5-2022

Evaluation of Cotton Management Decisions: Cover Crops, Weed Control, and Injured Stands

Savana D. Denton
University of Tennessee, Knoxville, sdavi172@utk.edu

To the Graduate Council:

I am submitting herewith a dissertation written by Savana D. Denton entitled "Evaluation of Cotton Management Decisions: Cover Crops, Weed Control, and Injured Stands." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant, Soil and Environmental Sciences.

Tyson B. Raper, Major Professor

We have read this dissertation and recommend its acceptance:

Thomas C. Mueller, Angela T. McClure, Christopher L. Main, Darrin M. Dodds

Accepted for the Council:

Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

Evaluation of Cotton Management Decisions: Cover Crops, Weed Control, and Injured Stands

A Dissertation Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Savana Davis Denton
May 2022

DEDICATION

To my husband, Drew. You never cease to make life interesting and are always dreaming big. I am so proud to be your wife. Thank you for your support, understanding, and sacrifice.

To my son, Beau. You are the brightest light in my life. There is no title on earth better than Mama. I am so thankful God chose your Daddy and I to be your parents.

ACKNOWLEDGEMENTS

I would first like to thank Tyson Raper for the time, patience, and advice he has given me during my time with the University of Tennessee. I could not have asked for a better boss or advisor. His constant support and understanding gave me the space to become a mother without having to worry about my research projects while I was away, and I am incredibly grateful. To my committee members: Drs. Tom Mueller, Angela McClure, Chris Main, and Darrin Dodds, thank you all for your input and guidance during my time here.

I would also like to thank the cotton research crew at WTREC. This research could not have been completed without your assistance. The station support provided by the crews at WTREC, Ames, and Milan is also greatly appreciated. A special thanks goes to Dr. Lori Duncan for her assistance with processing and analyzing reflectance data; your willingness to help is greatly appreciated.

Finally, I would like to thank my family and friends for their support throughout my academic career. You may not have always known what I was talking about or working on, but you still asked and that means the world. A special thanks goes to my parents for dropping what they were doing when I called to help me when I needed it, whether that was babysitting or box mapping, or both! I am grateful to have such amazing role-models in my life.

ABSTRACT

Herbicide-resistant weed species have altered the challenges faced by Tennessee cotton ***(****Gossypium hirsutum,* L.) producers. While the weed control and environmental benefits of cover crops have been well-documented, the integration of cover crops into cotton production systems has presented management challenges. In-season broadcast postemergence weed control options are limited in cotton. Furthermore, off-target movement of 2,4-D and dicamba can result in additional management challenges if susceptible cotton is injured. Studies were conducted from 2018 to 2021 to evaluate: 1) cotton response to cover crop termination timings and methods; 2) postemergence weed control programs in cotton without the use of glyphosate; 3) the relationship between auxin injury, in-season reflectance data, and yield penalties; and 4) the effects of synthetic auxin exposure on yield components of cotton. Cover crop termination timings and methods impacted early season cotton growth, but yields were ultimately not affected. Postemergence control of weed species was generally greater with multiple POST applications compared to a single POST application but no herbicide program provided greater than 80% annual grass control 21 d after late-POST application. Auxin related injury and yield penalties may be better predicted following exposure during vegetative growth compared to exposure during reproductive growth. Exposure to 2,4-D caused more severe impacts to cotton than exposure to dicamba, but auxin application rate and timing impacted yield components and partitioning. Results from these studies will support a more sustainable production system through improved management of

cover crops, selection of herbicide programs, and understanding of the scope and severity of off-target movement of 2,4-D and dicamba.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I: EFFECT OF COVER CROP TERMINATION TIMING AND METHOD ON COTTON DEVELOPMENT AND YIELD

Abstract

The sustainability movement has influenced the cotton *(Gossypium hirsutum* L.) industry by igniting the search for more sustainable cotton production practices. Cover crops have been promoted for use in agricultural systems due to both environmental and economic opportunities. Cotton growers in West Tennessee faced challenges in the 2015 and 2016 growing seasons with cover crop termination management which resulted in failed cotton stands. The objective of this experiment was to determine effects of various cover crop termination timing and method on cotton emergence, development, and yield. Field experiments were conducted from 2018 to 2020 in both small plot and on-farm scenarios across West Tennessee. Cover crop termination timings consisted of an at-planting termination, three weeks prior to planting, and both a broadcast and furrow-strip termination six weeks prior to planting. The cover crop termination methods consisted of chemical termination, mechanical termination using a roller-crimper, and chemical + mechanical termination.

Cotton emergence and early-season maturity were impacted by termination timing and method, where emergence and growth were impacted most severely following at-planting or mechanical terminations. Thrips injury was greater in cotton following a chemical + mechanical termination of the cover crop. Three-cornered alfalfa hopper damage was less prevalent in cotton when cover crops were terminated six weeks prior to planting in a broadcast method or with a roller-crimper. While early-season impacts were observed, end of season yield differences were not observed. Still, producers in short season environments should be acutely aware of the higher level of risk associated with

at-planting terminations or terminations without herbicides if season length is not conducive for compensation.

Introduction

Cotton (*Gossypium hirsutum* L.) producers in the United States strive to incorporate sustainable agronomic practices into production systems (Daystar et al., 2017). The integration of cover crops is a production practice that can provide environmental benefits and benefit the cash crop. Cover crops improve soil and water quality, reduce soil erosion and nutrient leaching, and cycle nutrients (Balkcom et al., 2016; Hartwig and Ammon, 2002; Kaspar and Singer, 2011). Additional benefits include a reduction in weed seed germination and an increase in beneficial insect populations (Balkcom et al., 2016; Hartwig and Ammon, 2002; Tillman et al., 2004). Even so, yield impacts on the cash crop are often inconsistent from year to year (Bauer and Busscher, 1996).

