The second factor consisted of either no herbicide, S-metolachlor, or mesotrione. Herbicide rates and manufacturers can be found in Table 1. All treatments were applied at 15 gal/ac immediately following corn planting. The experimental treatments were replicated 4 times. Visible crop injury was estimated at 14 and 28 days after application (DAA). At 28 DAA, crop height measurements of 3 random plants in each plot were measured to the crop canopy and then averaged. Crop density was counted as plants 3 ft of row 14 DAA. Yield was collected from the middle two rows of each plot using a smallplot combine, and weights were adjusted to 15.5% moisture.

#### **POST-Tolerance Study Setup and Data** Collection

All experiments followed the same treatments and design as their PRE-trial counterparts. However, for the POST experiment, treatments were applied when corn was 12 in. tall. Visible crop injury was estimated at 14 and 28 DAA. Crop height measurements of three random plants were measured to the crop canopy, recorded at 14 DAA, and then averaged. Yield was collected from the middle two rows of each plot using a smallplot combine, and weights were adjusted to 15.5% moisture. Planting and harvest dates for both years are listed in Table 2.

#### **Statistical Analysis**

*PRE-Study*. Data from the PRE trials were analyzed separately by year given the different environmental conditions from year to year. Crop height, crop density, and yield were converted to be relative to the nontreated plots. Then mean separations were analyzed for injury, relative crop height, relative crop density, and relative yield using Fisher's protected least significant difference ( $\alpha = 0.05$ ) to see if the main PSII-inhibiting herbicide or the additive herbicide had an effect.

*POST Study*. Data from the POST trials were analyzed the same as the PRE trials excluding relative crop density, which was not recorded for the POST trials.

#### **Results and Discussions**

In 2017, PRE herbicides were applied immediately after planting and received an activating rainfall of 1.3 in. two days later. In 2018, PRE herbicides were applied two days after planting and received an activating rainfall of 0.6 in. the night of the application.

#### Injury

In both years, corn injury 14 DAA was influenced by an interaction of the PSII herbicide and the additive herbicide (Table 3). Injury was in the form of interveinal chlorosis with some bleaching in mesotrione-containing treatments on new leaves. In 2017, applications of ametryn alone, ametryn plus mesotrione, and ametryn plus *S*-metolachlor caused 9%, 5%, and 7% injury, respectively (Table 4). However, in 2018, ametryn and ametryn plus mesotrione caused no observable injury. Fluometuron-containing treatments caused injury in both years with fluometuron plus mesotrione causing 10% injury in both years. In 2017, this was the highest injury observed for any treatment but did not differ from fluometuron alone, and ametryn alone. In 2018, it was higher than all other treatments.

Corn injury in 2018 was transient. By 28 DAA no differences were detected between treatments, and no treatment displayed injury higher than 3% (data not shown). However, corn injury 28 DAA in 2017 was not transient and was influenced by an interaction of PSII herbicide and herbicide added (Table 3). In 2017, some plots with injury of 5% or higher 14 DAA did not recover by 28 DAA (Table 4). For example, fluometuron alone, fluometuron plus mesotrione, and fluometuron plus *S*metolachlor exhibited 9%, 10%, and 5% injury, respectively, 14 DAA, and then 9%, 16%, and 9% injury, respectively, 28 DAA. However, treatments containing ametryn plus mesotrione, diuron plus mesotrione, prometryn plus mesotrione, diuron plus S-metolachlor were exceptions to this lack of recovery. Each of these treatments exhibited 5% injury 14 DAA and then exhibited no injury 28 DAA.

Overall, injury in both years and at both ratings was low (<20%). Excluding ametryn- and fluometuron-containing treatments, injury was <10% at 14 and 28 DAA, which would be considered acceptable to most growers.

#### **Relative Stand**

Relative stand did not differ among factor of PSII herbicides or by an interaction of PSII and herbicide added. (Table 3). Densities in nontreated plots were 8.1 and 7.7 plants per 3 feet of row in 2017 and 2018, respectively (data not shown).

#### **Relative Height**

In 2017, corn height was not affected by any factor. Although visible injury symptoms of interveinal chlorosis were not present by 28 DAA in 2018, height was influenced by the PSII herbicides that were applied (Table 3). Consistent with injury at 14 DAA, fluometuron-containing treatments (which caused the highest visible injury) also caused the greatest reduction in height (77% of the nontreated plots; Tables 4 and 5). Generally, any PSII herbicide that caused injury 14 DAA reduced height compared to the nontreated plots, except metribuzin- and simazine-containing treatments which did not reduce height compared to nontreated plots in 2018.

#### **Relative Yield**

Although various treatments may have caused visible injury and height reduction in 2017 and 2018, relative yield was not influenced by any factor (Table 3). Corn is a fairly vigorous crop with the ability to recover from early injury caused by other herbicides. Corn yield components develop at different stages giving corn the ability to compensate from adverse effects throughout the growing season (Milander, 2015). Ears per plant, kernels per ear and kernel weight are each primary yield components that are determined at different times after the V4 stage (Fageria et al., 2006). Since injury in 2017 and 2018 was minimal and in most treatments transient, the corn was likely able to compensate for any yield component affected by the herbicides later in the growing season.

#### **POST-Study**

*Rainfall.* Given that corn was already 12 in. tall during this application, the herbicides did not need to be activated to provide ideal performance. However, any herbicide that did reach the soil surface would have to be activated before providing residual activity. In 2017, 3.0 and 1.3 in. of rainfall were received 2 and 10 days after application, respectively. In 2018, rainfall events each totaling 0.6 in. were received 2 and 4 days after application.

#### Injury

In 2017 and 2018, corn injury 14 DAA was influenced by an interaction between PSII herbicide and herbicide added (Table 6). Injury was in the form of interveinal chlorosis with some bleaching in mesotrione-containing treatments on contacted leaves as well as new growth. In 2017, linuron plus Smetolachlor caused the highest level of injury (45%; Table 7). In general, linuron-containing treatments, along with diuron plus S-metolachlor and prometryn plus S-metolachlor, caused greater injury compared to most other treatments. In 2018, prometryn alone and in combination with S-metolachlor, caused 45% and 49% injury, respectively (Table 7). Ametryn plus S-metolachlor, linuron plus S-metolachlor, and prometryn plus mesotrione, each caused 38%, 38%, and 35% injury, respectively, all which were comparable. Atrazine-, fluometuron-, metribuzin-, and simazine-containing treatments each caused <15% injury in both years (Table 7).

In 2018, injury was transient, less than 10% (data not shown), and injury did not differ among treatments (Table 6). However, injury 28 DAA in 2017 was influenced by an interaction between PSII herbicide and herbicide added. Linuron plus *S*-metolachlor caused 29% injury in 2017 and was the

most injurious treatment (Table 7). Diuron plus S-metolachlor, linuron plus mesotrione, and prometryn plus S-metolachlor were comparable and caused 17%, 18%, and 18% injury, respectively. No other treatment caused greater than 10% injury.

Overall, injury was fairly moderate among treatments in both years, excluding fluometuron-, metribuzin-, and simazinecontaining treatments, all which caused injury <15% (Table 6). Levels of injury caused by these treatments would likely be tolerable to most growers.

#### **Relative Height**

In 2017 and 2018, height 14 DAA was influenced by an interaction between PSII herbicide and herbicide added. Generally, height followed the trend of injury. For example, in 2017, linuron plus S-metolachlor presented the highest injury (45%), and corn height following this treatment was only 77% of nontreated plots (Tables 7 and 8). In 2017, plots injured >10% also had heights that were reduced compared to nontreated plots. In 2018, the same was true, excluding plots treated with diuron plus mesotrione and plots treated with propazine alone (Tables 7 and 8). Overall, height 14 DAA generally followed the same trends as injury 14 DAA for a given year.

#### **Relative Yield**

In 2017 and 2018, relative yield was influenced by an interaction between PSII herbicide and herbicide added. Ametryn alone, ametryn plus mesotrione, diuron alone, diuron plus mesotrione, metribuzin alone, metribuzin plus *S*-metolachlor, propazine alone, simazine alone, and simazine plus *S*-metolachlor all yielded comparable to atrazine-containing treatments in 2017 (Table 7). In 2018, corn in plots treated with fluometuron plus mesotrione and *S*-metolachlor, metribuzin alone, metribuzin plus mesotrione or *S*-metolachlor, prometryn plus mesotrione, prometryn plus *S*-metolachlor, and simazine plus mesotrione all yielded comparable to atrazine-containing treatments.

These applications were made while the corn was 12 in. tall or about V5. During this time and the proceeding weeks, yield components such as ear per plant and kernels per ear were developing (Fageria et al., 2006; Uribelarrea et al., 2002). Likely, the chlorosis and stunting caused by certain herbicides affected the development of these yield components and therefore hindered yield in some treatments.

#### **Practical Applications**

#### **PRE-Study**

Determining which herbicides should be further tested to potentially replace atrazine should be based on a combination of each response variable. Likely, growers would be apt to avoid herbicides that injure their crop beyond a reasonable level even if yield is not impacted. Therefore, even though yield was not impacted for any PRE herbicide, certain ametryn- and fluometuron-containing treatments caused >10% injury and should therefore no longer be considered for this use in corn (Table 3). Also, herbicides that reduce corn height should not be considered since height reduction may also concern growers or delay canopy closure which could negatively impact weed control (Anderson, 2008). Given the negative effects of reduced crop height, in addition to ametryn- and fluometuron-containing treatments, prometryn- and propazine-containing treatments should also be eliminated from further testing. Corn tolerance to diuron-, linuron-, metribuzin-, and simazine-containing treatments applied PRE should be further tested to validate the tolerance observed in this study. Weed control trials should also be conducted on these herbicides and herbicide combinations to ensure they will adequately replace atrazine.

#### **POST-Study**

The same factors should be considered for POST applications of these herbicides. Based on crop injury, relative crop height, and relative yield in 2017 and 2018, only metribuzinand simazine- containing treatments should be further assessed for crop tolerance and weed control when applied POST.

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Herbi	cide		
Common name Trade name		Rate	Manufacturer
		lb ai/ac	
ametryn	Evik	1.8	Syngenta Crop Protection, LLC
atrazine	Aatrex 4L	1.0	Syngenta Crop Protection, LLC
diuron	Direx	0.5	ADAMA
fluometuron	Cotoran	1.0	ADAMA
linuron	Linex	0.69	Tessenderlo Kerley, Inc.
mesotrione	Callisto	0.81	Syngenta Crop Protection, LLC
metribuzin	Tricor 4F	0.25	United Phosphorus Limited
prometryn	Caparol	2.0	Syngenta Crop Protection, LLC
propazine	Milo-Pro	0.5	Albaugh, LLC
simazine	Princep 4L	2.0	Syngenta Crop Protection, LLC
S-metolachlor	Dual II Magnum	1.25	Syngenta Crop Protection, LLC

 Table 1. Herbicides, rates, and manufacturers for preemergence and postemergence corn trials in 2017 and

 2018 at the University of Arkansas System Division of Agriculture's Milo J. Shult Agricultural Research and

 Extension Center in Fayetteville.

Table 2. Planting, herbicide application, and harvest dates for preemergence (PRE)-and postemergence (POST)-corn trials at the University of Arkansas System Division of Agriculture's Milo J. Shult Agricultural Research and Extension Center in Fayetteville in 2017 and 2018.

			Dates of significance					
Trial	Year	Planting	Herbicide application	Harvest				
PRE	2017	May 26	May 26	October 26				
	2018	April 20	April 22	October 8				
DOOT	0017	A 1140	M 40					
POST	2017	April 12	May 18	September 21				
	2018	April 20	May 20	October 8				

Table 3. Significance of *P*-values for interactions and main factors of photosystem II (PSII) herbicide and herbicide added on corn injury, relative stand, relative height, and relative yield by year for preemergence corn trials

			utais.			
		Corn i	njury	Corn relative stand	Corn relative height	Corn relative yield
Year	Factor	14 DAA <sup>†,‡</sup>	28 DAA	14 DAA	28 DAA	
				(%)		
2017	PSII herbicide	<0.0001*	<0.0001*	0.4403	0.0667	0.1341
	Herbicide added	0.0359*	0.1969	0.6312	0.1849	0.2123
	PSII herbicide* Herbicide added	0.0305*	<0.0001*	0.2601	0.0633	0.8833
2018	PSII herbicide	0.0038*	0.1331	0.8979	<0.0001*	0.1304
	Herbicide added	0.9924	0.5905	0.6933	0.5604	0.0952
	PSII herbicide* Herbicide added	0.0292*	0.1846	0.7074	0.4607	0.0904

<sup>†</sup> Abbreviations: DAA, days after application.

<sup>‡</sup> Asterisks represent significance at  $\alpha$  = 0.05.

		Corn injury					
	-		14 DA	<b>A</b> †		28 E	DAA
PSII herbicide	Herbicide added	20	017	20	18	20	17
				9	%		
ametryn	None	9	ab‡	0	d	11	b
	Mesotrione	5	С	0	d	0	d
	S-metolachlor	7	bc	6	bc	10	b
atrazine	None	0	d	0	d	0	d
	Mesotrione	0	d	0	d	0	d
	S-metolachlor	0	d	0	d	0	d
diuron	None	0	d	0	d	0	d
	Mesotrione	5	С	0	d	0	d
	S-metolachlor	0	d	0	d	0	d
fluometuron	None	9	ab	7	b	9	bc
	Mesotrione	10	а	10	а	16	а
	S-metolachlor	5	С	5	bc	9	bc
linuron	None	0	d	0	d	0	d
	Mesotrione	0	d	0	d	0	d
	S-metolachlor	0	d	0	d	0	d
metribuzin	None	0	d	0	с	0	d
	Mesotrione	4	cd	0	С	0	d
	S-metolachlor	5	С	5	bc	6	С
prometryn	None	7	bc	3	С	0	d
	Mesotrione	5	С	3	С	0	d
	S-metolachlor	5	С	5	bc	6	С
propazine	None	0	d	3	С	0	d
	Mesotrione	0	d	3	С	0	d
	S-metolachlor	4	cd	3	С	0	d
simazine	None	0	d	5	bc	0	d
	Mesotrione	5	С	0	d	6	С
	S-metolachlor	0	d	5	bc	8	bc

Table 4. Corn injury as influenced by interactions between photosystem II (PSII) herbicide and herbicide
added in 2017 and 2018 applied preemergence.

<sup>†</sup> Abbreviations: DAA, days after application.

<sup>‡</sup> Means within a factor followed by the same letter are not significantly different according to Fisher's protected least significant difference ( $\alpha = 0.05$ ).

Table 5.	Relative corn height as influenced by photosystem II (PSII) herbicide
	in 2018 applied preemergence.

in zo to applied preemergence.						
Photosystem II (PSII) herbicide	Relative corn height					
	% of nontreated					
ametryn	86 c					
atrazine	96 ab					
diuron	100 a					
fluometuron	77 d					
linuron	98 ab					
metribuzin	96 ab					
prometryn	89 c					
propazine	91 bc					
simazine	98 ab					

<sup>a</sup> Means within a factor followed by the same letter are not significantly different according to Fisher's protected least significant difference ( $\alpha = 0.05$ ).

yield by year for postemergence corn trials.									
		Corn	injury	Corn relative height	Corn relative yield				
Year	Factor	14 DAA <sup>a,b</sup> 28 DAA		14 DAA					
				(%)					
2017	PSII herbicide	<0.0001*	<0.0001*	0.0030*	<0.0001*				
	Herbicide added	0.0001*	0.0143*	0.0030*	0.0001*				
	PSII herbicide*	0.0072*	0.0009*	0.0051*	0.0006*				
	Herbicide added								
2018	PSII herbicide	<0.0001*	0.8141	<0.0001*	<0.0001*				
	Herbicide added	<0.0001*	0.8262	<0.0001*	<0.0001*				
	PSII herbicide*	<0.0001*	0.6551	0.0003*	<0.0001*				
	Herbicide added								

Table 6. Significance of <i>P</i> -values for interactions and main effects of photosystem II (PSII)
herbicide and herbicide added on corn injury, relative stand, relative height, and relative
vield by year for postemergence corn trials

<sup>a</sup> Abbreviations: DAA, days after application. <sup>b</sup> Asterisks represent significance at  $\alpha = 0.05$ .

Table 7. Corn injury and yield as influenced by interactions between photosystem II (PSII) herbicide a	nd
herbicide added in 2017 and 2018 applied postemergence.	

				Co	rn inj	ury					
		14 DAA <sup>a,b</sup>			28	28 DAA		Corn relative yield <sup>c</sup>			
PSII herbicide	Herbicide added	20	)17	20	)18	2	017		2017	2	018
					%				% of no	ntreated	
ametryn	none	0	h	13	fq	6	cde	85	abcdef	83	defa
,	mesotrione	4	qh	16	f	6	cde	81	bcdefg	78	fqh
	S-metolachlor	0	bc	38	bc	5	cde	71	hij	81	efgh
atrazine	none	4	d	4	i	6	cde	94	а	96	abc
	mesotrione	4	d	4	i	6	cde	89	abc	96	abc
	S-metolachlor	4	d	8	hi	6	cde	91	ab	99	ab
diuron	none	10	def	4	i	9	cd	82	bcdefg	56	j
	mesotrione	4	gh	14	fg	5	cde	84	abcdef	67	i
	S-metolachlor	22	b	29	de	17	b	73	ghij	66	i
fluometuron	none	5	fg	15	f	3	е	66	j	56	j
	mesotrione	8	efg	7	hij	9	cd	69	ij	93	abcd
	S-metolachlor	6	efgh	7	hij	8	cd	57	k	87	cdef
linuron	none	21	bc	6	hij	9	cd	78	defghi	68	i
	mesotrione	26	b	6	hij	18	b	80	cdefgh	73	hi
	S-metolachlor	45	а	38	bc	29	а	69	ij	82	defgh
metribuzin	none	0	h	4	i	6	cde	89	abc	90	abcde
	mesotrione	4	gh	6	hij	6	cde	77	fghi	96	abc
	S-metolachlor	8	efg	9	gh	5		80	cdefgh	88	cdef
prometryn	none	15	cd	45	ab	10	с	66	j	74	ghi
	mesotrione	11	de	35	cd	7	cd	76	fghi	100	a
	S-metolachlor	29	bc	49	а	18	b	71	hij	95	abc
propazine	none	0	h	14	fg	6	cde	87	abcde	58	j
	mesotrione	0	h	5	hij	6	cde	67	j	72	hi
	S-metolachlor	0	h	25	е	6	cde	71	hij	43	k
simazine	none	0	h	4	i	7	cd	87	abcde	88	cdef
	mesotrione	0	h	4	i	4	de	77	efghi	89	abcdef
	S-metolachlor	0	h	7	hij	4	de	88	abcd	38	k

<sup>a</sup> Abbreviations: DAA, days after application.

<sup>b</sup> Means within a column followed by the same letter are not significantly different according to Fisher's protected least significant difference ( $\alpha$  = 0.05). ° Corn yield in 2017 and 2018 averaged 163 and 187 bu./ac in the nontreated plots, respectively.

		Relative corn height <sup>c</sup>					
			14 DAA	a,b			
PSII herbicide	Herbicide added	20	017	20	2018		
			% of nontr	reated			
ametryn	None	92	abc	86	def		
	Mesotrione	92	abc	86	def		
	S-metolachlor	90	abcd	83	etg		
atrazine	None	96	ab	99	ab		
	Mesotrione	96	ab	99	ab		
	S-metolachlor	96	ab	98	abc		
diuron	None	93	abc	91	bcde		
	Mesotrione	97	а	93	abcde		
	S-metolachlor	77	gh	82	efg		
fluometuron	None	95	abcd	89	cdef		
	Mesotrione	91	abcd	89	cdef		
	S-metolachlor	90	abcd	96	abcd		
linuron	None	87	cdef	89	cdef		
	Mesotrione	83	defg	88	def		
	S-metolachlor	74	h	73	g		
metribuzin	None	89	abcde	100	а		
	Mesotrione	90	abcd	97	abcde		
	S-metolachlor	90	abcd	93	abcde		
prometryn	None	88	bcdef	79	fg		
	Mesotrione	81	efg	83	efg		
	S-metolachlor	80	fgh	73	g		
propazine	None	95	abc	93	abcde		
	Mesotrione	93	abc	90	cdef		
	S-metolachlor	94	abc	62	h		
simazine	None	90	abcd	90	cdef		
	Mesotrione	92	abcd	83	ef		
	S-metolachlor	95	abcd	92	abcde		

Table 8. Relative corn height as influenced by photosystem II (PSII)
herbicide in 2017 and 2018 applied postemergence.