Compared to other row crops, cotton is particularly susceptible to early season stressors (National Cotton Council of America, 2007). Large quantities of surface biomass often results in inconsistent seed placement and poor seed to soil contact. Unless rainfall occurs soon after planting, inadequate seed placement is likely to cause germination issues. Cover crop residues can slow cotton growth which leaves cotton susceptible to early season pests and less able to recover from residual herbicide injury. In most cases cotton recovers from early season stress, but increased risks associated with a delayed crop linger throughout the season and these risks can be particularly impactful in short-season environments.

Stand establishment and early season growth are priorities in the northern Cotton Belt, as there is a narrow planting window (Butler et al., 2020). Delayed or inadequate emergence may lead to replanting outside of the optimal window. Replanted cotton may grow in less-than-ideal conditions were cotton reaches critical growth stages during periods of increased drought stress, higher temperature, and increased pest pressure. An early fall can also wreak havoc on a delayed crop that is not given the opportunity to completely mature. Even the decision to replant to soybean (*Glycine max* L.) can cause issues associated with herbicide plant-back restrictions and wasted fertilizer inputs. Either way, the producer is faced with economic strain due to increased input costs.

Adoption of cover crops into cotton production systems has not been widespread, mainly due to associated costs and previous failed attempts. A 2017 University of Tennessee survey of row crop producers found that 29% of respondents planted a cover crop in 2016, while 22% of respondents had planted a cover crop previously but chose not to continue the practice (Campbell, 2018). Reasons given for not continuing to plant cover crops were 1) planting difficulties, 2) increased cost of production, 3) termination difficulties, and 4) yield reductions. Other areas of the U.S. Cotton Belt report similar issues integrating cover crops into the cotton production system (Roesch-McNally et al., 2017). This research focuses on the effects of cover crop termination timing and method on cotton emergence, development, and yield.

Termination Timing

There are risks and benefits from incorporating a cover crop program, which are greatly impacted by termination timing decisions. Premature cover crop termination reduces the biomass accumulation that would occur in the spring and decreases the amount of time residue persists into the growing season (Balkcom et al., 2016). Delaying termination increases cover crop biomass levels but can also increase the likelihood of failed cover crop termination, failed or delayed cotton emergence, and the opportunity of a 'green bridge' for insect pests. Additionally, the timing of cover crop termination impacts soil moisture (Hargrove and Frye, 1987; Kornecki et al., 2009; Wortman et al., 2012), soil temperature (Balkcom et al., 2016), and weed suppression by mulching (Webster et al., 2013; Wiggins et al., 2016) or allelopathic effects (Price et al., 2008).

Strip termination is an alternative termination method that may reduce the risks and increase the benefits of a cover crop. Using this method, a band of cover crop is terminated prior to planting where the cash crop will be planted. Then, around the time of cash crop planting, the remaining living cover crop is terminated. Theoretically, this allows for a clean seed bed for cash crop planting as well as a greater chance of receiving benefits from the accumulated cover crop biomass. Limiting the quantity of biomass over the furrow reduces binding of planter row cleaners, allows for proper operation of gauge wheels and double disk openers, and eases furrow closing by press wheels. Limited research has been conducted to evaluate the efficacy of strip terminations in cover crop systems.

The University of Tennessee currently recommends terminating cover crops at least two weeks prior to cotton planting. Terminations three weeks prior to planting are more economically sound as delayed terminations at, or around the time of planting carry far greater risks. The strip termination method could lend itself to being both economically and environmentally sound in that there is reduced risk at planting because of the terminated strips but increased benefits of cover crops can be observed in the row middles.

Termination Method

Achieving adequate cover crop termination is another important factor in managing a cover crop. Creating a uniform seed bed for the cash crop to be planted into can reduce the chances of planting difficulties where planting units get bound or tangled in residue or 'hairpinning' occurs where residue is pushed into the furrow along with seed (Kornecki and Price, 2010). Cover crops may be terminated naturally, chemically, or mechanically or by using some combination of these methods.

Non-hardy winter species and determinate species in southern latitudes terminate naturally when winter temperatures are low enough to kill them. Mild winters have increased frustrations at termination timing due to species in the mustard (*Brassicaceae*) family surviving the winter months. Radishes (*Raphanus sativus* L.) that do not winterkill are extremely difficult to terminate once flowering begins in the spring as there are no effective herbicide options for control (McClure et al., 2017). Chemical termination is a common method for producers and is an effective control option for most cover crop species (Kornecki et al, 2009). Mechanical termination is accomplished with various implements including mowers, undercutters, plows, disks, or roller-crimpers. Mowers do not lend themselves to providing a uniform seed bed and regrowth of the cover crop is common (Creamer and Dabney, 2002). Roller-crimpers; however, consistently provide a uniform mat of cover crop residue on the soil surface and the likelihood of regrowth minimal. The basis for using a roller-crimper is to create a layer of residue that will act as a mulch (Davis, 2010). As the roller-crimper moves across the field, the protruding fins crimp and crush the cover crop to terminate it (Kornecki and Price, 2010).

Adequate termination of cover crops is required because of the subsequent effects of cover crop regrowth on the cotton crop including reduced soil moisture content, delayed cotton emergence, and reduced cotton yield (Price et al., 2009; Singer et al., 2007). Currently, the University of Tennessee recommends a chemical application prior to the use of a roller-crimper (McClure et al., 2017). There is a short window for cover crops to accumulate biomass in the northern region of the Cotton Belt and biomass accumulation is highly variable year to year. For those reasons, the roller-crimper alone may not always guarantee adequate termination in this area of the Cotton Belt. Chemical termination of cover crops will provide the most consistent kill in comparison to mechanical terminations by way of the roller-crimper.

A great deal of research has been conducted on single species cover crop effects on the soil and subsequent cash crops (Bauer and Busscher, 1996; Mirsky et al., 2009; Price et al., 2008). However, management practices for single species cover crop programs may not work well for cover crop blends, which have increased in popularity due to government program support. To ensure a seamless integration of multi species ‘soil health’ blends into cotton production systems, more information is needed specifically in the realm of termination – both timing and method.