<sup>a</sup> Abbreviations: DAA, days after application.

<sup>b</sup> Means within a factor followed by the same letter are not significantly different according to Fisher's protected least significant difference ( $\alpha = 0.05$ ).

Height of corn in 2017 and 2018 in the nontreated plots averaged 20 and 18 inches, respectively.

## Herbicide Programs with and without Atrazine in Corn

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#### Abstract

The extensive use of atrazine by growers has led to traces of the herbicide being found in groundwater, surface water, and aquifers. Research was initiated in 2017 and 2018 to explore different corn herbicide regimes with little or no atrazine. Different preemergence herbicide treatments (Dual II Magnum at 1 pt/ac or 10 oz/ac Verdict), as well as various postemergence herbicide mixtures (Acuron Flexi at 2 qt/ac, Capreno at 3 oz/ac, Corvus at 5.6 oz/ac, or Resicore 48 oz/ac) were applied alone or in combination with atrazine at 1 pt/ac to Roundup Ready/Liberty Link corn directly after planting or at a 12-in. corn height. Each postemergence treatment was mixed with labeled rates of glyphosate and glufosinate to resemble practical treatments common in Arkansas. Palmer amaranth, broadleaf signalgrass, and pitted morningglory control never fell below 95%. Crop injury and yield data were analyzed by year given the two unique environments. Verdict injured corn 8% and 5% higher than Dual II Magnum 14 days after the preemergence application in 2017 and 2018, respectively. Averaged over preemergence herbicide and atrazine rate, Corvus injured corn 21% in 2017. In 2018, treatments of Dual II Magnum followed by (fb) Corvus caused 11% injury, which was higher than all other treatments. In both years, corn yield was influenced by an interaction between preemergence herbicide, herbicide premixture applied postemergence, and atrazine rate applied postemergence. Based on this research, the weeds assessed can be controlled without atrazine, and there are herbicide options available, although they may injure corn.

#### Introduction

Weed management in corn varies greatly depending on the geographical crop production region in the United States. Webster and Nichols (2012) found that the most troublesome weeds affecting corn in the southern U.S. include morningglories (Ipomoea ssp.), Texas millet (Panicum texana Buckl.), broadleaf signalgrass [Urochloa platyphylla (Munro ex C. Wright) R.D. Webster], johnsongrass (Sorghum halepense L.), sicklepod (Senna obtusifolia L.), nutsedges (Cyperus ssp.), and Palmer amaranth (Amaranthus palmeri S. Wats). But perhaps the most troublesome weed is Palmer amaranth. If left uncontrolled for 4 weeks, just one Palmer amaranth per 4 foot of row may reduce corn yields up to 4% (Smith and Scott, 2017). Palmer amaranth does not only impact yield. Bensch et al. (2003) reported that Palmer amaranth can grow up to 6 feet tall in less than 40 days in some environments, meaning lateseason infestations may interfere with crop harvest. Given the problems that weeds can cause at any point during the growing season, control should be season long.

Time, labor cost, and convenience are all reasons why growers have adopted herbicides as the main tool for weed control in corn (Armstrong et al., 1968; Pleasant et al. 1994). However, there are precautions that should be taken to reduce the risk of weeds evolving resistance. A key cause of herbicide resistance evolution is the reliance of growers on one site of action (SOA) (Norsworthy et al., 2012). Although many factors may contribute, research has shown that glyphosate-resistant horseweed (*Conyza canadensis* L.), common ragweed (*Am*- *brosia artemisiifolia* L.), and pigweed (*Amaranthus* ssp. L.) evolved resistance to glyphosate from consecutive applications over a 3- to 6-year time frame (Culpepper et al., 2006). From these findings, it is apparent that growers should use multiple SOAs in a growing season.

One way the crop protection industry has enabled growers to use multiple SOAs is through premixtures. An example of a premixture is Acuron Flexi<sup>®</sup>, which contains bicyclopyrone [Weed Science Society of America (WSSA) group 27], mesotrione (WSSA group 27), and S-metolachlor (WSSA group 15). This premixture can be applied preemergence or postemergence to corn and combines two SOAs and provides foliar and residual control of many broadleaf and grass weeds (Anonymous, 2016). By providing more than one effective SOA, selection pressure is taken off of a specific herbicide, thus slowing resistance evolution (Norsworthy et al., 2012).

Given the issues at hand, research was initiated in 2017 to explore weed control programs without the use of atrazine. The goal of this study was to provide growers with other reliable options in the absence of atrazine.

#### Procedures

#### **Experimental Sites**

In both 2017 and 2018, all field experiments were conducted on a Convent silt loam (Coarse-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts) at the University of Arkansas System Division of Agriculture's Milo J. Shult Agri-

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cultural Research and Extension Center (SAREC) in Fayetteville. The soil at Fayetteville consisted of 34% sand, 53% silt, and 13% clay, with an organic matter content of 1.5% and a pH of 6.8.

#### Study Setup and Data Collection

All experiments were planted to Pioneer hybrid 1197YHR that was planted at 32,000 seeds/ac into conventionally tilled and raised beds. Plot size was 12 ft wide by 20 ft long, and rows were spaced 36 in. apart. All corn trials were furrow irrigated and otherwise managed according to the Arkansas Corn Production Handbook (MP437). This study was designed as a randomized complete block consisting of three factors. The three factors were 1) preemergence herbicide, 2) herbicide premixture applied postemergence, and 3) rate of atrazine (0 or 1 pt/ac applied with premixture (Table 1). Treatments were intended to represent real-life herbicide programs that growers use in Arkansas corn production, either with or without atrazine, and therefore all received 22 oz Roundup Powermax and 29 oz/ ac Liberty with the postemergence application. Preemergence applications were made immediately following planting into a clean weed-free raised bed while postemergence applications were made when the corn was 12 in. tall. In 2017 and 2018, 1- to 2-in. Palmer amaranth average density at the postemergence application timing was 4 and 5 plants per square yard, respectively, 0.3- to 2-in. broadleaf signal grass average density was 16 and 25 plants per square yard, respectively, and 1- to 2-in. morningglory average density was 2 and 3 plants per square yard, respectively. All applications were made with a CO<sub>2</sub>-pressurized backpack sprayer at 15 gal/ac. Dates of planting, herbicide applications, and harvest for each year are shown in Table 2. Visible estimates of injury and Palmer amaranth, broadleaf signalgrass, and morningglory control were taken 21 days after the preemergence application (DAPRE) and 14 days after the postemergence application (DAPOST). The middle two rows of each plot were harvested at physiological maturity using a small-plot combine, and yield was adjusted to 15.5% moisture.

#### **Statistical Analysis**

Data were analyzed by year due to environmental differences each year caused by the different planting timings. Weed control ratings for any weed never fell below 95% at any time during the growing season; therefore, these data were not analyzed. Means were separated for corn injury ratings 14 DAPRE, 14 DAPOST, and yield using Fisher's protected least significant difference ( $\alpha = 0.05$ ) to see if preemergence herbicide, herbicide premixture, or atrazine had an effect.

#### **Results and Discussion**

#### **Preemergence Weed Control**

The two preemergence herbicides were activated via rainfall and provided exceptional control (>95%) of Palmer amaranth, broadleaf signalgrass, and pitted morningglory (data not shown). This study shows the utility of both Dual II Magnum and Verdict as preemergence control options to complement or supplement current preemergence herbicides in corn.

#### **Postemergence Weed Control**

Postemergence weed control did not fall below 95% for any treatment 14 DAPOST (data not shown). Various premixes and herbicides were included in different treatments to provide additional foliar activity on broadleaf and grass weeds; however, most of these premixes and herbicides also provide residual control. Corvus has been shown to control barnyardgrass, entireleaf morningglory, and Palmer amaranth greater than 90% for 4 weeks after application (Stephenson and Bond, 2012). The residual control of these herbicides is important to prevent weed competition until canopy formation to prevent weed seed germination (Gonzini et al., 1999). Although atrazine is the typical residual herbicide used for in-season weed control in corn, these results indicate that there are herbicides that can provide weed control comparable to atrazine-based weed control programs.

The introduction of glufosinate-resistant corn has been instrumental in control of glyphosate-resistant weeds. Although glufosinate controls most annual broadleaf weeds, it has been shown to be weak on grasses (Hamill et al., 2000). The inclusion of glyphosate likely eliminated grass weeds in all treatments as seen in other research (Shaw and Arnold, 2002). The excellent control shown by these herbicides in this study demonstrates a small percentage of effective herbicides that can be used to control weeds in the absence of atrazine.

#### **Crop Injury**

Preemergence Application. Corn injury 14 DAPRE was influenced by the preemergence herbicide applied (Table 3). No other herbicides had been applied at this point; therefore, injury data are presented by factor and year (Table 4). Applications of Verdict 10 oz/ac injured corn 13% and 8%, which was more than Dual II Magnum in 2017 and 2018, respectively (Table 3).

*Postemergence Application*. In 2018, corn injury was influenced by an interaction between the preemergence herbicide and the postemergence premixture (Table 3). However, in 2017, corn injury was not affected by an interaction between preemergence herbicide and postemergence premixture and therefore data are presented separately by factor (Tables 3 and 5).

In 2017, averaged over premixture and atrazine, corn that received Verdict preemergence was injured more than corn that received Dual II Magnum preemergence (Table 5). Given the higher injury that Verdict caused preemergence, corn may not have been able to recover in a timely manner and was therefore injured more. Averaged over preemergence herbicide and atrazine rate, Corvus injured corn 21% in 2017 (Table 5). In 2018, treatments of Dual II Magnum preemergence followed by (fb) Corvus POST caused 11% injury, which was higher than the other treatments in 2018. In general, Corvus-containing treatments were more injurious to corn 14 DAPOST.

#### Yield

In 2017 and 2018, corn yield was influenced by a threeway interaction between preemergence herbicide, postemergence premixture, and atrazine (Table 3). In 2017, corn in treatments containing the premixture of Acuron Flexi yielded the best except when combined with Dual II Magnum preemergence and atrazine postemergence (Table 6). In 2018, corn in treatments containing the premixture of Acuron Flexi yielded the best except when combined with Verdict preemergence and atrazine postemergence (Table 6). In 2018, corn in treatments that received Verdict preemergence fb Corvus with atrazine postemergence yielded lower than all other treatments (Table 6). Averaged over atrazine, corn injury for this treatment was also higher than injury from other treatments in 2018 (Table 5). Perhaps the light chlorosis triggered stress and hindered the corn from setting an ear and kernel count comparable to other corn plots. An overall trend by year was difficult to uncover and more research is needed to accurately assess the yield effects that were noted in this study.

#### **Practical Applications**

We achieved excellent weed control with all of the herbicide combinations and applications used in this study. However, the weed control achieved in this study is not an overall implication that atrazine is not needed in corn. The weed pressure in this study was light. This, in combination with the timely application, allowed weeds to be almost completely controlled in both years. This study is not intended to show that atrazine is not needed, but rather that it should be complemented often and supplemented occasionally with HPPD such as mesotrione, and Group 15 herbicides such as metolachlor, to lessen the likelihood of resistance evolution and environmental contamination.

Given the results from this study, in a similar environment, with similar weed pressure, atrazine may not be required to control certain weeds; however, these full season programs, as well as other full season programs, should be further tested before broad recommendations are made that are applicable to multiple environments.

#### Acknowledgments

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Table 1. List of commentations and falles used in helpfulde dealinerity with manufacturers.						
I rade name	Common name	Rate	Timing	Manufacturer		
Dual II Magnum	S-metolachlor	1 pt/ac	PRE <sup>†</sup>	Syngenta Crop Protection		
Verdict	Saflufenacil + dimethenamid	10 oz/ac	PRE	BASF Crop Protection		
Acuron Flexi	bicyclopyrone + mesotrione + <i>S</i> -metolachlor	2 qt/ac	POST	Syngenta Crop Protection		
Capreno	thiencarbazone-methyl + tembotrione	3 oz/ac	POST	Bayer Cropscience		
Corvus	thiencarbazone-methyl + isoxaflutole	5.6 oz/ac	POST	Bayer Cropscience		
Resicore	acetochlor + mesotrione + clopyralid	48 oz/ac	POST	Dow AgroSciences		
Roundup PowerMax	glyphosate	22 oz/ac	POST	Bayer Cropscience		
Liberty	glufosinate	29 oz/ac	POST	BASF Crop Protection		
Aatrex	Atrazine	1 pt/ac	POST	Syngenta Crop Protection		

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<sup>†</sup> Abbreviations: PRE, preemergence; POST, postemergence.

Table 2. Planting, herbicide application, and harvest dates for corn trials at the University of Arkansas System Division of Agriculture's Milo J. Shult Agricultural Research and Extension Center (SAREC) in Fayetteville in 2017 and 2018.				
Dates of significance				
Year	Planting	Preemergence	Postemergence	Harvest
2017	May 26	May 26	June 16	October 25
2018	April 20	April 20	May 20	October 8

		by year for corn tri	ais.	
Year	Factor	14 DAPRE <sup>†,‡</sup>	14 DAPOST	Yield (bu./ac)
2017	PRE	<0.0001*	0.0386*	0.0011*
	POST		<0.0001*	<0.0001*
	Atrazine		0.5467	<0.0001*
	PRE*POST		0.1195	0.0014*
	PRE*Atrazine		0.7326	<0.0001*
	POST*Atrazine		0.2785	<0.0001*
	PRE*POST*Atrazine		0.8323	<0.0001*
2018	PRE	<0.0001*	0.0054*	0.0448*
	POST		0.0003*	<0.0001*
	Atrazine		0.7094	0.2255
	PRE*POST		0.0001*	<0.0001*
	PRE*Atrazine		0.3849	<0.0001*
	POST*Atrazine		0.9838	0.0029*
	PRE*POST*Atrazine		0.7771	0.0002*

Table 3. Significance of <i>P</i> -values for interactions and main effects of PRE
herbicide, POST premixture herbicides, and atrazine on corn injury and yield
by year for corn trials.

<sup>†</sup> Abbreviations: PRE, preemergence; POST, postemergence; DAPRE, days after

preemergence application; DAPOST, days after postemergence application.

<sup>‡</sup> Asterisks represent significance at  $\alpha = 0.05$ .

Iniury
2017 and 2018.
Table 4. Influence of preemergence herbicide on corn injury in

		Injury
Year	Preemergence herbicide	14 DAPRE <sup>†,‡</sup>
		%
2017	Verdict	13 a
	Dual II Magnum	5 b
2018	Verdict	8 a
	Dual II Magnum	3 b

<sup>†</sup> Abbreviations: DAPRE, days after preemergence application.

<sup>‡</sup> Means within a year followed by the same letter are not significantly different according to Fisher's protected least significant difference ( $\alpha = 0.05$ ).

		Injury	
Year	Factor	14 DAPOST <sup>†,‡</sup>	
		%	
2017	PRE§		
	Verdict	9 a	
	Dual II Magnum	6 b	
	POST		
	Acuron Flexi	3 b	
	Capreno	2 b	
	Corvus	21 a	
	Resicore	3 b	
2018	PRE*POST		
	Verdict		
	Acuron Flexi	0 b	
	Capreno	2 b	
	Corvus	3 b	
	Resicore	1 b	
	Dual II Magnum		
	Acuron Flexi	1 b	
	Capreno	4 b	
	Corvus	11 a	
	Resicore	3 b	

Table 5. Influence of preemergence herbicide and postemergence
premixture on corn injury in 2017 and 2018.

<sup>†</sup> Abbreviations: DAPOST, days after postemergence application.

<sup>‡</sup> Means within a factor followed by the same letter are not significantly dif-

ferent according to Fisher's protected least significant difference ( $\alpha = 0.05$ ).

PRE data averaged over POST and atrazine in 2017; POST data averaged over PRE and atrazine in 2017.

		_	Yield				
Factor		Atrazine <sup>†</sup>	201	7		201	8
PRE <sup>‡</sup>					bu./ac		
Dual II Ma	gnum						
P	POST						
А	curon Flexi	-	214	a§		276	а
		+	195	b		260	ab
C	Capreno	-	154	d		200	def
		+	188	bc		229	cde
C	Corvus	-	154	d		216	def
		+	188	bc		246	bc
F	Resicore	-	153	d		227	cde
		+	185	bc		225	de
Verdict							
A	curon Flexi	-	216	а		278	а
		+	216	а		234	cde
C	Capreno	-	196	b		237	b
	•	+	181	с		225	de
C	Corvus	-	177	С		217	def
		+	152	d		178	g
F	Resicore	-	158	d		219	de
		+	196	b		236	cd

#### Table 6. Influence of preemergence herbicide and postemergence premixture on corn yield in 2017 and 2018.

<sup>†</sup> Atrazine applied at 1 pt/ac.

<sup>‡</sup> Abbreviations: PRE = preemergence application, POST = postemergence application.

§ Means within a factor followed by the same letter are not significantly different within a year according to Fisher's protected least significant difference ( $\alpha = 0.05$ ).

## Irrigation Timing, Fertigation, and Tillage Effects on Corn Yield

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#### Abstract

Three studies were conducted on irrigated corn at the University of Arkansas System Division of Agriculture's Rice Research and Extension Center near Stuttgart on a DeWitt silt loam soil to evaluate i) no-till, cover crops, and tillage treatments (2017 to 2019); ii) weekly versus sensor-based irrigation timing and iii) urea (46% N) versus urea-ammonium nitrate (UAN, 32% N) with surge irrigation (2018–2019). In addition, a weekly versus sensor-based trial was conducted at the Lon Mann Cotton Research Station near Marianna, Arkansas in 2019. The cover-crop and no-till treatments included no-tillage and a cover crop planted in the fall to a mix of cereal rye, tillage radishes, winter peas, and black oats. The tillage study concluded that there was no significant difference between tillage and no-till, but planting corn no-till into cover crops resulted in a significant yield reduction both years. Another study evaluated the yield and water use differences of a calendar (once a week irrigation) and schedule based on soil moisture sensors. The sensor-based method in the first year used half of the water of the calendar method but resulted in a significant yield reduction (P =0.02) of 20 bu./ac. However, in 2019, no significant difference in yield was observed at the two locations. Yield and water use differences between these two methods are inconclusive. The objective of the fertigation study was to compare the benefit of surge irrigation to conventional continuous furrow irrigation (control), this comparison was done with urea. Additionally, another treatment was implemented to compare using 32% UAN injected into a surge valve to distribute the fertilizer referred to as fertigation. If fertigation could be shown to be successful, it could provide for an alternative method to apply late-season nitrogen. No differences in yield were found between the urea treatments, but yields were significantly reduced both years for the UAN treatments. Results of the study indicate that yields observed for a field under no-till, with surge irrigation under current Division of Agriculture fertilizer recommendations with urea, and a sensor-based irrigation plan are comparable or better than the conventional weekly irrigated and fertilized tillage system.

#### Introduction

Halvorson et al. (2006) found that irrigated no-till systems had the potential to replace continuous tillage systems in the central Great Plains in a continuous irrigated corn (*Zea mays* L.) system. They found a 16% average higher yield in a continuous tillage system than in the no-till system, but the lower yield in the no-tillage system may have been a result of slower early spring development and delayed tasseling. Sainju and Singh (2001) found that yields between chisel plow (tillage) and no-till corn in central Georgia could be maintained by terminating the cover crop 2 weeks earlier in the spring, due to nitrogen sequestering by the residue. Habbib et al. (2016) found that after four years of conversion from tillage to a no-till cover crop system, the nitrogen use efficiency, grain yield, and grain nitrogen content increased in corn.