Materials and Methods

Experiments were conducted from 2018 to 2020 on producer's fields and at research and education centers across West Tennessee to determine the effects of various cover crop termination timing and method on subsequent cotton emergence, development, and yield. Producer field sites were located near Humboldt, TN (Calloway silt loam; Fine-silty, mixed, active, thermic Aquic Fraglossudalfs and Grenada silt loam; Fine-silty, mixed, active, thermic Oxyaquic Fraglossudalfs) from 2018 to 2020, Henderson, TN (Guyton silt loam; Fine-silty, siliceous, active, thermic Typic Glossaqualfs) from 2018 to 2019, and Trenton, TN (Memphis silt loam; Fine-silty, mixed, active, thermic Typic Hapludalfs) from 2018 to 2019. Research centers were located near Milan, TN at the Milan AgResearch and Education Center (MREC; Grenada silt loam; Fine-silty, mixed, active, thermic Oxyaquic Fraglossudalfs) in 2018, and at the West Tennessee AgResearch and Education Center (WTREC) in Jackson, TN (Lexington silt loam; Fine-silty, mixed, active, thermic Ultic Hapludalfs) from 2018 to 2020. Experimental units in Humboldt, Henderson, and Trenton were 12 m by 24 m and 4 m by 9 m in MREC and WTREC. Treatments were arranged in a randomized complete block design and replicated three times at Humboldt, Henderson, and Trenton and four times at MREC and WTREC.

A Natural Resource Conservation Service (NRCS) approved cover crop blend was seeded into experimental locations during the fall of the previous year. Cover crop blend components varied slightly from year to year on producer's fields due to recommendations from NRCS. In general, species included cereal rye (*Secale cereale*

L.), wheat (*Triticum aestivum* L.), crimson clover (*Trifolium incarnatum* L.), hairy vetch (*Vicia villosa* Roth), and Austrian winterpea (*Pisum sativum* L. ssp. *sativum* var. *arvense*). Experiments located on research and education centers consisted of cereal rye, wheat, crimson clover, hairy vetch, and Austrian winterpea seeded at 21, 28, 6, 6, and 8 kg ha^{-1}, respectively.

Termination timing applications were triggered six weeks prior to planting, three weeks prior to planting, and at planting (Table 1, all tables and figures are located within the appendix). The target planting date for all experiments was 01 May. At six weeks prior to planting, both a broadcast termination and a strip termination were implemented. The strip termination was achieved by spraying a 20 cm band where the seed furrow would be and the remaining green cover crop in the row middles was terminated at planting. Experiments conducted at both research and education centers also included a termination four weeks prior to planting and a fallow treatment.

Termination method treatments included a chemical termination, a mechanical termination, and a chemical and mechanical termination. Termination method treatments were applied approximately one month prior to the target plant date. Chemical termination was accomplished with a broadcast herbicide application consisting of glyphosate and dicamba. Mechanical termination was accomplished with a roller-crimper. For the chemical + mechanical termination, experimental units were first rolled and then chemically terminated.

All termination applications consisted of glyphosate (RoundUp PowerMax, Bayer Crop Science, St. Louis, MO) at 1.5 kg ae ha^{-1} and dicamba (Xtendimax with Vaporgrip

Technology, Bayer Crop Science, St. Louis, MO) at 0.8 kg ae ha^{-1}. Broadcast applications were applied with a MudMaster Multi-Purpose Sprayer (Bowman Manufacturing, Newport, AR) operating at a pressure of 276 kPa with TTI 11004 (TeeJet Technologies, Springfield, IL) nozzles and an application volume of 140 L ha^{-1}. Strip termination applications were accomplished with tractor-mounted sprayers at equivalent application pressure and volume to ensure that cover crops would be terminated where the cotton crop would later be seeded. Equipment utilized for strip terminations varied across environment due to the use of producer equipment but in general, drop nozzles were fabricated onto an existing spray boom to achieve strip termination.

Prior to each termination timing, cover crop biomass samples were collected from experimental units to be treated. Biomass samples were collected from two, 0.25 m^2 locations within each experimental unit and combined for a representative sample of the plot. Samples were dried in a forced-air dryer at 41°C for 72 hours to achieve a constant mass. Cover crop biomass samples were also collected at planting in the termination timing treatments.

Cotton was seeded as close to the target planting date as possible, taking into consideration environmental conditions (Table 1). Planting decisions, in terms of cotton variety and seeding rate, were left up to the discretion of the producer but followed recommended guidelines put forth by UT Extension (Raper, 2016). Planter attachments varied slightly between locations. Row cleaners were either Martin-Till® floating row cleaners with razor wheels (Martin Industries, Elkton, KY) or Yetter floating row cleaners with no-till coulters (Yetter Manufacturing Co., Inc., Colchester, IL). Closing wheels consisted of either rubber or cast iron closing wheels or double disc closing wheels followed by a single press wheel. Maintenance herbicide applications typically applied at or around the time of cotton planting were held off until approximately 21 days after planting to evaluate weed suppression provided by cover crops and their residue. A weed suppression rating was collected at 21 days after planting. After this rating, herbicide applications were applied as needed in season for weed control to prevent yield impacts from uncontrolled weed species.

At 21 d after planting, cotton emergence was evaluated by counting the number of emerged plants in 12 m row. The number of plants that had reached two leaf stage (BBCH12) within the same 12 m row section was recorded. The number of plants at BBCH12 was divided by the total number of emerged plants resulting in a percentage of cotton at BBCH12. Weed and insect pressure was monitored, and data were collected if warranted for each location. At all on-farm locations in 2018 and 2019, thrips injury was rated on a scale of 0 – 5 (0 = no injury, 5 = plant death), slug damage was rated on a scale of 0 – 100 (0 = no damage/defoliation, 100 = plant death), and the number of plants that exhibited injury from three-cornered alfalfa hoppers were counted in the center two rows of each plot. Early season soil moisture levels were monitored as well with ECH_2O EC-5 sensors that measure volumetric water content of the soil (Decagon Devices, Pullman, WA)

At cutout, plant height, total node count, node of first fruiting branch (FFB), and node above cracked boll (NACB) were collected. Experimental units were also rated to capture percent of open bolls approximately 14 d prior to defoliation (0 – 100; 0 = no open bolls, 100 = bolls completely open). Two rows were mechanically harvested from each experimental unit with a spindle cotton picker modified for small plot research. Seed cotton was weighed and ginned to collect yield measurements and lint turnout percentage. Lint samples were collected and sent to the USDA Fiber Classing Office in Memphis, TN for fiber quality analysis.