Spencer et al. (2019) compared Irrigation Water Management (IWM) practices for furrow irrigation in Arkansas and Mississippi on paired grower fields that implemented IWM practices and those that did not. The implementation of the IWM practices reduced total water use by 39.5%, increased grain yield by 6.5 bu./ac, and increased irrigation water use efficiency by 51.3%. Similar results were reported by Henry and Krutz (2016) on 14 on-farm comparisons, and via side by side comparisons at 4 research stations. Their data shows a 3–5% increase in yields (around 8 bu./ac) and water use was decreased by 40%.

#### Procedures

The Pioneer corn hybrid P1662AM was planted at the University of Arkansas System Division of Agriculture's Lon Mann Cotton Research Station in Marianna on 38-in. row spacing furrow irrigated field on a soil mapped as a Memphis silt loam soil in 2019. Plots were four rows wide and 550 ft long and the middle two rows were harvested for yield. The same hybrid was planted at the Rice Research and Extension Center near Stuttgart on 30-in. row spaced furrow irrigated field on a soil mapped as a DeWitt silt loam soil in 2017 through 2019. Plots in Stuttgart were 1200 ft long and 8 rows wide, the middle 4 rows were harvested for yield. Planting dates were in late April or early May, generally towards the end of when local farmers were finishing planting corn. This was done to increase the probability that irrigation treatment effects could be created. The study area was in continuous corn for the 3-year period of the study. Plots were randomized with three replications in a split plot design and irrigated using lay-flat pipe (Delta Plastics, Little Rock, Ark.). Field preparation, fertilization, planting and herbicide/pesticide treatments were practiced according to

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the University of Arkansas System Division of Agriculture's Cooperative Extension Service (CES) recommendations. An average plant density of between 31,000 to 34,000 plants/ac was established at both locations over the 3 years, however the stand in the cover crop and tillage treatments at Stuttgart were less due to the inability to completely close the slot in these treatments with a conventional planter closing system (Kinze, Williamsburg, Iowa).

Irrigation treatments included sensor-based irrigation and calendar-based or weekly irrigation. Granular matric potential soil moisture sensors were installed at 6-, 12-, 18-, and 30-in. depths in all sensor base irrigation plots. Treatments were replicated 4 times. Sensors were read and logged with a 900M Watermark monitor data loggers (Irrometer, Riverside, Calif.) in Marianna. At the site near Stuttgart, Agsense telemetry units (Huron, S.D.) were used.

Weather parameters were recorded with a WatchDog 2900 ET Weather Station (Spectrum Technologies, Aurora, III.) installed adjacent to the field in Marianna. In Stuttgart, a Davis Weather-link Station was used (Vernon Hills, III.).

Sensor-based irrigation was scheduled using the CES mobile app, "Soil moisture calculator" using a 50% allowable depletion and a silt loam with a pan soil type. The app calculates the remaining available water and irrigation decisions were based on this information. In Stuttgart, the effective rooting zone was assumed to be 30 in.; in Marianna, because of the presence of a fragipan, the rooting zone was assumed to be 24 in. and was based on sensor responses.

The calendar-based irrigation method included irrigating every Monday unless rain provided adequate soil water. The weekly-based irrigation method was applied in accordance with local farmer decisions about irrigation in the area. Thus if farmers around the station were irrigating, the calendar treatments were irrigated. Grain yield data were analyzed using Analysis of Variance (ANOVA) using Sigmaplot (Systat Software, Inc, San Jose, Calif.) and Tukey's Honest Significant Difference (HSD) test, unless otherwise noted.

For the fertigation study conducted in 2018 and 2019, nitrogen was applied two different ways. A total of 200 pounds of nitrogen was applied to each study. The entire study area received 100 pounds of ammonium sulfate (21 pounds of nitrogen) and 79 pounds of urea at planting. The treatment effects were applied during the second application of nitrogen at the 4-6 leaf stage. First, nitrogen was applied with a drop spreader (Gandy 10T, Owatonna, Minn.) as urea on continuous flow irrigation and as urea on surge flow irrigated treatments. The last treatment was fertigated once with 32% urea-ammonium nitrate (UAN) through a surge irrigation valve during the first irrigation of the season. The UAN can be injected into a P and R Surge valve (Lubbock, Texas) during the soak cycle using a proprietary program. In 2018 it was applied during the soak phase in accordance with the surge valve program. However, in 2019, it was applied during the advance phase because of the low yields observed in 2018. The UAN was pumped from a liquid tank through pressure compensated drip emitters to deliver the UAN to the split plots. All of the plots were irrigated within a day of each other, the surge treatments were irrigated and the continuous treatments plugged, then the continuous treatments were irrigated while the surge treatments were plugged. The fertigation study and tillage study were irrigated when the sensor-based treatments in the irrigation study were irrigated, thus these studies were irrigated at the same time as the soil moisture sensor-based irrigation protocols.

#### **Results and Discussion**

The three studies were analyzed separately. The plots were side by side, but since they were irrigated and treated differently, they were analyzed separately. Results are separated by studies, sensor-based irrigation versus calendar method to test the difference in yield between sensors and the calendar scheduling methods. The tillage study was conducted to test the difference between no-till and cover crop treatment effects on yield. Finally the third study was conducted to evaluate the feasibility of fertigating (applying fertilizer during irrigation) corn with liquid fertilizers.

#### **Sensor-Based Irrigation**

At Stuttgart in 2018 the field experienced a few and infrequent rainfalls, irrigation treatments showed a significant difference (P = 0.02) in yield between the calendar method and sensor-based method (Table 1). In 2018 the weekly treatment used 24.3 ac-in./ac versus 11.8 ac-in./ac or about half of the water of the calendar-based treatments, but yields were 20 bu./ac less where irrigation was based on soil moisture sensors. During 2019, both Stuttgart and Marianna experienced significant rainfall during the growing season such that both studies resulted in the irrigation plots being irrigated just twice and three times for the calendar-based irrigation treatments during the 2019 season. The amount of irrigation water applied in 2019 is not known due to a flowmeter failure in Stuttgart. In 2019, the experiment was also established in Marianna, with no significant difference in yield observed between the two scheduling methods and irrigated the same number of times as the Stuttgart study (flow meter data missing). The results (Table 1) do not show a significant difference in yield (P = 0.35) between sensor and calendar-based irrigation scheduling, in 2019. Preliminary yield results do not show conclusive evidence of differences between the two methods in 2019.

#### Tillage, No-Till and Cover Crop Effect Results

In Stuttgart, a study was conducted to compare tillage, no-till, and cover cropping systems. A standard treatment of full tillage included disking, field cultivation, and a bedder roller was evaluated compared to no-till, and no-till including a cover crop. Herbicide applications were the same for all treatments, except the cover crop treatment had an additional glufosinate application (40 oz/ac) to ensure cover crop termination. In 2018 the cover crop was terminated 10 days before planting, but in 2019 the cover crop was terminated after planting. The cover-crop was planted in the fall prior to corn planting and was a mix of cereal rye, tillage radishes, winter peas, and black oats. Treatment difference were only analyzed by each year. The data was analyzed using JMP<sup>®</sup>, Version 15 (SAS Institute, Inc., Cary, N.C.) 1989–2019 using analysis of variance and Tukey's honestly significant difference (HSD) and Dunnetts mean comparison tests. In 2017, the experiment was conducted over a previous gypsum and tillage study, where no differences were observed from the effect of gypsum or deep tillage treatment effects (data not shown) so only the deep tillage data is shown for 2017 since it was the same area used in 2018 and 2019.

In 2018 and 2019, the planter (Kinze, Williamsburg, Iowa) was modified with dimple closing wheels (Yetter Manufacturing, Colchester, Ill). Plant stands in the cover crop and no-till study were slightly reduced, which resulted in some skips in the no-till and cover crop treatments more than in the tillage study. This was a result of poor slot closing from the stock rubber tire closing wheels designed for tilled soil. Stand counts for the treatments were not estimated, but the stand differences in 2017 versus 2018 and 2019 are a likely factor in the significant yield difference in 2017. Planting the no-till and cover crop treatments presented some challenges as it is difficult to plant exactly on top of the bed after the first year. The beds erode over time and silt-in, making it difficult, even with a tractor equipped with Real Time Kinematic tractor guidance, to keep tractor tires and bed centers perfectly aligned. In fresh beds, tractor tires can fit the bed due to the loose soil; but under no-till conditions, beds are firm and slightly misaligned due to erosion, making slight imperfections that can lead to a deviation of several inches from the centerline. The combined effect of the narrower beds and solid nature of them, generally placed the corn on the edge of the bed rather than the center. Equipment improvements are needed to force the planter to center on a bed or regrove the furrows so they are consistent and result in corn rows that are nearly perfectly centered on beds. In some plot areas of the no-till and cover crop, this effect was dramatic and led to small areas that resulted in skips due to plants being drowned out.

In 2016, the study area was used to test gypsum and deep tillage treatment effects. After 2017, the tillage study was conducted in the treatment zone that had been deep tilled in 2016 (no gypsum) and only the 2017 deep tillage data is reported for 2017. In 2017 there was a difference observed between tillage and the cover crop treatments (P = 0.038), but not between the cover crop treatment and no-till (P = 0.54) or no-till and tillage (P=0.30), as shown in Table 2. In 2018 and 2019, when the slot closure problem was resolved with dimple closing wheels, no difference between the treatments was observed although the *P*-value is near the 0.05 significance level and is approaching significance. However, when both years are combined and the Dunnetts test is used instead of the Tukey's HSD, the cover crop treatment is different from the tillage (P = 0.020) and no-till (P = 0.029). Thus, one can conclude that there is a significant difference between the cover crop no-till treatment and tillage and no-till; however no significant difference could be found across the 3-year study between the no-till treatment and tillage. More study into cover crops and their impact on yield is warranted.

#### Fertigation and Surge Irrigation Results

The results for the fertilizer and surge study are presented in Table 3. Yields were analyzed separately for each year of the study. In 2018, continuous flow + urea and Surge + urea treatment yields were not significantly different (P=0.11), However, surge irrigation where UAN was applied during the soak cycle was different (P = 0.04). In 2019, the same result was found, even though UAN had been applied during the soak phase. In 2018, UAN was applied in the advance phases, while in 2019 it was applied in the soak phase; but a reduction in grain yield occurred both years for the UAN fertigated treatment. Based on this data, it is not advisable to fertigate corn with surge irrigation as significant yield losses are possible. In contrast, fertigation in rice has shown no yield penalty (Pickelmann et al., 2018).

#### **Practical Applications**

To date, these series of studies have shown no significant difference in corn yields between tillage and no-till systems. More work is needed to determine yield and water use differences between sensor-based and weekly calendar-based methods. Additionally, when fertigation was attempted, a yield reduction was found and is thus not recommended; however when surge irrigation is used with existing urea fertilization recommendations, a yield improvement may be possible.

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Table 1. Irrigation treatment yields in bushels per acre (bu./ac) between soil moisture sensor and calendarbased scheduling at the University of Arkansas System Division of Agriculture's Rice Research and Extension Center near Stuttgart and the Lon Mann Cotton Research Station, Marianna, 2018–2019.

Year	ar Location Sensor-based Scheduling		
		(bu./ac)	(bu./ac)
2019	Marianna	178 (a)†	163 (a)
2019	Stuttgart	237 (a)	225 (a)
2018	Stuttgart	167 (a)	187 (b)

<sup>†</sup> Subscripts denote significant difference for the row ( $\alpha$  = 0.05).

Table 2. Tillage treatment yields in bushels per acre (bu./ac) by year at the University of Arkansas Syste	em
Division of Agriculture's Rice Research and Extension Center near Stuttgart, 2017–2019.	

Year	Tillage/Conventional	No-Till	Cover-Crop and No-Till
	(bu./ac)	(bu./ac)	(bu./ac)
2019	217.1 (a)†	223.8 (a)	195.9 (b)
2018	165.6 (a)	157.3 (a)	147.3 (b)
2017	158.0 (a)	138.0 (ab)	124.0 (b)

<sup>†</sup> Subscripts denote significant difference for the row ( $\alpha$  = 0.05).

# Table 3. Corn fertilizer yields in bushels per acre (bu./ac) comparing fertilizer urea and urea-ammonium nitrate (UAN) and surge irrigation at the University of Arkansas System Division of Agriculture's Rice Research and Extension Center near Stuttgart, 2018–2019.

Year	Surge Irrigation + UAN in soak cycle		
	(bu./ac)	(bu./ac)	(bu./ac)
2019	207.0 (a)†	220.0 (a)	198.0 (b)
2018	162.9 (a)	152.7 (a)	113.4 (b)

<sup>†</sup> Subscripts denote significant difference for the row ( $\alpha = 0.05$ ).

## Corn Response to Sulfur Fertilizer Source and Rate in Arkansas

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#### Abstract

Corn (Zea mays L.) is an important row crop in Arkansas. In 2018, approximately 645,000 acres of corn were harvested in Arkansas. Sulfur (S) is an important nutrient in corn nutrition. However, there is limited information on S fertility under current Arkansas corn production conditions. The objectives of this research were to: 1) evaluate the effect of S fertilizer source and rate on corn grain yield, 2) investigate the relation between corn ear-leaf S and grain yield, and 3) quantify the amount of S removed in corn grain in Arkansas. Twelve replicated corn S fertilization experiments were conducted at commercial farms and the University of Arkansas System Division of Agriculture research stations from 2017 to 2019 on soils typically used for corn production. Sulfur fertilization significantly increased corn grain yield in 4 of the 11 tests reported suggesting that the native soil S at the nonresponsive sites was enough to support optimal corn grain yield. At S responsive sites, there was no significant S-source by S-rate effect, indicating that all preplant sources behaved similarly. Generally, sand content of the S responsive sites was higher than nonresponsive sites, and the soil organic matter content was lower in S responsive sites than in the nonresponsive sites. Soil organic matter has been known to supply S, and sandier soils are more prone to S leaching. Averaged across the 4 S responsive sites and all S rates, S fertilization increased the corn grain yield by 6%. The relation between concentration of S in the corn earleaf and relative corn grain yield (in selected site-treatments) suggested that corn ear-leaf S concentration is a potential predictor of corn S status. Under the current Arkansas production practices, corn grain yields of 225 and 250 bu./ac will remove 12.6 and 14.0 lb S/ac from soil respectively.

#### Introduction

Corn (Zea mays L.) is a major row crop in Arkansas. In 2018, approximately 645,000 acres of corn were harvested in Arkansas. Between 1992 and 2018, the average corn grain yield in Arkansas increased from 130 to 181 bu./ac, which represents a substantial increase in S removal from the soil nutrient reserves. Sulfur plays an important role in many plant physiological processes such as protein and chlorophyll synthesis. Therefore S deficiency can negatively impact corn grain yield and quality, thus reducing the growers' profits.

During the last three decades, increasing environmental regulations (to reduce man-made S emissions), increasing corn yields, and use of highly concentrated macronutrient fertilizers have necessitated supplemental S fertilizer application in many soils. Unfortunately there is virtually no published information on the effect of S source and rate on corn grain yield and grain S removal rates under the current Arkansas production conditions. Leaf analysis has been used as a diagnostic criteria for predicting in-season S fertilization need in some states with varying degrees of success. However much of the data supporting leaf analysis is dated due to introduction of modern corn hybrids. In the absence of any Arkansas-based data, the University of Arkansas System Division of Agriculture currently recommends the application of 20 lb S/ac for fields with a history of S deficiency. This approach is based on professional judgment and is currently the best available alternative to prevent significant yield potential losses due to S deficiency.

Arkansas growers are facing low commodity price and high fertilization costs. There is a need to improve the S fertilizer use efficiency to help the growers improve their profit margins. The most cost effective research approach is to begin by evaluating the effect of S fertilizer source and rate on corn and improve diagnostic methods for detecting in-season S deficiency. Such information is needed to evaluate and if needed revise current S fertilization recommendations. The specific objectives of this project were to 1) evaluate the effect of S fertilizer source and rate on corn grain yield, 2) investigate the relation between corn ear-leaf S and relative grain yield, and 3) quantify the amount of S removed in the harvested corn grain in Arkansas.

#### Procedures

Twelve replicated S-fertilization trials were conducted between 2017 to 2019 at the commercial farms and the University of Arkansas System Division of Agriculture research stations, as represented in Table 1. Prior to S application, a composite soil

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sample was taken from the 0-to 6-in. depth of all replications of each test. Each composite soil sample consisted of a total of 5 or 6 cores collected from the top of the bed and bed-shoulder in an alternating sequence. Selected properties of the soils were measured by methods used by the Division's soil testing laboratory.

In 2017, the S sources were: elemental S (ES) (90% S), ammonium sulfate (AS) (%24 S), Gypsum (% 21 S), sulfate of potash and magnesia (also known as K-MAG) (%22 S), MES15 (15% S), and MESZ (10% S). Elemental S and ammonium sulfate were each applied at four rates equivalent to 20, 30, 40 and 50 lb S/ac. Gypsum, K-Mag, MES15, and MESZ were applied at a single rate of 20 lb S/ac. In 2018 and 2019 ES, AS, Gypsum, K-MAG and MESZ were each applied at 10, 20, and 30 lb S/ac. The experimental design in 2017 was a randomized complete block and in 2018 and 2019, was a factorial of 5 Ssources and 3-S rates, plus a control of 0 S (check plot). Each plot was 25 ft long and 10 to 12.6 ft wide allowing for 4 rows of corn spaced 30 or 38 in. apart depending on the location. The S treatments were applied to the plot surface area before planting and the treatments were mechanically incorporated into the top 3- to 4-in. of the soil. The beds were then pulled with a hipper and corn was planted on the top of the bed. All the other nutrients were applied at the rates to ensure that S was the only nutrient limiting the corn grain yield. At 6 sites we took corn ear-leaf samples (20 leaves/plot) when the corn was at early silk stage. Leaf samples were dried, ground, and analyzed for S. Corn was furrow irrigated by the cooperative producer or the research station staff as needed. Corn management closely followed the Division of Agriculture Cooperative Extension Service recommendations.

The middle two rows of each plot were harvested with a plot combine for sites at the Division research stations. For trials located in commercial fields, one 12-ft section in each of the two center rows was hand-harvested and later placed through a combine. The calculated grain yields were adjusted to a uniform moisture content of 15.5% before statistical analysis. Corn grain samples were collected from 410 plots at selected site-years and analyzed for S. When appropriate, means were separated by the least significant difference (LSD) method and interpreted as significant when  $P \le 0.10$ .

#### **Results and Discussion**

The results of 11 experiments are reported, because one experiment was damaged and we were not able to collect meaningful data. Soil pH was 5.7 to 7.4, Mehlich-3 extractable P, K, and S were 19 to 79, 80 to 258, and 8 to 38 mg/ kg respectively (38 to 158, 160 to 516, and 16 to 78 lb/ac of P, K, and S respectively). These values are within the range of properties of soils commonly used for corn production in Arkansas. Sulfur fertilization significantly (P < 0.10) increased corn grain yield in 1 out of the 4 tests in 2017 and 3 out of 4 sites in 2018, but did not increase corn grain yield in any of the 4 sites in 2019 (Tables 2–3). Corn grain yield in the non-S responsive sites ranged from 96 to 250 bu./ac depending on the location. The Arkansas average corn grain yield in 2018 was 181 bu./ac, which suggests that in some Arkansas soils, the native supply of S can support above-average corn grain yield. Developing soil and plant diagnostic criteria to identify such soils can improve our growers' profits. At the 4 S responsive sites, the grain yield of corn that did not receive any S (0 S, check plot) was 158 to 194 bu./ac, while the average grain yield of corn that received any S was 167 to 204 bu./ac depending on the location. Averaged across the 4 S responsive sites and all S rates, S fertilization increased the corn grain yield by 6%. In general the 4 S responsive sites had either low soil organic matter or higher sand content as compared to the nonresponsive sites. Sulfur leaching is more prevalent in sandy soils and soil organic matter is a potential source of S. At the S responsive sites, there was no significant S-source by S-rate effect suggesting that under the conditions of these tests, the S sources had similar effects on corn grain yield.

The relationship between concentration of S in the corn earleaf samples and relative corn grain yield from selected treatments of 6 site-years suggests that maximal corn grain yields were produced when the corn ear-leaf S concentration was 0.24% to 0.25%, albeit some scatter in the data (Fig. 1). This suggest that corn ear-leaf S concentration is a potential suitable predictor of corn S status. However, additional data from a large number of sites are needed to test the reproducibility of this trend.

Average and median S concentration in corn grain samples, from 410 plots, were 0.1%. The data indicate that corn grain yields of 175, 200, 225, 250 bu./ac will remove 9.8, 11.2, 12.6 and 14.0 lb of S/ac from the soil, respectively (Fig. 2). Corn producers, crop consultants, and other advisory professionals can use this data to make more informed corn S management decisions. The information is also valuable for research and extension professionals for developing more efficient corn S fertilizer recommendations.

#### **Practical Applications**

Sulfur fertilization significantly (P < 0.10) increased corn grain yield in 4 of the 11 studies. Averaged across the 4 S responsive sites and all S rates, S fertilization increased the corn grain yield by 6%. An important implication of the results is that native soil S can sustain optimal corn grain yields in some Arkansas soils. Corn ear-leaf analysis can be a potential diagnostic tool for identifying soils where a yield benefit from S fertilization will not be expected. Research on developing diagnostic criteria to identify soils that do not need S fertilization will help the growers to improve their profit margin by applying S-fertilizer only when a potential yield benefit can be expected. Our research indicates that under current cropping conditions in Arkansas, corn crops of 175, 200, 225, 250 bu./ac will remove 9.8, 11.2, 12.6 and 14.0 lb of S/acre from the soil.

#### Acknowledgments

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Table 1. Test identification code, study year and location, soil series, corn hybrid, planting, fertilization, and harvest dates, and soil organic matter (SOM) for 11 corn sulfur fertilization trials conducted in Arkansas during 2017–2019.

					Planting	Harvest	
Site code	Year	County	Soil Series	Corn Hybrid	date	date	SOM
							(%)
CLZ73	2017	Clay	Collins Silt Loam	Croplan 6640	8-April	6-Sept.	2.62
GRZ73	2017	Greene	Fontaine silt loam	Pioneer P1197	16-April	8-Sept.	1.16
LEZ73	2017	Lee	Convent Silt Loam	Croplan 66265	18-May	18-Sept.	1.70
CHZ83	2018	Chicot	Henry silt loam	Agventure 8714	1-May	19-Sept.	-
CLZ83	2018	Clay	Beulah silt loam	Pioneer P1197	11-April	9-Sept.	1.20
GRZ83	2018	Greene	Fontaine silt loam	DeKalb 6208	06-May	13-Sept.	1.20
LEZ83	2018	Lee	Loring silt loam	Croplan 6265	06-May	13-Sept.	1.86
GRZ93	2019	Greene	Fontaine silt loam	Dekalb 6744	27-March	7-Sept.	1.30
MSZ93	2019	Mississippi	Steele loamy sand	Terral 28BHR	29-April	8-Sept.	1.98
PHZ93	2019	Phillips	Loring silt loam	Dynagro D57VC51	23-April	29-Aug.	1.48
POZ93	2019	Poinsett	Dundee silt loam	Progeny 7115	10-April	28-Aug.	1.38

Table 2. Corn grain yield response to sulfur (S) fertilizer source and rate at two commercial farms in Clay (CLZ73), and Greene (GRZ73) counties and the University of Arkansas System Division of Agriculture's Lon Mann Cotton Research Station in Lee County (LEZ73) during 2017.

		Study site		
S-Source	S-rate	CIZ73	GRZ73	LEZ73
	lb S/ac	Co	rn grain yield (bu./	'ac)
none	0	215	194 cd†	96
Elemental S	20	215	212 ab	94
Elemental S	30	208	204 abc	96
Elemental S	40	224	198 bcd	107
Ammonium sulfate	20	217	209 ab	105
Ammonium sulfate	30	239	204 abc	106
Ammonium sulfate	40	236	199 bcd	100
Ammonium sulfate	50	221	185 d	104
Gypsum	20	222	213 ab	99
K_MAG	20	217	204 abc	93
MES15	20	235	215 a	104
MESZ	20	250	207 abc	110
<i>P</i> value		0.55	0.10	0.12

<sup>†</sup> Means followed by the same letter are not significantly different at P = 0.10.

Table 3. Corn grain yield response to elemental sulfur (ES), ammonium sulfate (AS), gypsum (GYP), sulfate of potash and magnesia (KMAG) each applied at three rates (10, 20, and 30 lb S/ac) and a control (0 sulfur) in Chicot (CHZ83), Clay (CLZ83), Greene (GRZ83), Lee (LEZ83) county in 2018; and Greene (GRZ93), Philips (PHZ93), Poinsett (POZ93), Mississippi (MSZ93) counties in 2019.

		Study site							
Sulfur source	Sulfur rates	CHZ83	CLZ83	GRZ83	LEZ83	GRZ93	PHZ93	POZ93	MSZ93
	lb S/ac			C	orn grain yiel	d (bu./ac)			
None	0	132	178 cde <sup>†</sup>	162 d	158 hig	237	203	219	178
ES	10	118	186 bcde	167 cd	161 fgh	241	231	204	179
ES	20	130	187 bcde	207 ab	163 efgh	245	222	210	182
ES	30	123	210 ab	<b>FNT</b> <sup>‡</sup>	166 cdefgh	246	215	226	174
AS	10	107	169 e	189 abc	174 abcde	242	216	237	178
AS	20	136	186 bcde	172 cd	184 a	247	212	240	176
AS	30	132	174 de	FNT	180 ab	244	213	221	181
GYP	10	118	189 bcde	184 abcd	149 i	234	225	225	177
GYP	20	135	191 bcde	169 cd	160 ghi	235	220	221	170
GYP	30	119	210 ab	FNT	167 cdefg	236	222	214	178
KMAG	10	131	191 bcde	177 cd	163 efgh	241	214	230	174
KMAG	20	122	191 bcde	190 abc	162 efgh	244	215	223	167
KMAG	30	133	198 bc	FNT	159 ghi	231	224	223	189
MESZ	10	134	169 e	160 d	175 abcd	233	229	225	182
MESZ	20	128	196 bc	209 a	153 hi	252	229	207	180
MESZ	30	132	221 a	FNT	179 abc	232	232	223	178
P-value		NS	0.02	0.037	0.0003	NS§	NS	NS	NS

<sup>+</sup> Means followed by the same letter are not significantly different at P = 0.10.

<sup>‡</sup> FNT, fertilizer not tested at this site.

§ NS, means are not significant at P = 0.10.



Fig. 1. Relationship between sulfur (S) concentration in corn ear leaf at early silk stage and relative corn grain yield for selected S treatments in 6 S fertilization trials conducted in Arkansas during 2017–2019. Each data point is the average of 4 replications.



Fig. 2. Corn grain sulfur removal rates at four corn grain yield levels based on the median corn grain S concentration in grain samples collected from 410 experimental plots of corn S fertilization trials conducted in Arkansas during 2017–2019.

## Nitrogen Sufficiency Level Guidelines for Pre-Tassel Fertilization in Arkansas

C.L. dos Santos,<sup>1</sup> T.L. Roberts,<sup>1</sup> and L.C. Purcell<sup>1</sup>

#### Abstract

Corn (*Zea mays* L.) is one of the primary cereal crops worldwide with a yearly production of approximately 39 billion bushels. The U.S. is the main global corn producer, contributing approximately 35% of the total world production. Corn yield has increased 140% over the last four decades in part because of a 40% increase in nitrogen (N) input over the same period. However, the excessive application of N for corn production raises environmental and economic concerns, emphasizing the need for agricultural practices that lead to an efficient use of nitrogen. The objective of this study was to develop a prediction system for in-season N application using plant tissue analysis. Eight site-years were planted with Pioneer hybrid 1197YHR. The treatment structure was composed of 14 N fertilization regimes with season total N rates ranging from 0 to 225 lb N/acre and with split-applications at preplant, V10, V12, and VT timings. At phenological stages V10 and V12, the uppermost fully collared leaves were sampled; while at the VT stage, the ear-leaf was collected. Relative grain yield (RGY) was positively associated with ear-leaf N concentration (LN) at V10, V12, and VT stages. Regression equations for three growth stages did not differ significantly from each other. Therefore, a single regression relationship was developed between RGY and LN concentration. This equation can be used to predict the need for additional midseason N fertilization between the V10 to VT growth stages to maximize yield.

#### Introduction

Nitrogen (N) rate and timing of application recommendations for corn production vary greatly among states in the U.S. In Arkansas, for instance, recommendations are to apply 20% to 25% of the total N rate before planting. The remaining 75% to 80% of the N rate should be applied as sidedress between the V6 and V8 stages (Ritchie et al., 1989) or be split in 50% to 65% of the N rate in sidedress and 15% to 25% of the total N rate as a pre-tassel application between V10 and VT (Slaton et al., 2013a, 2013b). The amount of N to be applied is defined by soil texture and yield goal. For example, for a loamy soil and a yield goal up to 175 bu./acre, the recommended N rate is 175 lb N/acre. For the same soil, but with the yield goal above 175 bu./acre, the recommendation is 220 lb N/acre. In contrast, for a clayey soil, with the yield goal of 175 bu./acre, the recommendation is 230 lb N/acre and for the yield goal above 175 bu./ acre, the recommendation is 290 lb N/acre (Slaton et al., 2013a).

Many strategies can be put into effect to improve N use efficiency (NUE); for instance, matching crop demand and N fertilizer supply, splitting N fertilizer applications, minimizing application during the wet season, and changing fertilizer sources to match the environmental conditions (Mosier et al., 2004). Splitting the recommended N rate between preplant and in-season applications allows a more precise assessment of a crop's N requirement (Scharf et al., 2002). The majority of N is absorbed by the crop after the V8 stage (Russelle et al., 1983), and N applied before V8 is exposed to potential losses, lowering overall NUE. Synchronizing the crop's time of greatest N requirement with N fertilization reduces the probability of losses (Magdoff, 1991). Furthermore, in-season N applications as late as V12 to VT have resulted in no significant yield losses, indicating that there is a large window of opportunity to apply N in-season to corn, especially in irrigated systems (Russelle et al., 1983). Diverging from the global average NUE of 33% (Ladha et al., 2005), furrow-irrigated corn in Arkansas has been reported to recover 81% to 91% of the N fertilizer applied at V6 and more than 80% when fertilizer was applied in a two-way split (preplant and sidedress application at V6 to V8) (Roberts et al., 2016).

The search for new in-season N status assessment tools led to the development of the Illinois Soil Nitrogen Test (ISNT) (Khan et al., 2001; Mulvaney et al., 2001). Williams et al. (2007) described that the ISNT is based on an alkaline digestion of a soil sample, followed by colorimetric analysis of the NH<sub>3</sub>-N released during the digestion. The hydrolysates are fractionated in total hydrolysable-N, hydrolyzable NH<sub>4</sub>-N, amino sugar and NH<sub>4</sub>-N, amino acid-N, and amino sugar-N. The test was used to classify Illinois soils as responsive to N fertilization (ISNT result < 225 ppm) or nonresponsive (ISNT result > 235 ppm; Khan et al., 2001). Calculations of the economic optimum N rate (EONR) for the average fertilizer cost and corn price ratio correlated negatively and strongly with the EONR, indicating the ability to predict corn response to N (Williams et al., 2007). In contrast, the results from 80 site-years of corn response to N trials found it incapable of distinguishing responsive from

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nonresponsive soils in Wisconsin (Osterhaus et al., 2008). This study correlated the ISNT values with soil organic matter (SOM) and suggested that the ISNT is measuring a constant fraction of the SOM rather than readily mineralizable-N. However, there is no test for predicting the need for a pre-tassel N application rate using plant tissue-N concentration. This research aimed to identify the sufficiency level of tissue-N concentration to serve as a guideline for pre-tassel fertilization.

#### Procedures

In 2017, two field studies were conducted at two University of Arkansas System Division of Agriculture research stations: the Pine Tree Research Station (PTRS; 35.12 N, 90.92 W) and the Milo J. Shult Agricultural Research and Extension Center (SAREC; 36.09 N, 94.17 W). In 2018 and 2019, three field studies were conducted each year at three research stations, one field at each of the following locations: the SAREC (36.09 N, 94.17 W), PTRS (35.12 N, 90.92 W), and the Rohwer Research Station (RRS; 33.80 N, 91.26 W). The experimental design was a randomized complete block with four replications and fourteen different treatments, which represented different timings and rates of N (Table 1). All the experimental trials were on silt loam soils. Plots were planted with the Pioneer hybrid 1197YHR at the seeding rate of 40,000 seeds/acre and consisted of four rows 30-ft long with row spacings of 36, 30, and 38 inches for the SAREC, PTRS, and RRS, respectively. Preplant N rates (0 or 30 lb N/acre) were applied to the field and incorporated into the soil prior to the corn sowing. The sidedress rate was applied to the field between stages V6 and V8 and meant to simulate deficient, optimal, and above optimal N status of the corn prior to pre-tassel N applications. The pretassel application was applied to the field in one of the following stages: V10, V12, or VT.

The uppermost leaf with a visible collar was collected from five plants in the two middle rows of each plot at V10 and V12 stages, and five identifiable ear-leaves were sampled at the VT stage prior to N fertilization. The leaf samples were oven-dried at 150 °F until a constant weight, ground, sieved via a 20-mesh screen, and analyzed for total N using combustion (Campbell, 1992) at the Univesity of Arkansas System Division of Agriculture's Fayetteville Agricultural Diagnostic Lab.

Plots were trimmed to 20 ft in length at maturity, the two center rows were harvested using a small plot combine, and yield was corrected to 15.5% moisture. Relative grain yield (RGY) was calculated as the ratio between the yield of an individual plot and the maximum yield attained within each environment and multiplied by 100.

Statistical analysis was conducted in R 3.5.2 (R Core Team, Vienna, Austria). The relationships between RGY and ear-leaf N concentration (LN) at different growth stages (V10, V12, and VT) were investigated by fitting a segmented regression (Eq. 1) between RGY and LN values at each growth stage (Table 2). Where  $\theta_0$  is the intercept,  $\theta_1$  is the increment in RGY per one unit change in *LN*, and  $x_1$  is the joint point of the regression.

$$RGY = \begin{cases} \theta_0 + \theta_1 \times LN, & LN < x_1 \\ \theta_0 + \theta_1 \times x_1, & LN \ge x_1 \end{cases}$$
 Eq. 1

#### **Results and Discussion**

The relationship between RGY and LN at the V10 stage (Fig. 1) was characterized by a segmented regression model, where RGY increased linearly between LN values of 1% and 2.98% and plateaued for LN values between 2.98% and 3.67%. Similarly, at the V12 growth stage (Fig. 2) RGY increased linearly between LN values of 1% and 2.97% and plateaued for LN values between 2.97% and 3.67%. Likewise, at the VT growth stage (Fig. 3) RGY increased linearly between LN values of 1% and 3.67%. Likewise, at the VT growth stage (Fig. 3) RGY increased linearly between LN values of 1% and 3.19% and plateaued for LN values between 3.19% and 3.67%. The joint points 2.98%, 2.97%, and 3.19% represent the minimum adequate LN concentration at the V10, V12, and VT stages, respectively, at which maximal yields would be produced without supplemental N fertilization.

Using the confidence interval-hypothesis test equivalence, the coefficients from all three regressions (RGY and LN at V10, V12, and VT stages) do not differ from each other since their confidence intervals overlap. Therefore, all three growth stages were included in one analysis, investigating the relationship between RGY and LN between V10 and VT stages (Fig. 4). The relationship between RGY and LN between V10 and VT stages was characterized by a nonlinear model, where RGY increased linearly between LN values of 1% and 2.96% and plateaued for LN values between 2.96% and 3.67%. The joint point of the regression  $(2.96 \pm 0.08\%)$  represents the minimum adequate LN concentration between the V10 and VT stages at which no midseason N fertilization would be required to produce maximal yields. The LN sufficiency concentration between V10 and VT stages of 2.96% agrees well with previous literature reported by Greub et al. (2018) who reported a sufficiency level of 3.1% at R1.

#### **Practical Applications**

Tissue analysis can be used to assess N sufficiency for corn produced in Arkansas, which would assist growers in determining the potential need for a pre-tassel N application. By providing this information to growers, there is a possibility of salvaging yield with a pre-tassel N application, in cases where N sufficiency levels are below optimum (< 2.96% N). Furthermore, there is also the possibility to prevent N overfertilization, in cases where N sufficiency levels are equal or above optimum levels ( $\geq$  2.96% N). Additionally, this tool also provides a wide window for leaf collection to monitor plant N sufficiency, since in the environments where these studies were conducted, the time between the V10 and VT stages ranged from 21 to 28 days.

#### Acknowledgments

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	•			
		Pre-tassel		
Treatment	Pre-plant	Sidedress	Pre-Tassel	application timing
		(Ib N/acre)		
1	0	0	0	-
2	30	50	0	-
3	30	50	45	V10
4	30	50	45	V12
5	30	50	45	VT
6	30	100	0	-
7	30	100	45	V10
8	30	100	45	V12
9	30	100	45	VT
10	30	150	0	-
11	30	150	45	V10
12	30	150	45	V12
13	30	150	45	VT
14	30	190	0	-

Table 1. Nitrogen rates and times of application for different treatments.

	relative grain yield and leaf tissue nitrogen concentrations at the V10, V12, and VT growth stages.								
Growth Stage	R <sup>2</sup>	Number of observations	Coefficient	Estimate	95% Confidence limits				
			Intercept	-37.06	-48.61	-25.51			
V10	0.86	167	Slope	42.28	39.17	45.39			
			Joint point	2.98	2.88	3.08			
			Intercept	-17.39	-26.93	-7.85			
V12	0.79	148	Slope	34.92	31.08	38.76			
			Joint point	2.97	2.79	3.14			
			Intercept	-28.18	-36.35	-20.01			
VT	0.83	184	Slope	35.76	33.86	39.67			
			Joint point	3.19	3.07	3.30			

Table 2. Regression coefficients using a linear plateau model for the relationships between elative grain yield and leaf tissue nitrogen concentrations at the V10, V12, and VT growth stage



Fig. 1. Relationship between relative grain yield (%) and leaf nitrogen concentration at the V10 growth stage (%). The red dotted line represents the joint point and the grey shaded area represents the confidence interval at  $\alpha$  = 0.05.



Fig. 2. Relationship between relative grain yield (%) and leaf nitrogen concentration at the V12 growth stage (%). The red dotted line represents the joint point and the grey shaded area represents the confidence intervals at  $\alpha$  = 0.05.



Fig. 3. Relationship between relative grain yield (%) and leaf nitrogen concentration at the VT growth stage (%). The red dotted line represents the joint point and the grey shaded area represents the confidence intervals at  $\alpha$  = 0.05.



Fig. 4. Relationship between relative grain yield (%) and leaf nitrogen concentration between the V10 and VT growth stages (%). The red dotted line represents the joint point and the grey shaded area represents the confidence intervals at  $\alpha = 0.05$ .

## Dark Green Color Index as a Midseason Nitrogen Management Tool in Corn Production Systems

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#### Abstract

In Arkansas, nitrogen (N) recommendations for corn (*Zea mays* L.) are based on soil texture and yield goal. Producers apply the N in a two- or three-way split to decrease N losses from mechanisms such as volatilization, leaching, and denitrification. These split application strategies allow for an in-season assessment of corn N status before applying fertilizer, ultimately narrowing the gap between fertilizer-N supply and crop demand. Several remote sensing techniques have been employed as tools for N status assessment in corn. One assessment tool is the Dark Green Color Index (DGCI) that measures the intensity of greenness, which has been correlated with leaf-N concentration (LN) and relative grain yield (RGY). The present research evaluated aerial DGCI measurements as a tool for crop N assessment and as a guideline for pre-tassel N fertilization. Data from eight site-years utilized 14 N fertilization regimes with the season-total N rates ranging from 0 to 225 lb N/acre and with split-applications at preplant, V10, V12, and VT timings. At the growth stages V10, V12, and VT, leaf samples were collected for TN analysis and red, green and blue (RGB) digital images were captured from the field at 100 ft above ground level with an unmanned aerial system (UAS). Images were processed to create an orthomosaic and data were extracted from orthomosaics to measure DGCI. A multiple regression using DGCI of individual plots and the DGCI values well (R<sup>2</sup> = 0.89). These results indicate that DGCI is a simple and effective tool for assessing the need for additional N fertilizer applied to corn in-season.