Data were analyzed in SAS (v. 9.4, SAS Institute Inc., Cary, NC) using the PROC GLIMMIX procedure. Termination timing and termination method were separate experiments and therefore were analyzed separately. Location and replication were set as random effects to evaluate termination management decisions across multiple environments (Blouin et al., 2011; Gbur et al., 2012). Data were subjected to analysis of variance and means were separated using Fisher's Protected LSD at the 0.05 level of significance.

Results and Discussion

Cover Crop Biomass

Overall, cover crop biomass accumulation was limited across all site years (Table 2). In most instances, cotton was grown in the growing season prior to experimental initiation causing cover crop planting to be delayed into November. The delayed planting of the cover crop shortened the window for growth prior to winter which attributed to low overall biomass accumulation. Sykes et al. (2021) performed cover crop variety testing in TN and observed approximately a 50% reduction in cover crop biomass accumulation among top performers when cover crops were planted in early November compared to early October. Average cover crop biomass accumulation from November planted cover crops in the variety testing program conducted by Sykes et al. (2021) was 938 and 2,731 kg ha^{-1} for April and May terminations, respectively.

Timing of cover crop termination impacted cover crop biomass at the time of termination (Table 2; $p < 0.0001$) as well as at-planting (Table 2; $p = 0.0049$). Accumulated cover crop biomass ranged from 417 to 524 kg dried plant material ha^{-1} for terminations at four and six weeks prior to planting which was less that biomass accumulated at three weeks prior to planting (746 kg ha^{-1}). Cover crop biomass levels at termination were highest in the at-planting treatments (1,175 kg ha^{-1}). When comparing cover crop biomass at the time of cotton planting, cover crops terminated at-planting (1,923 kg ha^{-1}) accumulated more biomass than any other termination timing treatment (171 – 684 kg ha^{-1}). In some site-years, cotton planting was delayed following the at-planting termination causing the difference in cover crop biomass accumulation of the at-planting termination treatment. The fallow treatment did have plant material present at the time of planting, which consisted of winter annual weed species that emerged.

In cover crop termination method treatments, no differences were observed for cover crop biomass levels at termination (Table 2; $p = 0.5142$). Cover crop biomass levels ranged from 834 to 965 kg dried plant material ha^{-1}. Cover crop biomass was not collected at-planting for termination method treatments. However, the cover crop at the time of termination had not yet reached an adequate growth stage or biomass level for adequate termination from a roller-crimper alone based on visual observations; small grains included within the cover crop mixes had typically not reached the anthesis stage during any of the conducted experiments. Similarly, Ashford and Reeves (2003) observed reduced kill rates of wheat, rye, and black oats (*Avena strigose* Schreb.) at flag leaf stage and anthesis with the roller-crimper alone compared to either chemical or mechanical + chemical termination methods.

Cotton Emergence and Early Season Stressors

Both cover crop termination timing and method affected cotton emergence (Table 3; $p < 0.0001$). Cotton emergence data was collected and analyzed from on-farm locations only. When cover crop termination was delayed until cotton planting, cotton emergence was reduced 21 d after planting (56,487 plants ha^{-1}) compared to all other termination timings (70,456 – 75,552 plants ha^{-1}). Reduced emergence may be partially due to allelopathy; Shekoofa et al. (2020) evaluated allelopathic effects of cover crop extracts from various termination timings and observed the greatest suppression of cotton germination to be from cover crops terminated at planting. Cotton emergence following mechanical termination was reduced (53,098 plants ha^{-1}) compared to emergence following either chemical (71,831 plants ha^{-1}) or mechanical + chemical termination (70,732 plants ha^{-1}). In contrast, Price et al. (2009) did not observe differences in cotton population between a rolled cover crop and a rolled + chemically sprayed termination. Again, it is possible that the failure of the cover crop in our studies to reach an adequate growth stage and biomass level for proper crimping may have caused the discrepancies between results from Price et al. (2009) and those noted in these experiments.

Cover crop termination timing impacted early season cotton development (Table 3; $p < 0.0001$). Cotton planted into a living stand of cover had less of the stand reaching

BBCH12 21 d after planting than cover crop terminations that took place prior to planting (7% versus 16 – 20%, respectively). Cover crop termination method did not impact early season development (Table 3; $p = 0.1249$).

Cover crop termination timing did not impact observed thrips injury (Table 3; $p = 0.0953$). Cover crop termination method affected observed thrips injury; plots that were both mechanically and chemically terminated had higher thrips injury (2.1) in comparison to either termination method alone (1.6 – 1.8) (Table 3; $p < 0.0001$). Results noted differ from those observed by Toews et al. (2010), who reported an inverse relationship between thrips density and ground cover from both cereal and legume cover crop species. In general, slug damage – measured as percent defoliation – was miniscule across cover crop termination timings and methods (Table 3; $p \geq 0.0783$). Percent slug damage ranged from 2.4 to 4.1% and 3.0 to 5.1% for termination timing and method treatments, respectively.

Cover crop termination timing and method impacted the percentage of three-cornered alfalfa hopper damaged plants (Table 3; $p \leq 0.0223$). Cover crops terminated six weeks prior to planting with a broadcast application resulted in four percent of emerged cotton with three-cornered alfalfa hopper damage which was less than cotton following cover crops terminated at-planting, three weeks prior to planting, and six weeks prior to planting in strips (10, 9, and 12%, respectively). Balkcom et al. (2016) reported that delaying cover crop termination has the potential to create a 'green bridge' for insect pests. It is suspected that the movement of insect pests off the cover crop and onto the emerging cash crop likely drove the increases in injury. Cover crops that were mechanically terminated with the roller-crimper (2%) resulted in less three-cornered alfalfa hopper damaged cotton than cover crops that were chemically terminated or chemically and mechanically terminated (17 – 18%). This response is not well understood, and it may be an anomaly in the data.

There were no differences in grass or broadleaf weed control due to cover crop termination timing or method (Table 3; $p \geq 0.1124$). However, Webster et al. (2013) observed reduced weed pressure with the presence of cover crop biomass due to increased competition for light, nutrients, and water. Across all locations, termination timing, and termination method, broadleaf weed control ranged from 59 to 80% 21 d after planting. Grass weed control ranged from 29 to 72%. The lack of weed control observed may be due to low levels of biomass previously discussed or inconsistent cover crop stand due to a cover crop blend being planted (Raper et al., 2019). The need for in-season weed control remains due to variability in cover crop stand and suppression (Wiggins et al., 2016).

Cotton Growth and Lint Yield

Cover crop termination timing had no effect on cotton height or NACB (Table 4; $p \geq 0.8040$). Cover crops terminated at-planting resulted in cotton with more nodes (20.0) than cotton following cover crops terminated three weeks prior to planting or six weeks prior to planting in strips (18.9 – 19.2) ($p = 0.0231$). In terms of FFB, cotton following cover crops terminated at-planting set the FFB higher on the plant (6.6) than cotton following cover crops terminated six weeks prior to planting (6.1) (Table 4; $p = 0.0322$). In terms of percent open bolls, cotton maturity was delayed when cover crops were terminated at-planting (29%) compared to all other termination timings (39 – 42%) (Table 4; $p = 0.0042$). Late-season cotton growth and maturity were not impacted by termination method as no differences were observed in terms of plant height, total nodes, FFB, NACB, and percent open bolls (Table 4; $p \geq 0.1987$).

Cotton lint yields were not impacted by either cover crop termination timing or termination method (Table 5; $p \geq 0.1354$). Lint yields ranged from 1,047 to 1,182 and 1,049 to 1,165 kg ha^{-1} for cover crop termination timing and termination method, respectively. Kornecki and Price (2010) also did not observe yield differences between mechanical termination or mechanical termination with the addition of glyphosate, but Price et al. (2009) observed reduced yield following mechanical only termination in comparison to a chemical + mechanical termination. Cotton is an incredibly adaptive plant for its growing conditions and can compensate for adverse environments, especially those incurred early-season, and still produce adequate yield (Toews et al., 2010).

Conclusions

Although differences were observed in cotton emergence and growth due to cover crop termination timing and method, yield differences were not noted. Still, these results highlight the higher level of risk associated with delayed cover crop termination. If these studies had been conducted in a short-season environment, the potential of decreased stands, delayed maturity, and cotton yield impacts can be substantial. Insect damage and injury was also impacted by cover crop termination timing and method. Early and complete terminations of cover crops resulted in fewer TCAH damaged plants. Terminations with the roller-crimper were inconsistent in terms of kill rate which is due to the lack of biomass accumulated in the spring in this region of the Cotton Belt. Although the strip termination did not provide any added yield benefits, this method of termination has the potential to maximize benefits of cover crops while reducing risk in terms of cotton growth and development.

Acknowledgements

The authors would like to thank the Research and Education Administration and Staff at Milan and West Tennessee for their assistance and support in completing these trials. The authors would also like to thank the producers Matt Griggs, Marti Hinson, and Rusty and Jeff Harris for providing the land to conduct these research trials. This project was funded through a grant from Tennessee Natural Resources Conservation Service and the authors would like to thank Brad Denton, Matthew Denton, Dave McMillen, Greg Bran, and Mike Hubbs for their assistance in acquiring funding for this project.

References

Ashford, D.L., & Reeves, D.W. (2003). Use of a mechanical roller crimper as an alternative kill method for cover crop. *American Journal of Alternative Agriculture, 18,* 37-45. https://doi.org/10.1079/AJAA200232

Balkcom, K.S., Duzy, L.M., Kornecki, T.S., & Price, A.J. (2016). Timing of cover crop termination: management considerations for the southeast. *Crop, Forage, and Turfgrass Management, 1,* 1-7. https://doi.org/10.2134/cftm2015.0161

Bauer, P.J., & Busscher, W.J. (2004). Winter cover and tillage influences on coastal plain cotton production. *Journal of Production Agriculture, 9,* 50-54.

Blouin, D.C., Webster, E.P., & Bond, J.A. (2011). On the analysis of combined experiments. *Weed Technology, 25,* 165-169. https://doi.org/10.1614/WT-D-01-00047.1

Butler, S, Raper, T.B., Buschermohle, M., Tran, L. , & Duncan, L. (2020). Making the cotton replant decision: a novel and simplistic method to estimate cotton plant population from UAS-calculated NDVI. *Journal of Cotton Science, 24,* 104-111.

Campbell, K.M. (2018). *Tennessee row crop producer survey on willingness to adopt best management practices* [Master's thesis, University of Tennessee]. Tennessee Research and Creative Exchange. https://trace.tennessee.edu/utk_gradthes/5066/

Creamer, N.G., & Dabney, S.M. (2002). Killing cover crops mechanically: review of recent literature and assessment of new research results. *American Journal of Alternative Agriculture, 17,* 32-40. https://doi.org/10.1079/AJAA20014

Davis, A.S. (2010). Cover-crop roller-crimper contributes to weed management in no-till soybean. *Weed Science, 58,* 300-309. https://doi.org/10.1614/WS-D-09-00040.1

Daystar, J.S., Barnes, E., Hake, K., & Kurtz, R. (2017). Sustainability trend and natural resource use in U.S. cotton production. *BioResources, 12*, 362-392. https://doi.org/10.15376/biores.12.1.362-392

Gbur, E.E., Stroup, W.W., McCarter, K.S., Durham, S., Young, L.J., Christman, M., West, M., & Kramer, M. (2012). *Analysis of generalized linear mixed models in the agricultural and natural resource sciences*. ASA, CSSA, and SSSA.