#### Introduction

Nitrogen (N) application recommendations in corn production systems vary among states in the U.S. In Arkansas, N is recommended based on soil texture and yield goal (Slaton et al., 2014a; 2014b). In a clayey soil and with a yield goal below 175 bu./acre, the recommended rate is 230 lb N/acre. In contrast, on the same soil, the recommended rate is 290 lb N/acre, when the yield goal is above 175 bu./acre. If the soil texture is loamy, the recommended N rates are 175 and 220 lb N/acre for yield goals below and above 175 bu./acre, respectively.

The majority of N uptake by corn occurs after the V8 growth stage (Russelle et al., 1983), which exposes N applied before V8 to potential loss mechanisms. Thus, synchronizing the time of greatest N requirement by the corn crop with N fertilization application timings reduces the possibility of losses (Magdoff, 1991). In Arkansas, recommendations are that the N rate should be split into two or three applications. In a two-way split strategy, 20% to 25% of the recommended N rate is applied at preplant and the remaining 75% to 80% is applied as a sidedress application, between the V6 and V8 growth stages. In the three-way split, 20% to 25% of the recommended N rate is applied at preplant, and 50% to 65% should be applied between the V6 and V8 growth stages as a sidedress application, and 15% to 25% of the N is applied between the

V10 and VT growth stages (Slaton et al., 2014a). In addition to matching corn N demand and supply, splitting the N rate also allows in-season implementation of N-status assessment tools (Scharf et al., 2002).

Several tests have been developed to assess corn N requirement using soil and plant analysis, such as the Presidedress Nitrate Test (PSNT) (Magdoff et al., 1984), the Pre-Plant Nitrate Test (PPNT) (Bundy and Malone, 1988), and the Illinois Soil Nitrogen Test (ISNT) (Khan et al., 2001; Mulvaney et al., 2001). In addition to the chemical tests, several sensors have also been employed in the field as in-season evaluation tools. Chlorophyll meters, such as the SPAD-502 meter (Konica Minolta, Tokyo, Japan), have been employed to assess N levels. The more chlorophyll that is present in the leaf, the higher the leaf-N concentration, which can be related to potential lateseason N applications to corn (Samborski et al., 2009).

The normalized difference vegetation index (NDVI) has also been used to evaluate corn N status using sensors such as the Crop Circle ACS-210 (Holland Scientific, Lincoln, Neb.) and Green Seeker (N Tech Industries, Inc., Ukiah, Calif.). These sensors calculate NDVI based on red ( $650 \pm 10$  nm) and near infrared (NIR,  $750 \pm 15$  nm) reflectance from the crop canopy. The NDVI value ranges between -1 and 1 (Eq. 1). With higher values being correlated with sufficient leaf-N concentrations (Schlemmer et al., 2013).

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$$NDVI = \frac{NIR - red}{NIR + red}$$
 Eq. 1

The dark green color index (DGCI) has also been utilized to assess the plant N status. The DGCI method requires the red, green and blue (RGB) from standard digital camera values to be converted to hue, saturation, and brightness values. The DGCI value ranges from 0 (yellow) to 1 (dark green) as shown in Eq. 2 (Karcher and Richardson, 2003).

*...* 

001

$$DGCI = \frac{60}{-60} + (1 - Saturation) + (1 - Brightness)$$
Eq. 2

Rorie et al. (2011) found that yield, leaf-N concentration (LN) and chlorophyll concentration were directly correlated with DGCI. Purcell et al. (2015) developed calibration curves for the amount of N to apply that would recover 90% to 95% RGY from DGCI values measured between the V6 and V10 growth stages on the uppermost collared leaf.

Rhezali et al. (2018) compared grain yield resulting from N application based on DGCI calibration curves (Purcell et al., 2015) with the University of Arkansas System Division of Agriculture's Cooperative Extension Service N rate recommendations (Slaton et al., 2014a). Overall, N rates recommended with the DGCI method ranged from 82 to 100 lb N/acre less than the recommended Extension rate while maintaining yield in all cases. The authors concluded that the DGCI method was capable of predicting the in-season N requirement for corn and future research should focus on simplifying the method and extending it to aerial platforms so it can be used directly in the field. This research aimed to employ aerial DGCI measurements as guidelines for pre-tassel fertilization.

#### **Procedures**

Between 2017 and 2019, eight field studies were conducted at three University of Arkansas System Division of Agriculture research stations: the Pine Tree Research Station (PTRS; 35.12 N, 90.92 W), Milo J. Shult Agricultural Research and Extension Center (SAREC; 36.09 N, 94.17 W), and the Rohwer Research Station (RRS; 33.80 N, 91.26 W). The PTRS and SAREC stations contained field studies in all three years, while the RRS did not have studies in 2017. The experimental design was a randomized complete block with 4 replications and 14 different combinations of N rate and time of application, aiming to cause different levels of N sufficiency (Table 1). Experimental units were planted on silt loam soils with the Pioneer hybrid 1197YHR at the seeding rate of 40,000 seeds/ acre. Each experimental unit was 30-ft long with 4 rows that were spaced 36 (SAREC), 30 (PTRS), and 38 (RRS) inches apart. For treatments 2 through 14, the preplant N rate was applied to the field and incorporated into the soil prior to planting. Sidedress N rates were applied to experimental units when the crop was between the V6 and V8 growth stages. The different sidedress rates were meant to mimic suboptimal, optimal, and above-optimal N rates prior to pre-tassel fertilization. Pre-tassel fertilization was applied to experimental units between the V10 and VT growth stages according to the treatment plan (Table 1).

The RGB images were collected at the V10, V12, and VT growth stages with a DJI Phantom 4 Pro using the camera that comes as standard equipment on the UAS (25.4-mm 20-megapixel CMOS sensor). Images were collected at 100 ft above ground level and with 80% overlap between the pictures. An orthomosaic of the individual images was built using Professional Agisoft MetaShape<sup>©</sup> (http://www.agisoft.com). The DGCI values of individual plots were determined from orthomosaic images using Field Analyzer<sup>©</sup> (https://www.turfanalyzer.com/field-analyzer) software.

The uppermost fully developed leaf blade from five corn plants were sampled from the two middle rows of each plot at the V10 and V12 growth stages, and five identifiable earleaves were sampled at the VT stage prior to N fertilization. The samples were oven-dried at 150 °F until constant weight, ground, and sieved via a 20-mesh screen, and analyzed for total N using combustion (Campbell, 1992) at the University of Arkansas System Division of Agriculture's Fayetteville Agricultural Diagnostic Laboratory.

Plots were trimmed to 20 ft in length at maturity, the two center rows were harvested using a small plot combine, and yield was corrected to 15.5% moisture. Relative grain yield was calculated as the ratio between the yield of an individual plot and the maximum yield attained within each environment.

To account for the difference in light intensity or quality among different images, DGCI measurements from experimental units with high N were used as a reference or high N check. The reference DGCI value was calculated for each trial and growth stage as the average DGCI for treatment 14 (30 lb N/acre at preplant, and 190 lb N/acre at sidedress).

Statistical analysis was conducted in R v. 3.5.2 (R Core Team, Vienna, Austria). The relationship between DGCI, reference DGCI, and LN was investigated by fitting a generalized linear model, assuming a gamma distribution, in which DGCI was the response variable, and LN and reference DGCI were predictors. Additionally, the relationship between RGY, DGCI, and reference DGCI was investigated by fitting a generalized linear model, assuming a gamma distribution, in which RGY was the response variable, and DGCI and reference DGCI were predictors.

#### **Results and Discussion**

The relationship between DGCI, LN, and reference DGCI (Fig. 1) as characterized by multiple regression was strong showing that DGCI increased between LN values of 1% and 3.5% and between reference DGCI values of 0.60 and 0.79. The regression coefficients indicate that the quadratic relationship between DGCI and LN was maintained at different environmental light conditions, with different reference DGCI values, but, DGCI values increased linearly as reference DGCI values increased.

The relationship between RGY, DGCI, and reference DGCI (Fig. 2) as predicted by multiple regression was also relatively strong, where RGY increased linearly between DGCI values of 0.44 and 0.83. The regression coefficients indicate that when the reference DGCI values varied, the linear relationship between RGY and DGCI was maintained. However, a given

DGCI value would predict a range of RGY, depending upon the reference DGCI value. For example, a DGCI value of 0.65 when the reference DGCI is 0.80 predicts RGY of 28%, but when the reference DGCI is 0.60, RGY is predicted to be 97%.

Figure 3 presents the same relationships shown in Fig. 2 in a form more useful for producers. Using the example discussed above, the open circle in Fig. 3 shows a RGY of 97% when DGCI is 0.65 and the reference DGCI is 0.60; the closed rectangle shows a RGY of 28% when DGCI is 0.65 and the reference DGCI is 0.80.

#### **Practical Applications**

The assessment of N sufficiency using DGCI can help growers salvage corn yield in cases where the predicted RGY is low. Use of the DGCI to predict N sufficiency can also avoid unnecessary application of N in cornfields, ultimately reducing the risk of N movement into the landscape and reducing production costs. The advantage of the DGCI tool, when compared with plant tissue analysis is that the results are immediate, although this methodology requires the use of a high N area within the target area. The relationship between DGCI and RGY between the V10 and VT growth stages provides a relatively quick and simple tool to determine if RGY would respond to additional fertilizer N.

#### Acknowledgments

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		Nitrogen rate				
Treatment	Pre-plant	Sidedress <sup>a</sup>	Pre-Tassel	application timing		
		(Ib N/acre)				
1	0	0	0	-		
2	30	50	0	-		
3	30	50	45	V10		
4	30	50	45	V12		
5	30	50	45	VT		
6	30	100	0	-		
7	30	100	45	V10		
8	30	100	45	V12		
9	30	100	45	VT		
10	30	150	0	-		
11	30	150	45	V10		
12	30	150	45	V12		
13	30	150	45	VT		
14	30	190	0	-		

Table 1. Nitrogen rates and times of application for different treatments.

<sup>a</sup> Sidedress N applied between the V6 and V8 growth stages.



Fig. 1. Relationship between Dark Green Color Index (DGCI), Leaf Nitrogen Concentration (LN), and Reference Dark Green Color Index (DGCI =  $-0.409 + 0.197 \times TN - 0.028 \times TN^2 + 1.1 \times Reference DGCI$ , R<sup>2</sup> = 0.89).



Fig. 2. Relationship between Relative Grain Yield (%), Dark Green Color Index (DGCI), and Reference Dark Green Color Index ( $RGY = 43.66 + 399.02 \times DGCI - 343.92 \times Reference DGCI$ ,  $R^2 = 0.71$ ).



Fig. 3. Relationship between Relative Grain Yield (%, RGY), Dark Green Color Index (DGCI), and Reference Dark Green Color Index (*RGY* = 43.66 + 399.02 x *DGCI* -343.92 x *Reference DGCI*, R<sup>2</sup> = 0.71). The isolines represent RGY. Open circle shows a predicted RGY= 97% when DGCI is 0.65 and reference DGCI is 0.60; the filled square shows a predicted RGY= 28% when DGCI is 0.65 and reference DGCI is 0.80.

## Corn Grain Yield Response to Soil-Applied Phosphorus and Potassium in Arkansas

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#### Abstract

Corn (*Zea mays* L.) is an important row crop in Arkansas and phosphorus (P) and potassium (K) are two important nutrients in corn nutrition. Reliable soil-test-based fertilizer recommendations are the most cost effective tool for sound P and K fertilization. Information from replicated experiments on corn response to P or K fertilization is the cornerstone of reliable soil-test recommendations. Replicated field experiments were conducted to evaluate corn response to fertilizer P and K rate on soils typically used for corn production during 2017, 2018, and 2019. Phosphorus fertilization significantly (P < 0.05) increased corn grain yield at 4 site years where Mehlich-3 extractable soil-P was 27 ppm or less. At each of the 4 P-responsive sites, grain yield increase from P fertilization was approximately 20% as compared to the corn that did not receive any P. However, P fertilizer response was only observed when the soil pH was greater than 6.5. Potassium fertilization significantly increased corn grain yields increased as much as 39% from K fertilization compared to the corn that did not receive any K. However, at 8 of the site-years where the soil-test K levels were low (64–90 ppm), K fertilization did not influence corn grain yield.

#### Introduction

Corn (*Zea mays* L.) is a major row crop in Arkansas. In 2018, approximately 645,000 acres of corn were harvested in Arkansas. The equivalent of 60 lb  $P_2O_5$  and 45 lb  $K_2O/ac$  are removed from the soil by a grain yield of 175 bu./ac (International Plant Nutrition Institute, 2014). Between 1992 and 2018, the average corn grain yield in Arkansas increased from 130 to 181 bu./ac, which represents a substantial increase in P and K removal from the soil nutrient reserves. Many plant physiological processes such as energy transfer and carbohydrate metabolism depend on adequate P and K uptake. The deficiency of either nutrient will limit corn yield and reduce the growers' profits. Failure to replace the nutrients removed by the harvested grain with adequate fertilizer can lead to soil nutrient depletion and create yield-limiting situations.

Phosphorus deficiency in corn may result in stunting and purple discoloration of leaves (Sawyer, 2004). Early planted corn, and corn under no-till, have frequently been observed with phosphorus deficiency symptoms, particularly purpling of leaves, even on soils that have adequate levels of soil-test P. While P fertilizer applications may cause the plants to recover, warmer temperatures are often observed to stimulate recovery of the plants, with no yield effects from the deficiency. Potassium deficiency in corn results in chlorosis followed by death of older leaves around the margins, stunted growth, delayed maturity, lodging caused by weak straw, and low bushel weight (Sawyer, 2004). Leaf symptoms typically begin at the leaf tip and progress down the leaf.

Applying the right rates of P and K enables growers to maximize net returns from corn production and minimize nutrient loss into the surrounding landscape. Reliable soil-testbased fertilizer recommendations are the most cost-effective tool for applying the right amounts of P and K fertilizer. The University of Arkansas System Division of Agriculture's Soil Testing Laboratory categorizes soil-test P amounts determined by Mehlich III extraction less than 16 ppm as Very Low, between 16-25 ppm is considered Low, 26-35 ppm is considered Medium, 36-50 ppm is considered Optimum, and greater than 50 ppm is considered Above Optimum. Soil-test K amounts determined by Mehlich III extraction that are less than 61 ppm are considered Very Low, between 61-90 ppm is considered Low, 91–130 ppm is considered Medium, 131–175 ppm is considered Optimum, and greater than 175 ppm is considered Above Optimum. Although these thresholds have been established, more data is needed to determine the amount of fertilizer needed at each of these thresholds for optimum yields.

The development of reliable soil-test-based fertilizer-P and –K rate recommendations requires data from a large number of trials. Multiple site-years of research are needed to increase the reliability and applicability of soil-test correlation

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and calibration curves. The specific objective of this research was to evaluate corn grain yield response to soil-applied fertilizer-P or -K rates at multiple locations on soils typically used for corn production in Arkansas.

#### Procedures

Field trials were established at multiple locations during 2017, 2018, and 2019 to assess the yield response by corn from applications of different rates of P or K fertilizer. Selected agronomic information for the site-years where the P and K fertilizer studies were conducted are listed in Tables 1 and 2, respectively.

To evaluate P fertilizer response by corn, P was applied at rates of 0, 40, 80, 120, and 160 lb  $P_2O_5/ac$  as triple superphosphate in 4-row plots. Each plot was 25- or 40-ft long and 10- to 12.6-ft wide allowing for 4 rows of corn spaced 30 or 38 inches apart depending on the location. At on-farm locations, the P fertilizer was applied to the top of the bed and furrow within a week of corn planting. At experiment station locations (University of Arkansas System Division of Agriculture's Lon Mann Cotton Research Station and Pine Tree Research Station), the fertilizer treatments were applied pre-plant incorporated into the top 3 to 4 inches of the soil prior to establishing the beds. Blanket applications of muriate of potash and ZnSO<sub>4</sub> were applied to supply 90 to 120 lb K<sub>2</sub>O, ~5 lb S, and ~10 lb Zn/ac.

To evaluate K fertilizer response by corn, K was applied at rates of 0, 50, 100, 150, and 200 lb  $K_2O/ac$  as muriate of potash in 4-row plots. Plot establishment and maintenance was the same as described above for P fertilizer studies except blanket applications of triple superphosphate were applied to supply 80 to 90 lb  $P_2O_5$  per acre (instead of muriate of potash).

All experiments were fertilized with a total of 260 lb N/ac in single, double, or three-way split applications (e.g., preplant, 3 to 6-leaf stage and/or pre-tassel) depending on the location. Urea was incorporated preplant and topdressed at pre-tasseling while urea-ammonium nitrate (UAN) solution was knifed into the soil at 3-6-leaf stage. Corn was grown on beds and furrow irrigated as needed either by research station staff or by the cooperating producer. Corn management closely followed the University of Arkansas System Division of Agriculture's Cooperative Extension Service recommendations.

Composite soil samples were collected from the 0-to 6-in. depth of each replication prior to P and K fertilizer application for routine soil analysis. At on-farm locations, a composite soil sample was compiled from a total of 5 or 6 cores collected from the top of the bed and bed-shoulder in an alternating sequence. Soil samples were oven-dried, crushed, extracted with Mehlich-3 solution, and the concentrations of elements in the extracts were measured by inductively coupled plasma atomic emission spectroscopy at the University of Arkansas System Division of Agriculture's Soil Testing Laboratory located in Marianna, Arkansas. Soil pH was measured in a 1:2 (volume: volume) soil-water mixture. Results from soil analysis are presented in Table 3 for the P fertilizer rate studies and Table 4 for the K fertilizer rate studies. The middle two rows of each plot were harvested with a plot combine at maturity for sites on the experiment stations. For on-farm trials, one 12-ft section in each of the two center rows was hand-harvested at maturity and then shelled with a plot combine. The calculated grain yields were adjusted to a uniform moisture content of 15.5% before statistical analysis. Samples of grain were from each plot were analyzed for P and K content during 2017 and 2018 to assess nutrient removal by corn. When appropriate, means were separated by the least significant difference (LSD) method and interpreted as significant when  $P \le 0.05$ .

#### **Results and Discussions**

### Phosphorus

Corn grain yields as influenced by P fertilizer rate for 19 site-years are presented in Table 5. Corn yields increased significantly due to P fertilizer applications in a total of 4 site-years, including 2 site-years in 2017 (Arkansas71, Prairie71), and 2 site-years in 2019 (St.Francis93, St.Francis95). The increase in grain yields at the responsive sites ranged from 7% to almost 21%. At least 1 of the 4 site-years (Prairie71 in 2017) had visual P deficiency symptoms, including stunting and purpling, in the unfertilized control. The 4 site-years where yields were increased due to P fertilizer each had soil-test P values of 27 ppm or less (Table 3). However, other site-years, such as 2019 Lonoke91, also had relatively low soil-test P values and yet did not respond to P fertilizer. Relatively overall low yields were observed at Lee71 in 2017, Lee83 in 2018, and Lee91 in 2019. Poor stand establishment was observed at these site-years and likely contributed to lower than optimal yields.

Perhaps one of the important factors in P nutrition for corn is soil pH. In each of the 4 site-years where responses were observed, the soil pH was 6.5 or higher (Table 5). In contrast, nonresponsive site-years with low soil-test P had soil pH less than 6.5. Greater probability for response to P fertilizer when the soil pH is greater than 6.5 has been documented in rice because available soil P is reduced as soils become more alkaline (Slaton et al., 2002). When producing corn on soils with soil pH greater than 6.5, soil P and P fertilizer requirements should be monitored closely. Corn produced on soils with high soil pH and low soil-test P values will most likely result in yield response to P fertilizer.