Hargrove, W.L., & Frye, W.W. (1987). The need for legume cover crops in conservation tillage production. In J.F. Power (Ed.), *The role of legumes in conservation tillage systems: The Proceedings of a National Conference, University of Georgia, Athens, April 27-29* (pp. 1-5). Soil Conservation Society of America.

Hartwig, N.L. & Ammon, H.U. (2002). Cover crops and living mulches. *Weed Science, 50,* 688-699. https://doi.org/10.1614/0043-1745(2002)050[0688:AIACCA]2.0.CO;2

Kaspar, T.C., & Singer, J.W. (2011). The use of cover crops to manage soil. J.L. Hatfield & T.J. Sauer (Eds.), *Soil management: building a stable base for agriculture* (pp. 321-337). ASA.

Kornecki, T.S., & Price, A.J. (2010). Effects of different roller/crimper designs and rolling speed on rye cover crop termination and seedcotton yield in a no-till system. *Journal of Cotton Science, 14,* 212-220.

Kornecki, T.S., Price, A.J., Raper, R.L., & Arriaga, F.J. (2009). New roller crimper concepts for mechanical termination of cover crops in conservation agriculture. *Renewable Agriculture and Food Systems, 24,* 152-173. https://doi.org/10.1017/S1742170509002580

McClure, A., Steckel, L., Raper, T., Sykes, V., Montgomery, G., Kelly, H., Stewart, S., & Lee, J. (2017). Cover crop quick facts. UTIA Publication W417.

Mirsky, S.B., Curran, W.S., Mortensen, D.A., Ryan, M.R., & Shumway, D.L. (2009). Control of cereal rye with a roller/crimper as influenced by cover crop phenology. *Agronomy Journal, 101,* 1589-1596. https://doi.org/10.2134/agronj2009.0130

National Cotton Council of America. (2007). The First 40 Days and Fruiting to Finish. National Cotton Council of America. Memphis, TN. https://www.cotton.org/tech/physiology/upload/BMP_Doc.pdf.

Price, A.J., Arriaga, F.J., Raper, R.L., Balkcom, K.S., Kornecki, T.S., & Reeves, D.W. (2009). Comparison of mechanical and chemical winter cereal cover crop termination systems and cotton yield in conservation agriculture. *Journal of Cotton Science, 13,* 238-245.

Price, A.J., Stoll, M.E., Bergtold, J.S., Arriaga, F.J., Balkcom, K.S., Kornecki, T.S., & Raper, R.L. (2008). Effect of cover crop extracts on cotton and radish radicle elongation. *Communications in Biometry and Crop Science, 3,* 60-66.

Raper, T.B. (2016). 2016 Tennessee cotton quick facts. UTIA Publication W319.

Raper, T.B., McClure, M.A., Butler, S., Yin, X., & Blair, R. (2019). Impacts of single- and multiple-species cover crop on soybean relative to the wheat-soybean double crop system. *Crop, Forage, and Turfgrass Management, 5*. https://doi.org/10.2134/cftm2018.12.0104

Roesch-McNally, G.E., Basche, A.D., Arbuckle, J.G., Tyndall, J.C., Miguez, F.E., Bowman, T., & Clay, R. (2017) The trouble with cover crops: farmers' experiences with overcoming barriers to adoption. *Renewable Agriculture and Food Systems, 33,* 322-333.

Shekoofa, A., Safikhan, S., Raper, T.B., & Butler, S.A. (2020). Allelopathic impacts of cover crop species and termination timing on cotton germination and seedling growth. *Agronomy, 10*. https://doi.org/10.3390/agronomy10050638

Singer, J.W., Kohler, K.A., & McDonald, P.B. (2007). Self-seeding winter cereal cover crops on soybean. *Agronomy Journal, 99,* 73-79. https://doi.org/10.2134/agronj2006.0032

Sykes, V.R., Wilson, A., Palacios, F., Keadle, S.B., Bates, G., & McIntosh, D. (2021). Cover Crop Variety Tests in Tennessee 2021. UTIA Publication W1022.

Tillman, G., Schomberg, H., Phatak, S., Mullinix, B., Lagnight, S., Timper, P., & Olson, D. (2004). Influence of cover crops on insect pests and predators in conservation tillage cotton. *Journal of Economic Entomology, 97,* 1217-1232. https://doi.org/10.1603/0022-0493-97.4.1217

Toews, M.D., Tubbs, R.S., Wann, D.Q., & Sullivan, D. (2010). Thrips (Thysanoptera: Thripidae) mitigation in seedling cotton using strip tillage and winter cover crops. *Pest Management Science, 66,* 1089-1095. https://doi.org/10.1002/ps.1983

Webster, T.M., Scully, B.T., Grey, T.L., & Culpepper, A.S. (2013). Winter cover crops influence Amaranthus palmeri establishment. *Crop Protection, 52,* 130-135. http://doi.org/10.1016/j.cropro.2013.05.015

Wiggins, M.S., Hayes, R.M., & Steckel, L.E. (2016). Evaluating cover crops and herbicides for glyphosate-resistant Palmer amaranth (Amaranthus palmeri) control in cotton. *Weed Technology, 30,* 415-422. https://doi.org/10.1614/WT-D-15-00113.1

Wortman, S.E., Francis, C.A, Bernards, M.A, Drijber, R.A., & Lindquist, J.L. (2012). Optimizing cover crop benefits with diverse mixtures and an alternative termination method. *Agronomy Journal, 104,* 1425-1433. https://doi.org/10.2134/agronj2012.0185

Appendix

Table 1. Cover crop termination, cotton planting, and cotton harvest dates.

	Cover Crop Termination					Cotton	
	Fallow	6 wk PP [a]	4 wk PP	3 wk PP	At-planting	Planting	Harvest
2018							
WTREC	01 Nov 2017	21 March	05 April	19 April	25 May	04 June	17 Nov
MREC	01 Nov 2017	21 March	05 April	20 April	29 May	05 June	30 Oct
Griggs	--	22 March	--	30 April	11 May	11 May	05 Nov
Harris	--	22 March	--	18 April	18 May	15 May	12 Nov
Hinson	--	21 March	--	19 April	20 May	20 May	01 Nov
2019							
WTREC	15 Nov 2018	19 March	02 April	16 April	16 May	20 May	12 Nov
Griggs	--	19 March	--	12 April (Timing) 24 April (Method)	13 May	11 May	31 Oct
Harris	--	25 March	--	11 April	28 May	31 May	05 Nov
Hinson	--	02 April	--	24 April	20 May	20 May	02 Nov
2020							
WTREC	12 Nov 2019	20 April	05 May	11 May	30 May	01 June	05 Nov
Griggs	--	16 April	--	07 May	29 May	29 May	31 Oct

[a] Pre-Plant

Table 2. Effect of termination timing and method on cover crop biomass at time of termination and at planting.

	Cover Crop Biomass Levels			
	At Termination Application		At Planting	
Termination Timing	---------------------------- kg ha^{-1} (SE[c])		------------------------------	
Fallow	--		171 (819.9)	B
6 wk PP[a] Broadcast	417 (124.0)	C[b]	259 (334.7)	B
6 wk PP Strip	438 (132.2)	C	684 (454.8)	B
4 wk PP	524 (140.7)	C	395 (518.5)	B
3 wk PP	746 (124.0)	B	619 (304.5)	B
At-planting	1,175 (142.3)	A	1,923 (299.4)	A
p-value	< 0.0001		0.0049	
Termination Method				
Chemical	874 (153.3)		--	
Mechanical	965 (158.7)		--	
Chem + Mech	834 (157.8)		--	
p-value	0.5142		--	

[a] Pre-Plant

[b] Data pooled across environment. Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

[c] Standard Error

Table 3. Cover crop termination timing and method effect on cotton emergence, maturity, insect injury from thrips, slugs, and three-cornered alfalfa hoppers, and weed control 21 d after planting.

	---------------- Cotton ----------------		---------------------- Insects ----------------------			---- Weed Control ----	
	Emergence	Maturity	Thrips Injury	Slug Damage	TCAH[e] Injured Plants	Broadleaf	Grass
Termination Timing	plants ha^{-1} (SE[b])	% BBCH12[d]	----- 0-5 -----	----------------------------- %	-----------------------------		
Fallow	--	--	--	--	--	64 (9.8)	43 (18.8)
6 wk PP[a] Broadcast	75,552 (5,991.3) A[c]	20 (3.9) A	2.0 (0.19)	2.4 (0.88)	4 (4.8) B	68 (8.0)	51 (16.3)
6 wk PP Strip	72,236 (6,357.0) A	16 (4.0) A	2.0 (0.20)	2.6 (0.96)	12 (4.9) A	80 (9.2)	29 (20.1)
4 wk PP	--	--	--	--		62 (8.9)	33 (18.8)
3 wk PP	70,456 (5,991.3) A	17 (3.9) A	1.9 (0.19)	3.3 (0.88)	9 (4.8) A	71 (8.0)	48 (16.3)
At-planting	56,487 (5,991.3) B	7 (3.9) B	1.7 (0.19)	4.1 (0.88)	10 (4.8) A	75 (8.0)	72 (16.3)
p-value	< 0.0001	< 0.0001	0.0953	0.0783	0.0223	0.1861	0.1124
Termination Method							
Chemical	71,831 (5,093.5) A	18 (3.3)	1.8 (0.19) B	5.1 (1.8)	18 (11.0) A	59 (12.3)	--
Mechanical	53,098 (5,129.6) B	13 (3.3)	1.6 (0.19) B	4.8 (1.8)	2 (11.1) B	64 (12.4)	--
Chem + Mech	70,732 (5,092.8) A	17 (3.3)	2.1 (0.19) A	3.0 (1.8)	17 (11.0) A	62 (12.3)	--
p-value	< 0.0001	0.1249	< 0.0001	0.1864	0.0183	0.8025	--

[a] Pre-Plant
[b] Standard Error
[c] Data pooled across environment. Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.
[d] Second true leaf unfolded
[e] Three-Cornered Alfalfa Hopper

Table 4. Cover crop termination timing and method effect on cotton growth and maturity as measured by plant height, total nodes, first fruiting branch (FFB), nodes above cracked boll (NACB), and percent open bolls at defoliation.

	Plant Height	Total Nodes	FFB	NACB	Open Bolls
Termination Timing	------ cm (SE[b]) -----				--------- % ---------
Fallow	--	--	--	--	--
6 wk PP[a] Broadcast	104 (14.0)	19.6 (0.77) AB[c]	6.1 (0.14) B	3.8 (2.79)	39 (10.8) A
6 wk PP Strip	104 (14.1)	18.9 (0.79) B	6.1 (0.16) B	2.6 (3.17)	40 (11.1) A
4 wk PP	--	--	--	--	--
3 wk PP	106 (14.0)	19.2 (0.77) B	6.3 (0.14) AB	2.8 (2.79)	42 (10.8) A
At-planting	106 (14.0)	20.0 (0.77) A	6.6 (0.14) A	4.4 (2.79)	29 (10.8) B
p-value	0.8255	0.0231	0.0322	0.8040	0.0042
Termination Method					
Chemical	100 (11.8)	18.9 (0.48)	6.0 (0.22)	2.7 (2.02)	40 (8.7)
Mechanical	98 (11.8)	19.4 (0.49)	6.4 (0.23)	3.4 (2.02)	35 (8.8)
Chem + Mech	98 (11.8)	19.0 (0.48)	6.1 (0.22)	1.1 (2.02)	39 (8.7)
p-value	0.8340	0.4596	0.1987	0.2402	0.5662

[a] Pre-Plant
[b] Standard Error
[c] Data pooled across environment. Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 5. Effect of cover crop termination timing and method on cotton lint yield.