Phosphorus removed in corn grain at harvest from these studies averaged 0.38 lb  $P_2O_5$  per bushel of grain (Table 6). A slight trend for reduced amounts of P removed per bushel as yield increased was observed, but was not significant (data not shown). Based on these data, an average corn yield of 200 bu./ac would remove approximately 76 lb  $P_2O_5$ /acre. This number is similar to those previously reported (International Plant Nutrition Institute, 2014).

#### Potassium

Potassium grain yields as influenced by K fertilizer rate for 19 site-years are presented in Table 7. Corn yields increased significantly (P < 0.05) due to K fertilizer applications in a total of 5 site years, including 1 site-year in 2017 (Arkansas72), 1 site-year in 2018 (Clay82), and 3 site years in 2019 (Lee92, St.Francis92, St.Francis94). If the statistical significance is increased from 0.05 to 0.10 (P > F), an additional 3 site-years would result in significant yield increases resulting from K fertilizer. Of the responsive site-years, the soil-test K was 72 ppm or less. These site-years generally contained the lowest soil-test K levels of all site-years tested, indicating the highest probability for response to potassium fertilizer. Relatively low overall yields were observed at Cross82 in 2018 and Lee92 in 2019. Poor stand establishment was observed at these siteyears and likely contributed to lower than optimal yields. At the responsive site-years, the yield increase resulting from K fertilizer ranged from 26% to 39%.

Potassium removed in corn grain at harvest from these studies averaged 0.25 lbs  $P_2O_5$  per bushel of grain (Table 8). The amount of K removed was not related to K fertilizer or grain yields. Based on these data, an average corn yield of 200 bu./ac would remove approximately 50 lbs  $K_2O/ac$ , which is similar to previously reported data (International Plant Nutrition Institute, 2014).

## **Practical Applications**

Phosphorus fertilizer was required on 4 of 19 site-years while K fertilizer was required on 5 of 19 site-years. Although the response to P was generally on the soils with soil-test P in the low and very-low range, not all site-years with low or very low soil-test P were responsive to P fertilizer. The consistent factor was that soil pH was greater than 6.5 on the responsive site-years and less than 6.5 on the nonresponsive site-years.

The need for adequate K by corn is apparent from the low yields in the unfertilized controls in the responsive sites. Yield loss from inadequate K approached 40% in these studies, indicating the tremendous importance of K on corn yields. In contrast, the impact of insufficient P only ranged from 10% to 20%. Therefore, potential yield impact of inadequate K nutrition justifies the current K fertilizer recommendations.

Phosphorus and potassium are essential nutrients for corn production and can be limited in available forms in the soil enough to reduce yields. This data provides additional support for P and K fertilizer recommendations based on soil testing. The limited response to P fertilizer, except in fields with high soil pH, suggests that current fertilizer recommendations may need to be adjusted downward, particularly in the medium and low ranges. However, it may be necessary to evaluate soil pH and soil-test P to make fertilizer recommendations. More research is needed to further delineate the impact of soil pH on the P fertilizer response by corn.

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				Previous	Row	Plant	Harvest
Year	Location <sup>a</sup>	Hybrid	Soil Series	Crop	Width	Date	Date
					(in.)		
2017	Arkansas71	Armor 1550	Dewitt silt loam	Soybean	30	12-Apr	23-Aug
2017	Arkansas73	Armor 1550	Tichnor silt loam	Soybean	30	12-Apr	24-Aug
2017	Clay71	Croplan 6640	Crowley silt loam	Corn	30	8-Apr	7-Sep
2017	Clay73	Dekalb 66-87	Falaya silt loam	Soybean	30	13-Apr	6-Sep
2017	Lee71 <sup>b</sup>	Croplan 6274	Calloway silt loam	Grain Sorghum	38	3-May	21-Aug
2017	Mississippi71	Armor 1500	Sharkey silty clay	Soybean	38	17-Apr	11-Sep
2017	Prairie71	Croplan 6274	Calloway silt loam	Corn	30	9-Apr	21-Aug
2017	St.Francis71°	Croplan 6274	Calhoun silt loam	Corn	30	10-May	25-Aug
2018	Lee81 <sup>b</sup>	Croplan 6265SS	Convent Silt Loam,	Soybean	38	4-May	9-Sep
2018	Lee83 <sup>b</sup>	Croplan 6265SS	Convent Silt Loam,	Cotton	38	4-May	9-Sep
2018	Lonoke81	AgriGold 6659	Immanuel silt loam	Soybean	30	18-Apr	11-Sep
2018	St.Francis81°	Dyna-Gro D5751	Calhoun Silt Loam	Corn	30	4-May	11-Aug
2019	Cross91	Dekalb 64-32	Collins Silt Loam	Soybean	30	11-Apr	6-Sep
2019	Lee91 <sup>b</sup>	Pioneer P1197YHR	Memphis Silt Loam	Cotton	38	5-May	17-Sep
2019	Lonoke91	Dekalb 62-06	Stuttgart Silt Loam	Soybean	30	16-May	4-Sep
2019	Mississippi91	Dekalb 68-69	Foley-Calhoun-Bonn	Soybean	38	3-May	10-Sep
2019	St.Francis91°	Terral 28BHR18	Calloway silt loam	Corn	30	17-May	17-Sep
2019	St.Francis93°	Terral 28BHR18	Calloway silt loam	Soybean	30	17-May	17-Sep
2019	St.Francis95°	Terral 28BHR18	Calhoun silt loam	Corn	30	17-May	13-Sep

Table 1. Agronomic data for phosphorus fertilizer rate studies conducted between 2017 and 2019.

<sup>a</sup> Location designated by county, last digit of year, and test number in each county.

<sup>b</sup> University of Arkansas System Division of Agriculture's Lon Mann Cotton Research Station, Marianna.

<sup>c</sup> University of Arkansas System Division of Agriculture's Pine Tree Research Station, Colt.

Year	Location <sup>a</sup>	Hybrid	Soil Series	Previous Crop	Row Width	Plant Date	Harvest Date
_		-			(in.)		
2017	Arkansas72	Armor 1550	Dewitt silt loam	Soybean	30	12-Apr	23-Aug
2017	Arkansas74	Armor 1550	Tichnor silt loam	Soybean	30	12-Apr	24-Aug
2017	Clay72	Croplan 6640	Crowley silt loam	Corn	30	8-Apr	7-Sep
2017	Clay74	Dekalb 66-87	Falaya silt loam	Soybean	30	13-Apr	6-Sep
2017	Lee72 <sup>b</sup>	Croplan 6274	Calloway silt loam	Grain Sorghum	38	3-May	21-Aug
2017	Mississippi72	Armor 1500	Sharkey silty clay	Soybean	38	17-Apr	11-Sep
2017	Prairie72	Croplan 6274	Calloway silt loam	Corn	30	9-Apr	21-Aug
2017	St.Francis72 <sup>c</sup>	Croplan 6274	Calhoun silt loam	Corn	30	10-May	25-Aug
2018	Cross82	AgVenture 8714	Henry silt loam	Soybean	38	1-May	19-Sep
2018	Clay82	Pioneer 1197	Beulah Fine Sandy	Soybean	30	11-Aril	9-Sep
2018	Lee82 <sup>b</sup>	Croplan 6265SS	Memphis Silt Loam	Cotton	38	4-May	9-Sep
2018	Lonoke82	AgriGold 6659	Immanuel silt loam	Soybean	30	18-Apr	11-Sep
2019	Cross92	Dekalb 64-32	Collins Silt Loam	Soybean	30	11-Apr	6-Sep
2019	Lee92 <sup>b</sup>	Pioneer P1197YHR	Convent Silt Loam	Cotton	38	5-May	17-Sep
2019	Lee94 <sup>b</sup>	Pioneer P1197YHR	Convent Silt Loam	Soybean	38	5-May	17-Sep
2019	Lonoke92	Dekalb 62-06	Stuttgart Silt Loam	Soybean	30	16-May	4-Sep
2019	Mississippi92	Dekalb 68-69	Foley-Calhoun-Bonn	Soybean	38	3-May	10-Sep
2019	St.Francis92°	Terral 28BHR18	Calloway silt loam	Corn	30	17-May	13-Sep
2019	St.Francis94°	Terral 28BHR18	Calloway silt loam	Soybean	30	17-May	13-Sep

#### Table 2. Agronomic data for potassium fertilizer rate studies conducted between 2017 and 2019.

<sup>a</sup> Location designated by county, last digit of year, and test number in each county.

<sup>b</sup> University of Arkansas System Division of Agriculture's Lon Mann Cotton Research Station, Marianna.

<sup>c</sup> University of Arkansas System Division of Agriculture's Pine Tree Research Station, Colt.

Table 3	Table 3. Soil test data for P fertilizer rate studies conducted between 2017 and 2019.							
Year	Location	Soil pH	Р	K	Са	Mg	Cu	Zn
						opm		
2017	Arkansas71	6.7	22	75	1566	212	2.6	4.5
2017	Arkansas73	6.5	32	65	1955	284	2.3	4.1
2017	Clay71	6.6	44	89	1188	133	2.6	13.9
2017	Clay73	5.5	20	77	838	220	2.0	2.3
2017	Lee71	5.9	13	68	812	228	2.1	4.9
2017	Mississippi71	6.4	54	251	2645	560	4.2	4.3
2017	Prairie71	6.9	27	76	1135	145	1.7	4.4
2017	St.Francis71	6.3	30	118	1285	206	2.1	10.8
2018	Lee81	6.6	26	69	1129	304	2.0	2.1
2018	Lee83	7.2	43	102	1287	325	2.3	1.3
2018	Lonoke81	6.4	29	81	865	108	1.5	1.9
2018	St.Francis81	6.3	22	88	1592	303	2.0	11.2
2019	Cross91	6.3	30	131	810	134	1.7	7.1
2019	Lee91	7.3	54	127	1255	365	1.7	1.6
2019	Lonoke91	6.1	13	68	866	121	1.3	1.0
2019	Mississippi91	6.9	25	66	1401	162	1.5	2.3
2019	St.Francis91	6.9	12	65	1264	216	1.3	1.7
2019	St.Francis93	7.2	15	111	1292	319	1.7	1.3
2019	St.Francis95	6.9	10	65	1620	301	1.4	2.5

Table 4. Soil test data for K fertilizer rate studies conducted between 2017 and 2019.

Year	Location	Soil pH	Р	K	Ca	Mg	Cu	Zn
					pp	om		
2017	Arkansas72	6.3	25	60	1985	288	2.3	4.0
2017	Arkansas74	6.9	20	70	1516	212	2.3	3.5
2017	Clay72	6.4	40	101	1133	136	1.9	14.0
2017	Clay74	5.8	17	72	765	219	2.0	1.9
2017	Lee72	5.8	18	74	861	231	2.3	5.1
2017	Mississippi72	6.4	51	305	3186	697	4.3	4.1
2017	Prairie72	7.0	15	66	1232	151	1.7	3.2
2017	St.Francis72	7.0	25	107	1276	222	1.9	1.9
2018	Cross82	7.2	55	64	1197	270	1.8	2.2
2018	Clay82	6.8	59	66	594	97	2.4	3.8
2018	Lee82	7.2	42	113	1295	340	2.2	1.3
2018	Lonoke82	6.4	20	78	828	107	1.6	1.5
2019	Cross92	7.2	32	109	812	133	1.7	6.6
2019	Lee92	6.4	19	64	1069	246	1.4	1.7
2019	Lee94	6.1	24	97	1057	415	1.8	1.1
2019	Lonoke92	6.0	7	71	966	143	1.1	1.0
2019	Mississippi92	7.1	34	90	1440	162	1.8	2.5
2019	St.Francis92	7.1	25	64	1373	238	1.6	4.7
2019	St.Francis94	6.9	20	72	1276	235	1.1	5.9

				Grain Yield			
			P Fertilize	er Applied (Ib	P₂O₅/acre)		_
Year	Location	0	40	80	120	160	<i>P</i> > F
				bu./ac			
2017	Arkansas71	221	211	239	203	213	0.0303
2017	Arkansas73	201	215	227	213	212	0.4643
2017	Clay71	249	265	214	223	240	0.1649
2017	Clay73	210	230	213	226	230	0.3112
2017	Lee71	136	138	140	139	134	0.951
2017	Mississippi71	254	256	262	233	251	0.1247
2017	Prairie71	152	157	187	187	180	0.0178
2017	St.Francis71	178	188	178	180	182	0.8994
2018	Lee81	150	163	173	175	159	0.0959
2018	Lee83	122	136	124	134	139	0.1494
2018	Lonoke81	196	212	229	211	209	0.2659
2018	St.Francis81	129	145	151	131	140	0.7521
2019	Cross91	280	293	270	290	285	0.41
2019	Lee91	123	119	121	125	120	0.95
2019	Lonoke91	204	207	200	206	210	0.76
2019	Mississippi91	185	211	201	194	212	0.13
2019	St.Francis91	176	177	181	199	195	0.26
2019	St.Francis93	182	191	192	205	221	0.0045
2019	St.Francis95	140	167	167	177	167	0.01

# Table 5. Corn grain yield response from varying rates of phosphorus fertilizer at multiple locations in studies conducted between 2017 and 2019.

 Table 6. Phosphorus removal in corn grain in P rate studies conducted during 2017 and 2018.

 Corn Grain Phosphorus Content

		com Grain Phosphorus coment								
		P Fertilizer Applied (lb P2O5/ac)								
Year	Location	0	40	80	120	160				
				Ib P2O5/bu						
2017	Arkansas71	0.39	0.351	0.351	0.426	0.359				
2017	Arkansas73	0.369	0.374	0.408	0.39	0.428				
2017	Clay71	0.317	0.321	0.346	0.414	0.356				
2017	Clay75	0.390	0.369	0.398	0.405	0.403				
2017	Prairie71	0.385	0.405	0.421	0.372	0.367				
2018	Lee81	0.398	0.380	0.403	0.398	0.395				
2018	Lee85	0.415	0.413	0.403	0.390	0.400				
2018	Lonoke81	0.323	0.313	0.321	0.333	0.303				
2018	St.Francis81	0.398	0.482	0.407	0.393	0.402				

				Grain Yield			
			K Fertiliz	zer Applied (It	o K <sub>2</sub> O/ac)		_
Year	Location	0	50	100	150	200	<i>P</i> > F
				bu./ac			
2017	Arkansas72	143	218	234	225	226	0.0089
2017	Arkansas74	187	190	207	206	197	0.536
2017	Clay72	234	237	248	230	234	0.9632
2017	Clay74	226	229	211	221	231	0.5019
2017	Lee72	144	149	144	143	147	0.7794
2017	Mississippi72	258	249	248	244	250	0.4705
2017	Prairie72	173	189	189	175	173	0.4759
2017	St.Francis72	148	174	173	176	192	0.1779
2018	Cross82	110	149	160		174	0.0593
2018	Clay82	141	192	188	170	183	0.0054
2018	Lee82	115	113	115	117	111	0.8626
2018	Lonoke82	178	197	198	182	196	0.3758
2019	Cross92	261	278	254	261	264	0.58
2019	Lee92	98	117	126	127	129	0.003
2019	Lee94	154	147	148	146	148	0.55
2019	Lonoke92	164	185	197	196	191	0.09
2019	Mississippi92	211	213	214	215	227	0.09
2019	St.Francis92	140	149	173	185	176	0.009
2019	St.Francis94	120	138	189	190	155	<0.0001

Table 7. Corn grain yield response from varying rates of potassium fertilizer at
multiple locations in studies conducted between 2017 and 2019.

Table 8. Potassium removal in corn grain in K rate studies conducted during 2017 and 2018.
Corn Grain Potassium Content

		K Fertilizer Applied (lb K <sub>2</sub> O/ac)									
Year	Location	0 50 100 150 200									
				Ib K <sub>2</sub> O/bu							
2017	Arkansas72	0.222	0.218	0.231	0.23	0.238					
2017	Clay72	0.242	0.270	0.274	0.254	0.274					
2017	Prairie72	0.231	0.225	0.228	0.242	0.247					
2017	St.Francis72	0.242	0.273	0.257	0.249	0.257					
2018	Cross82	0.286	0.288	0.308	0.285	0.278					
2018	Clay82	0.261	0.274	0.273	0.246	0.27					
2018	Lonoke82	0.223	0.212	0.223	0.235	0.226					

# Drying and Fungal Deactivation of Corn Using Infrared Heating Technology

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# Abstract

Infrared (IR) drying of corn has become more widely investigated as an alternative drying method with advantages of energy efficiency and fungal deactivation. However, there is need to scale up the technology to achieve high-throughput (HT) drying suitable for industrial applications. This study evaluates the effectiveness of a continuous flow IR heating system to simultaneously dry and decontaminate corn over various drying bed thicknesses (0.6, 1.1, and 1.8 in.). These bed thicknesses and a conveyor speed of 0.377 ft/s corresponded with a drying throughput of 635, 1058, and 2116 lb/h. Additionally, the effect of varying the IR emitters' angles (30 (E-30) and 0 (E-0) degrees) on the effectiveness of corn drying and decontamination was examined. Although IR heating was able to dry and decontaminate corn at the initial moisture content (MC) of  $\approx$ 21% wet basis (w.b.) at all drying bed thicknesses, moisture removal was most effective at the least bed thickness (0.6 in.). At 0.6-in. bed thickness plus tempering (holding for 24 h between 122 and 140 °F) resulted in a total fungal count (TFC) reduction of 3.1 and 4.6 log (CFU/g) using IR emitters at 30 (E-30) and zero (E-0) degree angles, respectively. However, increasing the bed thickness to 1.1 in. resulted in a TFC reduction of 4.8 and 4.6 log (CFU/g) using E-30 and E-0, respectively. These results could help guide the design of HT corn drying and decontamination systems.

## Introduction

A significant amount of corn is produced in the southern end of the Corn Belt, mid-southern U.S.; in this region, mostly, in-bin drying of harvested corn is accomplished by supplying natural convection-heated air (Wilson et al., 2017a). Natural air-drying systems implement one or more fans to mechanically propel dry air from the bottom to the top of the corn bin. However, this system has drawbacks, such as longer drying durations, weather dependency, and non-uniform drying patterns. Longer durations and incomplete drying subject the corn to prolonged storage at the high moisture content (MC), resulting in increased fungal growth and reduced corn quality (Mohammadi Shad et al., 2019).

Infrared (IR) heating has been proposed as an alternative means to not only dry grain to a safe storage MC, but also inactivate microorganisms (Wang et al., 2014); IR heat causes thermal denaturation of proteins and nucleic acids in microorganisms and thereby deactivates the microbes (Hamanaka et al., 2011). Also, Wang et al. (2014) found that using IR heating to decontaminate rice grains from *Aspergillus flavus* resulted in a shorter heating duration. Furthermore, studies have shown that IR heating with subsequent tempering treatment increased fungal inactivation and moisture removal from grains (Pan et al., 2008; Wilson et al., 2017a).

The objectives of this study were (1) to evaluate the effectiveness of IR heating, including tempering, in reducing corn MC and total fungal count (TFC) during high-throughput drying, and (2) to evaluate and compare the effects of using

IR emitters at 30 (E-30) and zero (E-0) degrees angle on corn MC and fungal load reductions during high-throughput drying.

#### Procedures

### **Corn Samples**

Freshly harvested corn at initial MC ranging from 21% to 23% wet basis (w.b.) was obtained from the University of Arkansas System Division of Agriculture's Milo J. Shult Agricultural Research and Extension Center for this study. The corn was cleaned and stored at 39.2 °F until used. All samples were equilibrated at 77 °F for 24 h before used.

#### **Infrared Heating Equipment**

The pilot-scale IR system previously described by Wilson et al. (2015; 2017b) was customized to mimic corn drying in an industrial setup. The customized IR system allows variable parameters such as IR emitters' angle of inclination (Fig. 1).

#### **Experimental Design**

The IR heating system parameters used in this study are shown in Table 1. The IR heating chamber was filled with corn on three different bed thicknesses (0.6, 1.1, and 1.8 in.). As shown in Fig. 2, the intermittent IR heating (using E-0 and E-30) for each bed thickness was performed for 15 passes. Then samples were transferred to sealed containers and tempered at

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temperatures that varied between 122 °F and 140 °F. After 24 h, samples were then dried using IR heating for an additional 15 passes. The corn MCs were measured for the control after the first 15 IR heating passes before tempering (P15BT); after the tempering (AT) step; and after the 15 additional IR heating passes (P15AT). For TFC analysis, samples were taken in the following sequence: at the control condition, after the first five (P5), second five (P10), and third five (P15) IR heating passes, AT step, and fourth five (20), fifth five (P25), and sixth five (P30) IR heating passes. Control samples received neither IR heat treatment nor tempering.

#### Measurements of Corn Moisture Content

The MCs of corn samples were determined by using a calibrated AM 5200 Grain Moisture Tester (PERTEN Instruments, Hagerstown, Sweden); and sample procedures followed standards established by the American Society of Agricultural Engineers (ASAE) S352.2. Corn MC measurements were done in triplicate. The difference between the initial MC (control) and the MC after treatments was calculated and expressed as the percentage point of moisture removal.

#### **Total Fungal Count Analysis**

At each fungal sampling and treatment specified in the experiments, 2 samples of corn were taken. The standard procedure of the Association of Official Analytical Chemists International (AOAC, 2002) was used for determining the TFC of samples. The fungal count plates were placed in an incubator set at 77  $^{\circ}$ F for 120 h.

#### **Statistical Analysis**

A statistical software, JMP Pro v. 14.0 (SAS Institute, Inc., Cary, N.C.) was used to carry out analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) test for comparing more than two means. Also, 2-sided Dunnett's test was done for multiple comparisons of means. Statistical results were considered to be significant when P < 0.05.

#### **Results and Discussion**

# Treatments Using IR Emitters Inclined at Thirty-Degree Angles

The changes in MC of treated corn samples at different IR drying steps are shown in Table 2. The differences in percentage points of moisture removed amongst bed thicknesses were significant (P < 0.05); after P30, a total of 8.2, 5.6, and 3.0 percentage points of moisture were removed at bed thicknesses of 0.6, 1.1, and 1.8 in., respectively. After P15BT, only 4.5, 3.0, and 1.8 percentage points of moisture were removed at 0.6-, 1.1-, and 1.8-in. drying bed thicknesses, respectively. Corn dried at 0.6-in. bed thickness with tempering could meet safe storage MC (<14%). Wilson et al. (2015) showed that IR heating removed 8.5 and 4.8 percentage points MC from corn at an initial MC of 28% and 20%, respectively. Also, Khir et al. (2011) found that the IR drying of single-layered rice resulted in a higher MC reduction than that of thick-layered rice.

After P30 including tempering (i.e., P15BT – T – P15AT), TFC reductions of 3.1, 4.8, and 3.9 log (CFU/g) for 0.6-, 1.1-, and 1.8-in. bed thicknesses, respectively, were achieved (Fig. 3). The TFC of corn treated at bed thicknesses of 0.6 and 1.1 in. were not significantly different, but significantly different from bed thicknesses of 1.8 in., after P30. According to Dunnett's test, the TFC of corn after P10 was significantly different than the initial TFC for 0.6- and 1.1-in. bed thickness (Fig. 4). In line with this study, IR heating of corn followed by tempering at 122 °F for 4 h significantly reduced initial TFC by 3.8, 3.8 and 4.5 log (CFU/g) for corn treated at initial MC of 20%, 24%, and 28%, respectively (Wilson et al., 2017b).

# Treatments Using IR Emitters at Zerodegree Angle

After P15BT, the corn MC decreased by 4.9, 2.4, and 2.0 percentage points from initial MC, and then the MC decreased by another 4.4, 3.5, and 2.2 percentage points following P15AT for bed thicknesses of 0.6, 1.1, and 1.8 in., respectively. The most considerable reduction in corn MC was observed at a bed thickness of 0.6 in., while the least reduction in corn MC was observed at a bed thickness of 1.8 in. (Table 3).

Infrared heating using E-0, compared to using E-30, accelerated TFC reduction when corn samples were dried at 0.6in. bed thickness (Fig. 4). According to Dunnett's test, TFC for 0.6-in. bed thickness was significantly reduced from the initial count after P5 (2.5 minutes of heating). However, for 1.1-in. bed thickness, it took P15 to achieve a significant reduction in the initial TFC. For 1.8-in. bed thickness, significant TFC reduction was achieved only after P15AT.

# Comparison of Treatments Using IR Emitters at Thirty- and Zero-Degree Angles

Table 4 summarizes the effects of using E-0 and E-30 at the three bed thicknesses on corn MC removal and TFC reduction. Fifteen minutes of IR heating with intermediate tempering resulted in TFC reductions of 4.8 and 4.6 log (CFU/g) with E-30 and E-0, respectively, for 1.1-in. bed thickness; and a reduction of 4.6 log (CFU/g) with E-0 for 0.6-in. bed thickness. In agreement with this study, IR heating, including tempering, of rice resulted in a significant TFC reduction of  $3.11 \log (CFU/g)$ (Oduola et al., 2020). The TFC reduction after P30 (including tempering) using E-0 was the same for 0.6- and 1.1-in. bed thicknesses (4.6 log (CFU/g)), and both were significantly greater than TFC reduction for 1.8-in. bed thickness. While TFC reduction was not different between both emitters' angles for 1.1- and 1.8-in. thicknesses, the E-0 was more effective for 0.6-in. bed thickness compared to E-30. The greatest moisture removal was observed at 0.6-in. bed thickness for both emitters' angles.

# **Practical Applications**

This study provides insight into the use of IR heating of corn under conditions that are scalable to achieve a commercial drying process. Infrared heating significantly reduced the TFC and MC of corn. Larger scale IR equipment can be designed using the same IR parameters as in this study; the scaled-up IR equipment will allow industries and farmers to prevent spoilage of corn due to high MC and fungal growth. However, the efficiency of the IR heating technology can be increased by optimizing the IR heating process, including adding a vibrator to increase grain exposure time to IR heat. Hence, food security and safety will be improved, and profit will be maximized.

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Parameters	IR emitters i degree ang	nclined at 30- le, Tempered	IR emitters at 0-degree angle, Tempered		
Conveyor belt speed (ft/s)	0.377		0.377		
Intermittent IR drying duration (s)	30		30		
IR intensity [BTU/(h.m <sup>2</sup> )]	1.89 × 10-4	1	1.89 × 10 <sup>-4</sup>		
Product-to-emitter gap size (in.)	17		17		
Thickness (in.) [Feeding rate (lb/h)]	0.6	[635]	0.6	[635]	
	1.1	[1058]	1.1	[1058]	
	1.8	[2116]	1.8	[2116]	
Tempering	Yes		Yes		
IR emitters' angle (degrees)	30		Zero		

Table 1. Experimental	design for th	e pilot-scale infrared	d (IR) heating.
			· ( ) ··· J

Drying bed thickness (in.)	Initial MC	MC at tempering	MC after tempering	Final MC	Total drying duration
	(%)	(%)	(%)	(%)	(min)
0.6	21.2	16.7	17.4	13.0	15
1.1	20.9	17.9	19.0	15.3	15
1.8	20.9	19.1	20.0	17.9	15

Table 2. Corn moisture contents (MC) at initial, after 15 infrared (IR) passes (at tempering), after tempering, and after last 15 IR passes (final) of high-throughput drying with three bed thicknesses (IR emitters are at 30-degree angles).

Table 3. Corn moisture content (MC) at initial, after 15 infrared (IR) passes (at tempering), after tempering, and after last 15 IR passes (final) of high-throughput drying with three bed thicknesses (IR emitters are at 0-degree angle)

Drying bed thickness (in.)	Initial $\mathbf{MC}^{\dagger}$	MC at tempering	MC after tempering	Final MC <sup>‡</sup>	Total drying duration
	(%)	(%)	(%)	(%)	(min)
0.6	21.2	16.3	17.1	12.7	15
1.1	21.0	18.6	20.0	16.5	15
1.8	21.2	19.2	20.0	17.8	15

<sup>†</sup>15 IR heating passes were done to achieve MC from initial to tempering MC.

<sup>‡</sup>15 IR heating passes were done to achieve MC from time after tempering to the final MC.

Table 4. Tukey's honestly significant difference test for the effect of drying bed thickness and emitters status after infrared (IR) heating on the least square means of moisture removal and fungal count reduction expressed in log colony forming (CFU) per gram of corn sample.

Drying bed thickness (in.)	IR heating with emitters inclined at 30-degree angle	IR heating with emitters at 0- degree angle	IR heating emitters inclined at 30-degree angle	IR heating with emitters at 0-degree angle
	Moisture remova	al (% point)†	Points of total fungal red	uction (log (CFU.g <sup>-1</sup> ) <sup>†</sup>
0.6	8.2 aA	8.6 aA	3.1 bB	4.6 aA
1.1	5.6 aB	4.5 bB	4.8 aA	4.6 aA
1.8	3.0 aC	3.5 aB	3.9 aAB	3.9 aB

<sup>†</sup> Different lowercase letters mean a significant difference in a row; different uppercase letters indicate significant difference in the same column.



Emitter at 0-degrees angle

Emitter inclined at 30-degree angle

Fig. 1. Schematic image of catalytic infrared emitter including the heating element, catalyst, and insulation.



Fig. 2. Flow chart of the infrared (IR) heating experiments.



Fig. 3. The effect of infrared (IR) heating with emitters inclined at 30 degrees followed by tempering on the total fungal count [in terms of log colony forming units (CFU) per gram] of corn samples dried at bed thicknesses of 0.6, 1.1, and 1.8 in. after every five passes of drying, including tempering. \*Mean passes after tempering [Total fungal count for different steps (IR passes and tempering) for each thickness having different capital letters were significantly different at P < 0.05].



Fig. 4. The effect of infrared heating with emitters at zero degrees followed by tempering on the total fungal count (in terms of log colony forming units (CFU) per gram) of corn samples dried at bed thicknesses of 0.6, 1.1, and 1.8 in., respectively, after every five passes of drying and tempering. \*Mean passes after tempering [Total fungal count for different steps (IR passes and tempering) for each thickness having different capital letters were significantly different at *P* < 0.05].</p>

# Deterrence of Aspergillus flavus Regrowth and Aflatoxin Accumulation on Shelled Corn Using Infrared Heat Treatments

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# Abstract

The objectives of this study were to determine the suitable combinations of infrared (IR) heating duration and intensity, followed by tempering treatments to maximize the deactivation of aflatoxin-producing mold spores, specifically *Asper-gillus flavus* (*A. flavus*). Corn samples at moisture content of 24% wet basis were inoculated with spore suspension of *A. flavus* and incubated to allow microbial attachment on the kernels. Corn samples were then heated using IR energy and then tempered for 4 h. Following the treatments, the samples were placed in conditions favorable for mold regrowth. Treatments of non-tempered samples for 210 s at the lowest intensity  $[4.33 \times 10^3 \text{ BTU/(h.m^2)}]$  resulted in *A. flavus* load reductions of 5.9 log CFU/g. Treatments of non-tempered samples at the medium  $[1.11 \times 10^4 \text{ BTU/(h.m^2)}]$  and highest intensity  $[2.35 \times 10^4 \text{ BTU/(h.m^2)}]$  for 210 s resulted in complete deactivation of *A. flavus*. No fungal regrowth or aflatoxin persistence was observed on samples treated for 210 s at the lowest, medium, and highest IR intensities.

#### Introduction

Contamination of corn with mycotoxin-producing mold spores such as Aspergillus flavus (A. flavus) is a persistent problem in the southern states (Williams et al., 2008). As a result, the development of effective drying and fungal deactivation strategies to maintain grain quality and prevent the growth of mycotoxin-producing fungi has become a priority for the grain industry (Mohammadi Shad et al., 2019b). This research explored novel interventions using infrared (IR) heating to deter A. flavus contamination and regrowth, and aflatoxin accumulation on shelled corn. The IR heating or drying involves a heat transfer by radiation between a hot element and a material at a lower temperature that needs to be heated or dried. The advent of catalytic type of infrared (CIR) emitters that maximize heating of water in food materials by producing IR energy at peak wavelength offers new avenues for industrializing the IR heating technology for drying and decontamination of corn. Infrared heating, compared to conventional convective air heating, has merits of high heat delivery and rapid product surface heating characteristics. Also, the energy fluxes associated with IR heating may simultaneously dry corn and inactivate harmful mold spores while maintaining the corn quality (Wilson, 2016).

The objective of this study was to investigate the feasibility of utilizing a lab-assembled IR dryer equipped with catalytic infrared (CIR) emitters to heat high moisture content (MC) corn kernels followed by tempering treatments (holding the sample temperature at 158 °F for 4 h) to decontaminate the kernels by deactivating fungal growth of the heat-tolerant *A. flavus* and to detoxify aflatoxins on the grain during storage. Specifically, the study sought to determine the impacts of processing variables such as IR heating duration, IR intensity, and tempering on *A. flavus* deactivation and aflatoxin detoxification.

#### Procedures

Corn (Pioneer hybrid PI 1319 YHR/PI 2088) samples were harvested with initial moisture content (IMC) of  $24 \pm$ 0.6% (w.b.) in a commercial producer's field in Northeastern Arkansas; all mentioned MC values are reported as wet basis (w.b.), unless stated otherwise. The IMCs of corn samples were determined using an AM 5200 Grain Moisture Tester (PERTEN Instruments, Hägersten, Sweden). After removing all foreign materials, the cleaned corn samples were immediately stored in a laboratory cold room set at 39.2 °F until the next experimental steps could proceed.

# Aspergillus flavus Spore Inoculum Propagation

Freeze-dried spores of *A. flavus* (strain ATCC 28539TM, American Type Culture Collection, Manassas, Va.) were procured in vials. Contents of each vial were mixed with 1.0 mL of sterile water, then transferred to a test tube containing 6 mL of sterile water. *A. flavus* was allowed to rehydrate for 3 h and cultured on potato dextrose agar. After 7 days of incubation at 77 °F, spores were detached by flooding the culture plates with 0.03% Tween 80 (Wilson, 2019). The initial volume with detached spores was designated as the initial inoculum concentration.

#### **Corn Inoculation**

A 1.1-lb corn sample was inoculated with the original inoculum of detached *A. flavus* spore suspension in a sterilized Erlenmeyer flask. The flasks were then covered with aluminum foil and kept in an incubator at 95 °F for 5 days. Uniform fungal attachment to corn kernels was obtained by manually shak-

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ing the flasks containing *A. flavus* spore suspension and corn samples every 12 h (Wilson, 2019). The pre-experiment initial least square mean of the *A. flavus* and aflatoxin concentration for the initial inoculum volume with detached spores was  $6.59 \pm 0.38 \log (CFU/g)$  and  $11.44 \pm 1.77$  ppb, respectively. Inoculated samples were kept in a lab freezer for storage and retrieved 4 h before experiments for equilibration with ambient conditions.

#### **Infrared Treatments**

A catalytic IR dryer (Catalytic Drying Technologies LLC, Independence, Kan.) was used to dry corn (Fig. 1). Corn samples were dried using IR heating in 3 replicates at 6 different treatment durations. The lab-assembled IR dryer was equipped with a CIR emitter, which generated IR radiant energy through a catalytic reaction. The principle of the IR dryer is that the air across a platinum sheet embedded in the emitter assembly when combined with propane gas reacts by oxidation-reduction to yield IR energy, as well as small amounts of carbon dioxide and water vapor (Pan et al., 2008, 2011; Khir et al., 2011, 2012). The equipment had an effective heating area of 109.56 in.<sup>2</sup> (a circular emitter with a diameter of 11.8 in.). A radiometer was used to determine energy transfer (IR intensity) from the IR energy source to the product. For each treatment, 0.44 lb of inoculated corn samples were placed in a thin layer on the IR equipment's stage then treated at each IR intensity. Infrared intensities were increased by manually decreasing the gap distance between the product and emitter. The examined IR intensities (corresponding product-to-emitter gap distances) and treatment durations in the current study are indicated in Table 1. To determine the effect of adding a tempering step following treatments with IR, some IR-treated corn samples were tempered in sealed containers, holding the sample temperature at 158 °F for 4 h, without any extra heating and moisture removal.

## Aspergillus flavus Culturing

A 0.022-lb sample of corn was mixed with 90 mL sterile phosphate-buffered dilution water in a sterile stomacher bag. Then, it was masticated using a lab masticator (Silver Panoramic, iUL, S.A., Barcelona, Spain) set at 240 s and 0.5 strokes/s to be completely pulverized for total fungal load analyses. The homogenized sample was serially diluted using 9-mL sterile dilution buffer for the analysis (Mohammadi Shad et al., 2019a).

## Dichloran Supplemented Rose Bengal Agar

Rose Bengal Agar (RBA) is a selective medium used to detect and enumerate yeasts and molds in food samples. RBA base was liquefied in 500-mL bottles by autoclaving at 249.8 °F for 15 min. The medium was then allowed to cool to 113–122 °F then supplemented with 0.5 mL of dichloran, an inhibitor of mold spreading in fungal plating media. Then 1.5 mL of a stock solution of 2-parts streptomycin and 1-part chlortetracycline was added, after which it was poured into sterile Petri dishes and allowed to solidify. After the medium is cooled, 0.1-mL aliquots of sample solution were spread on the Petri plates using bent glass rods. The RBA plates were incubated (Thelco Model 4, Precision Scientific Instruments, Inc., Chicago, Ill.) at 77 °F for 120 h before counting. After incubation, the fungal Colony Forming Units (CFU) on each plate were counted (Wilson, 2019).

#### Aspergillus flavus Enumeration

The appropriate dilution factor, volume, and sample weight were considered to obtain the total CFU/g of each sample:

$$T_{cfu} = \frac{P_{cfu}}{D_r} \qquad \qquad \text{Eq. (1)}$$

Where,  $T_{cfu}$  = total colony forming units per gram of corn (CFU/g);  $P_{cfu}$  = colony forming units counted on plate per gram of corn (CFU/g);  $D_r$  = dilution factor (10<sup>-3</sup> to 10<sup>-6</sup> times).

#### Aflatoxin Measurement

Aflatoxin concentrations (ppb) were determined using a fluorometric test procedure (FluoroQuant Aflatoxin Test Kit, Romer Labs, Union, Mo.). This protocol called for 0.11 lb of corn sample to be blended in methanol: water (80:20) for 1 min then filtered using filter paper (Whatman number 1). Then, 1 mL of the filtrate and 1 mL of diluent were placed at the top of an extraction column. The resulting solution was mixed well by pipetting up and down two times. After, the column was placed in a cuvette. A plunger was placed on top of the column to push the extract through. Then, 0.5 mL of extracted sample, along with 1 mL of the developer was transferred to a clean cuvette. The cuvette was then capped, vortexed, and read using the FluoroQuant Aflatoxin Reader (FluoroQuant Aflatoxin Test Kit, Romer Labs, Union, Mo.).

#### **Fungal Regrowth**

To examine the fungal regrowth potential on treated corn samples, some batches of treated corn were placed in created favorable conditions for mold growth as following: the regrowth environment was created using saturated potassium chloride (KCl) solution, 0.13 lb of treated corn was placed in a 9.8  $\times$  9.8-in. square piece of cheese-cloth, then suspended in a jar above a salt solution of 0.18 lb KCl and 100 mL H<sub>2</sub>O, which creates 90% RH. Corn samples were incubated at 91.4 °F for 5 days. Subsequently, corn samples of 0.11 and 0.022 lb were taken out for aflatoxin testing and *A. flavus* enumeration, respectively. The same mentioned procedures were used for *A. flavus* and aflatoxin analyses.

#### **Statistical Analysis**

The experimental data were unbalanced with uneven observations per each factor level. For statistical analysis, a mixed model was applied using the software JMP Pro 14. Replication was assumed random, while intensity, treatment duration, and tempering were included as fixed effects. The data was run in the mixed model to produce a full factorial analysis of variance (ANOVA) using the default restricted maximum likelihood (REML) to evaluate the random covariance structure. The comparison of more than two fixed means was done using Tukey's honestly significant difference (HSD) test. All ANOVA F tests were considered to be significant when P < 0.05.

#### **Results and Discussion**

The pre-experiment initial LS means of the *A. flavus* and aflatoxin concentration for inoculated and non-treated samples (as control samples) were  $6.59 \pm 0.38 \log (CFU/g)$  and  $11.44 \pm 1.77$  ppb, respectively. Figure 2 shows the initial inoculum plated on RBA plates and subsequent dilutions. The *A. flavus* colonies were either yellow or green in color.

# Treatments vs. *Aspergillus flavus* and Aflatoxin Concentration

The impact of increasing infrared intensity and treatment duration on deactivation of A. flavus for non-tempered (a) and tempered (b) corn samples is located in Fig. 3. Table 2 shows a statistical analysis showing that the main effects of treatment duration, intensity, and tempering all had significant effects on the A. flavus concentration response. Additionally, the A. *flavus* concentration response was statistically impacted (P <0.05) by the two-way interactions of "treatment duration by tempering" and "intensity by tempering." Figure 4 shows the effect of increasing infrared intensity [BTU/(h.m<sup>2</sup>)] and treatment duration (s) on aflatoxin concentration of non-tempered (a) and tempered (b) corn samples. It should be noted that the fluorometric test procedure used (FluoroQuant Aflatoxin Test Kit, Romer Labs, Union, Mo.) has a limit of detection of 0.3 ppb and as a result 0.3 ppb is the lowest quantity of aflatoxin that can be distinguished from the absence of that substance (a blank value) with a confidence level of 99%. So, technically, the values of aflatoxin accumulation recorded after tempering the samples (Fig. 4b) are negligible. The aflatoxin response was statistically impacted by the effect of intensity and the two-way interaction of "intensity by tempering" (Table 3). For tempered corn samples, increasing IR intensity from  $4.33 \times 10^3$  to 1.11  $\times 10^4$  BTU/(h.m<sup>2</sup>) caused significant decreases in the load of A. flavus (P < 0.05). The A. flavus response decreased from  $1.72 \pm 0.14 \log (CFU/g)$  to  $0.42 \pm 0.2 \log (CFU/g)$ , as a result of increasing IR intensity from  $4.33 \times 10^3$  to  $1.11 \times 10^4$  BTU/  $(h.m^2)$  respectively. This same decreasing trend was seen for the aflatoxin concentration response as a result of increasing IR intensities. However, it was noted that for the non-tempered corn, no significant difference in the mold concentration occurred as a result of increasing IR intensity levels. The highest levels of aflatoxins were seen in IR-treated corn at the lowest intensity of  $[4.33 \times 10^3 \text{ BTU/(h.m^2)}]$ , followed by no tempering. This same trend was seen for A. flavus concentrations for IR-treated corn at  $[4.33 \times 10^3 \text{ BTU/(h.m^2)}]$  in tempered corn samples  $(1.83 \pm 0.17 \log (CFU/g))$ . Overall, the least effective treatment in reducing A. flavus occurred for corn samples, which were IR-heated for only 30 s then tempered; the mean

A. *flavus* concentration for this condition was  $3.99 \pm 0.28 \log$  (CFU/g) which significantly differed from all other condition combinations (P < 0.0001).

# Treatments vs. Regrowth of Aspergillus flavus and Aflatoxin Concentration

In the regrowth study (Fig. 5), the main effects of intensity and tempering were significant (P < 0.05) on A. flavus concentration. Increasing the IR intensity from  $4.33 \times 10^3$  to  $2.35 \times 10^4$  BTU/(h.m<sup>2</sup>) for both tempered and non-tempered corn samples resulted in a statistically significant reduction in A. flavus as indicated by the effects comparison test (Table 4). In addition, a significant two-way interaction of "intensity by tempering" and "duration by tempering" were also found to be significant (P < 0.05). Corn samples heated at the lowest IR intensity  $[4.33 \times 10^3 \text{ BTU/(h.m^2)}]$  without tempering had a significantly higher LS mean (1.31 log (CFU/g) of A. flavus concentration than all other treatments. However, as indicated above, increasing treatment duration of IR treatment resulted in statistically non-significant effects on the A. flavus concentration. In Fig. 6, the Fig. 6b indicates that corn samples were detoxified at all intensities and heating durations because 0.3 ppb is the lowest quantity of aflatoxin that can be distinguished from the absence of aflatoxin with a confidence level of 99%. The results of the regrowth study showed that aflatoxin production did not occur significantly in IR-treated corn when placed in regrowth conditions (Table 5). Contrary to the results of IR heating, other heating techniques such as with convectively heated air has proven to be insufficient in stopping fungal regrowth in treated crops (Wilson, 2016).

Infrared heating was effective in deactivating *A. flavus* on corn; incorporating a tempering step increased the efficiency of IR heating in deactivating *A. flavus* on corn. In order to completely curb aflatoxin accumulation on corn, the effect of IR heating on other molds, such as *A. parasiticus* and *A. no-mius* that are capable of producing aflatoxin should be studied. Therefore, the results in this study will be regarded as preliminary. The studied treatments provide an avenue to implement a scalable non-chemical approach to deactivate fungi on corn and contribute to an agriculturally sustainable practice that is friendlier to humans and other life forms and the environment.

#### Acknowledgments

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Fig. 1. Block diagram of laboratory assembled infrared dryer consisting of pressure gauge, fuel line, infrared emitter, stage and radiant energy loss barrier or insulation (aluminum foil).



Fig. 2. Original inoculum (10<sup>-1</sup>) of Aspergillus flavus and subsequent dilutions (10<sup>-2</sup> and 10<sup>-3</sup>) on Rose Bengal Agar plates.



Fig. 3. The effect of increasing infrared intensity [BTU/(h.m<sup>2</sup>)] and treatment duration (s) on inactivation of *Aspergillus flavus* concentration (log (CFU/g)) for non-tempered (a) and tempered (b) corn samples.



Fig. 4. The effect of increasing infrared intensity [BTU/(h.m<sup>2</sup>)] and treatment duration (s) on aflatoxin concentration (ppb) of non-tempered (a) and tempered (b) corn samples.



Fig. 5. The effect of increasing infrared intensity [BTU/(h.m<sup>2</sup>)] and treatment duration (s) on *Aspergillus flavus* concentration (log (CFU/g)) for non-tempered (a) and tempered (b) corn samples after subjecting to conditions favorable for regrowth of the microbe.



Fig. 6. The effect of increasing infrared intensity and treatment duration (s) on aflatoxin concentration of non-tempered (a) and tempered (b) corn samples after being subjected to conditions favorable for *Aspergillus flavus* regrowth.

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Product-to-Emitter	Infrared Intensity	
Gap Distance		Infrared Heating Duration
(in.)	[BTU/(h.m²)]	(s)
		0
4.3	2.35 x 104	30
8.7	1.11 x 10 <sup>4</sup>	60
14.2	4.33 x 10 <sup>3</sup>	90
		120

 
 Table 1. Table showing product-to-emitter gap distances, infrared intensities and treatment durations.

 

 Table 2. Effect test table showing the effect of tempering, infrared intensity and heating duration on Aspergillus flavus concentration.

Source	Log Worth	Prob > F
Tempering	4.439	0.00004*
Intensity [BTU/(h.m <sup>2</sup> )]	3.143	0.00072*
Intensity [BTU/(h.m <sup>2</sup> )]*Tempering	3	0.001*
Duration	2.636	0.00231*
Duration*Tempering	2.503	0.00314*

Asterisks (\*) indicate high statistical significance (P < 0.05).

Source	Log Worth	Prob > F
Intensity [BTU/(h.m <sup>2</sup> )]	1.896	0.01271*
Intensity [BTU/(h.m <sup>2</sup> )]*Tempering	1.83	0.0148*
Duration	0.682	0.20795
Duration*Tempering	0.63	0.23461
Tempering	0.003	0.99327

 Table 3. Effect test table showing the effect of tempering, infrared intensity and heating duration on Aflatoxin concentration.

Asterisks (\*) indicate high statistical significance (P < 0.05).

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Source	Log Worth	Prob > F
Tempering	3.099	0.0008*
Intensity [BTU/(h.m <sup>2</sup> )]*Tempering	2.836	0.00146*
Intensity [BTU/(*h.m <sup>2</sup> )]	2.593	0.00255*
Duration	0.724	0.1888
Duration*Tempering	0.654	0.22186

Table 4. Effect test table showing the effect of tempering, infrared intensity and heating duration on *Aspergillus flavus* regrowth.

Asterisks (\*) indicate high statistical significance (P < 0.05).

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Table 5. Effect	test table sh	owing the e	effect of ter	npering,	infrared
intensit	y and heating	duration o	n aflatoxin	regrowth	n.

Source	Log Worth	Prob > F
Intensity [BTU/(h.m <sup>2</sup> )]	1.209	0.06179
Intensity [BTU/[h.m <sup>2</sup> )]*Tempering	1.186	0.06517
Duration	0.488	0.32504
Duration*Tempering	0.475	0.3352
Tempering	0.047	0.89827

Asterisks (\*) indicate high statistical significance (P < 0.05).

# Runoff Water Quality from Corn Production: A Summary of Results from the Arkansas Discovery Program

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#### Abstract

The overall goal of the Arkansas Discovery Farms program is to assess the need for and effectiveness of on-farm conservation practices, document nutrient and sediment loss reductions, soil health and water conservation in support of nutrient management planning and sound environmental farm stewardship. Using state-of-the-art, edge-of-field runoff monitoring on several commercial, row crop farms in Eastern Arkansas, 268 water samples were collected from 15 different fields during 2013 to 2019 representing 20 site years. Median values across all sites and years for nitrate + nitrite-N (NO<sub>3</sub>), total nitrogen (TN), soluble reactive phosphorus (SRP), and total phosphorus (TP) were 0.45, 1.57, 0.13, and 0.53 parts per million (ppm), respectively. These results indicate relatively low concentrations that are similar to median values from streams in agricultural watersheds across the country. This implies that corn producers that cooperated in this study closely and consistently matched fertilizer needs to crop needs, so that there were only small amounts of fertilizer nutrients (P and N) available to be transported via runoff from the field following application. Overall, Discovery Farm studies have indicated that less than 5% of N and P applied as fertilizer leaves the field in surface runoff.

#### Introduction

Row crop producers in the Lower Mississippi River Basin (LMRB) are under increased scrutiny to demonstrate that current production systems are environmentally viable with respect to water quality and sustainability (Daniels et al., 2018). These concerns are manifested from regional issues such as hypoxia in the Gulf of Mexico (USEPA, 2018a) and critical groundwater decline in the Lower Mississippi Alluvial Valley aquifer (LMAV; Reba et al., 2017; Czarnecki et al., 2018). Nutrient enrichment remains a major impairment of water quality to the designated uses of fresh and coastal waters of the U.S. (Schindler et al., 2008). Nutrient runoff from cropland is receiving greater attention as a major source of nutrients from nonpoint sources (Dubrovsky et al., 2010). This is especially true in the Mississippi River Basin (MRB), as recent model estimates suggest that up to 85% of the phosphorus (P) and nitrogen (N) entering the Gulf of Mexico originates from agriculture (Alexander et al., 2008). These estimates are based on large-scale modeling within the MRB, with limited localized calibration or verification of the field losses of P and N. Furthermore, there have been few farm-scale studies of P and N loss, particularly the LMAV region of agriculture-dominant Arkansas and Mississippi (Dale et al., 2010; Kröger et al., 2012).

This scrutiny has prompted much activity aimed at reducing nutrients lost to the Gulf within the Mississippi River Basin, including the formation of the Mississippi River/Gulf of Mexico Hypoxia Task Force, a consortium of Federal agencies and States (USEPA, 2018a). This consortium developed an action plan to reduce nutrients entering the Gulf, which includes nutrient reduction strategies prepared by each member State (USEPA, 2018b).

Arkansas Discovery Farms are privately owned farms that have volunteered to help with on-farm research, verification, and demonstration of farming's impact on the environment and natural resource sustainability (Sharpley et al., 2015, 2016). The overall goal of the program is to assess the need for and effectiveness of on-farm conservation practices, document nutrient and sediment loss reductions, and water conservation in support of nutrient management planning and sound environmental farm stewardship. Edge-of-field monitoring (EOFM) of runoff from individual agricultural fields is critical to improving our understanding of the fate and transport of nutrients applied as animal manures and fertilizer to agricultural lands along the complex watershed continuum (Reba et al., 2013; Harmel et al., 2016; Sharpley et al., 2016).

Additionally, EOFM helps producers more clearly see how their management systems affect in-stream water quality and watershed functions (Sharpley et al., 2015). The objective of this paper was to provide a summary of nutrient loss from corn production across all years, locations, and production practices to provided quantification of nutrient losses from corn production.

#### **Procedures**

Edge-of-field runoff monitoring stations were established on several commercial farms in Arkansas, Jefferson, Phillips,

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Pope, and St. Francis counties of eastern Arkansas. During 2013 to 2019, 268 water samples were collected from 15 different fields equipped with EOFM stations representing 20 site years.

At the lower end of each field, automated, runoff water quality monitoring stations were established to 1) measure runoff flow volume, 2) collect water quality samples of runoff for water quality analysis, and 3) measure precipitation. Either a 60°, V-shaped, 8-in. trapezoidal flume that was pre-calibrated and gauged was installed at the outlet of each field or if an existing drainage pipe served as the outlet, it was instrumented (TRACOM, Inc., Alpharetta, Georgia). The ISCO 6712, an automated portable water sampler (Teledyne-ISCO, Lincoln, Nebraska), was used to interface and integrate all the components of the flow station. Where flumes were used, an ISCO 720 pressure transducer and flow module was used. For existing drainage pipes, an ISCO 750 flow velocity and flow module was utilized. All samples were analyzed at the University of Arkansas System Division of Agriculture's Arkansas Water Resources Laboratory (Arkansas Water Resources Center, Fayetteville, Arkansas), an EPA-certified laboratory, for total nitrogen (TN), nitrate + nitrite-N (NO<sub>3</sub>), total phosphorus (TP) and soluble reactive phosphorus (SRP).

#### **Results and Discussion**

The summary of nutrient concentrations for  $NO_3^-$ , TN, SRP and TP across all years and locations greatly varied while median values were relatively low (Table 1). The data indicated highly skewed data as expected as it represents all sites and years and the associated management practices. For this reason, the median values of 0.45, 1.57, 0.13, and 0.53 parts per million (ppm) for NO<sub>3</sub>, TN, SRP, and TP, respectively were used to describe central tendency rather than the mean. To put these values in perspective, Dubrovsky (2010) reported median concentrations of 4 ppm and 0.24 ppm of TN and TP, respectively for samples collected from agricultural watersheds from all over the United States during 1993-2004 by the United States Geological Survey (USGS). The median of TN data collected in Arkansas was lower than the USGS stream data; however, the median TP data collected in Arkansas was slightly higher. However, runoff volume from an individual field may be much lower than the volume of water in a major stream or river.

Nutrient concentrations also varied at a given site by year (Figs. 1 and 2), depicting the effect that the varying nature of hydrological events can have on nutrient losses.

# **Practical Applications**

Data from EOFM can help provide perspective on agriculture's impact on water quality in terms of nutrient losses. Our data indicates relatively low concentrations that are similar to median values from streams in agricultural watersheds across the country. This implies that corn producers that cooperated in this study closely and consistently matched fertilizer needs to crop needs, so that there were only small amounts of fertilizer nutrients (P and N) available to be transported via runoff from the field following application. To further illustrate this

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point, concentration of nutrients in runoff from corn production was similar to that from soybean (soybean in parenthesis) 0.45 (0.53), 1.57, (1.54), 0.13 (0.32), and 0.53, (0.44) ppm forNO<sub>3</sub>, TN, SRP, and TP, respectively (Daniels, 2020). Nitrogenfertilizer rates for corn can range from over 200 to 300 lb/ac ofN based on soil texture while no additional N fertilizer is appliedto soybean. Yet, there is little difference in concentration of Nin runoff between the two crops. This implies that somethingother than fertilizer rates may be controlling the concentrationof nutrients in runoff data. Overall, Discovery Farm studieshave indicated that less than 5% of N and P applied as fertilizerleaves the field in surface runoff. The fact that much of Arkansas' row crops are grown on long rows with very little slopehelps reduce energy associated with runoff so that transport isdampened or reduced.

#### Acknowledgments

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Parameter	Nitrate +Nitrite	Total Nitrogen	SRP <sup>a</sup>	Total Phosphorus
	(ppm)	(ppm)	(ppm)	(ppm)
Mean	1.5	2.9	0.2	0.8
S.D.	4.2	5.1	0.3	0.8
C.V. (%)	281	175	133	100
Min	0	0.03	0.003	0.060
Max	48.8	57.3	2.6	6.5
Median	0.45	1.57	0.13	0.53

 Table 1. Statistics of all concentration data from runoff water on Discovery Farms fields growing corn from 2013 through 2019 (number of samples included in analysis = 268).

SRP = soluble reactive phosphorus; PPM = parts per million.



Fig. 1. Mean Annual Nutrient Concentration in parts per million (ppm) in runoff from corn fields monitored at locations St. Francis 1 (Top) and St. Francis 2 (Bottom). SRP = soluble reactive phosphorus.



Fig. 2. Mean Annual Nutrient Concentration in parts per million (ppm) in runoff from corn fields monitored in Stuttgart. SRP = soluble reactive phosphorus.

# APPENDIX: CORN AND GRAIN SORGHUM RESEARCH PROPOSALS

2019-2020 Corn and Grain Sorghum Research Proposals						
Principal Investigator (PI)	Co-Pl	Proposal Name	Year of Research	Funding Amount		
				(US\$)		
S. Green	J. Massey, A. Hashem, and E. Brown	Timing cover crop termination to optimize corn yields and water-use efficiency	1 of 1	41,000		
R. Rorie	C. Rosenkrans	Development of evaluation of feral swine control measures for Arkansas	1 of 1	46,000		
N. Bateman	G. Lorenz, B. Thrash, and G. Studebaker	Evaluation of <i>Bt</i> traits for corn earworm control	1 of 1	20,000		
T. Faske	T. Kirkpatrick	Assess management options for corn nematodes in Arkansas	1 of 1	50,000		
J. Robinson		Development of an online course-nematology and sampling	2 of 2	15,000		
G. Lorenz	N. Joshi, N. Bateman, and G. Studebaker	Insect management in on-farm grain storage	2 of 3	20,000		
J. Kelley		Arkansas corn and grain sorghum research verification program	2 of 3	128,000		
L. Espinoza		Evaluation soil sampling methods for variable rate fertilization	2 of 3	22,000		
G. Atungulu	S. Sadaka and B. Bluhm	Development of drying and decontamination strategies to prevent mycotoxins in corn	3 of 3	52,000		
T. Barber	J. Norsworthy	Evaluation of various PSII herbicides for corn tolerance and effective weed control as potential replacements for Atrazine	3 of 3	72,000		
B. Blum		Gene editing: A new approach to overcome mycotoxins and environmental stress in Arkansas corn production	3 of 3	38,000		
M. Daniels	A. Sharpley	The Arkansas Discovery Farm Program	3 of 3	5000		
V. Ford		Crop enterprise budgets and production economics for corn and grain sorghum	3 of 3	10,000		
C. Henry	T. Spurlock	Improving irrigation scheduling and irrigation efficiency for corn production in Arkansas	3 of 3	164,000		
J. Kelley	J. Ross	Developing profitable irrigated rotational cropping systems for Arkansas	3 of 3	25,000		
J. Kelley		Overcoming yield limitations in corn	3 of 3	24,000		
M. Mozaffari		Increasing corn profit margins by improving sulfur fertilization practices	3 of 3	30,000		
J. Norsworthy	T. Barber	Evaluation of emerging weed control technologies in grain sorghum	3 of 3	18,000		
L. Purcell	T. Roberts	Managing corn N fertility based upon data from an unmanned aerial system	3 of 3	35,000		
T. Roberts		Developing best management practices for N fertilization in Arkansas corn production	3 of 3	71,000		
T. Spurlock	R. Stark	Detection, spread and economic impact of southern rust in SE Arkansas corn fields using remote sensing and spatial analysis technologies	3 of 3	26,000		
C. Wilson		Influence of phosphorus and potassium fertilizers on corn	3 of 3	31,000		
		Tot	al Funding:	943,000		



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