	Lint Yield
Termination Timing	kg ha^{-1} (SE[b])
Fallow	1,171 (168.9)[c]
6 wk PP[a] Broadcast	1,116 (154.2)
6 wk PP Strip	1,047 (157.0)
4 wk PP	1,121 (159.4)
3 wk PP	1,182 (154.2)
At-planting	1,130 (154.2)
p-value	0.3464
Termination Method	
Chemical	1,165 (176.1)
Mechanical	1,049 (176.6)
Chem + Mech	1,137 (176.1)
p-value	0.1354

[a] Pre-Plant
[b] Standard Error
[c] Data pooled across environment. Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

CHAPTER II: EVALUATION OF POSTEMERGENCE WEED CONTROL PROGRAMS IN COTTON WITHOUT THE ADDITION OF GLYPHOSATE

Abstract

Glyphosate has played an important role in agricultural production systems, especially after the release of glyphosate resistant crops. With increased usage and an overall reliance on the chemical, weed resistance to glyphosate has occurred and is now a major issue. The objective of this research was to investigate weed control levels provided by glufosinate, 2,4-D, and clethodim as an alternative to glyphosate. Multiple POST applications generally provided superior weed control in comparison to a single early-POST application. No programs provided greater than 80% annual grass control beginning 21 d after the mid-POST application. Applications of glufosinate or glufosinate + 2,4-D fb clethodim + glufosinate, glufosinate + 2,4-D, or clethodim + glufosinate + 2,4-D provided adequate broadleaf weed control throughout the rating period. While POST-only programs are an option, they are not a sustainable weed control practice. It remains important to incorporate residual herbicides into a weed control program as well as alternative weed control methods.

Introduction

Glyphosate resistant (GR) soybean (*Glycine max* (L.) Merr.), cotton (*Gossypium hirsutum* L.), and corn (*Zea mays* L.) were released in 1996, 97, and 98, respectively (Duke, 2005). Widespread adoption of GR crops occurred as glyphosate proved to be a simpler and more economical weed control option for producers (Culpepper, 2006; Owen and Zelaya, 2005). In 1995, prior to the release of GR crops, 12.5 million kg glyphosate were applied to agricultural areas in the U.S and has continually increased since

(Benbrook, 2016). Estimated annual usage of glyphosate in agricultural settings has exceeded 113 million kg since 2010 (USGS, 2021).

Shaner (2000) observed a general decrease in the amount of soybean and cotton hectarage treated with chemistry classes excluding glyphosate after the release of GR crops. The heavy reliance on glyphosate placed tremendous selection pressure on the chemistry which led to the development of GR weed species (Culpepper, 2006; Owen and Zelaya, 2005). A weed shift also occurred due to altered production practices that accompanied the adoption of GR crops (reduced tillage, reduced residual herbicide applications, and reduced rotation between modes of action) and producers encountered weed species that were naturally more tolerant to glyphosate (Culpepper, 2006; Shaner, 2000).

Of the weed species with developed resistance, Palmer amaranth (*Amaranthus palmeri* S. Wats) is one of the most troublesome weeds for row crop producers (Kruger et al., 2009; Van Wychen, 2016; Van Wychen, 2017). Palmer amaranth in TN has confirmed resistance to glyphosate (2006), ALS inhibitors (1994), and microtubule inhibitors (1998) as well as multiple resistance to glyphosate + ALS inhibitors (2009), glyphosate + PPO inhibitors (2015), and glyphosate + dicamba (2020) (Heap, 2021). Several grass species in TN have developed resistance to glyphosate including goosegrass (*Elusine indica* L.), Johnsongrass (*Sorghum halepense* L.), Italian ryegrass (*Lolium perenne* L. ssp. *Multiflorum* (Lam.) Husnot), annual bluegrass (*Poa annua*), junglerice (*Echinochloa colona)* and barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) (Heap, 2021). Along with resistance development, there have been reports of reduced herbicidal activity on some grass species when a combination of postemergence herbicides are applied (Mueller et al., 1989; Perkins et al., 2021). Decreased herbicidal activity on grass weed species has been attributed to antagonistic effects between commonly used postemergence herbicides such as glyphosate and dicamba (Perkins et al., 2021).

With the increase in GR-weed species, there has been a shift in weed control strategies to integrated weed management practices which include cover crop implementation, crop rotation, herbicide mode of action rotation, the use of residual herbicides, tillage, and the utilization of herbicide resistant (HR) crops (Sosnoskie and Culpepper, 2014; Webster et al., 2013). From 2019 to 2021, approximately 94 percent of TN cotton acreage was planted in cotton with resistance to glyphosate, glufosinate, and dicamba (XtendFlex™, Bayer Crop Science, St. Louis, MO) followed by approximately five percent of cotton acreage planted in cotton with resistance to glyphosate, glufosinate, and 2,4-D (Enlist™, Corteva Agriscience, Indianapolis, IN) (USDA-ARS, 2019 & 2020). The remaining one percent of TN cotton acreage was planted in cotton with resistance to glyphosate and glufosinate only (GlyTol® LibertyLink®, BASF Corporation, Research Triangle Park, NC).

The increasing number of GR-weed species has encouraged the agricultural community to find alternative methods for weed control outside of chemical control. While alternative methods can help to reduce weed populations, chemical control options still provide efficacious control at a relatively low cost per unit (Merchant et al., 2013). Typical chemical weed control programs in cotton include burndown applications, at planting applications, as well as single or multiple postemergence applications in-season which can include both postemergence and residual herbicides. Glyphosate is commonly used in an herbicide weed control program but due to the increase in GR species and the antagonistic nature of some postemergence herbicide combinations, it is necessary to investigate cotton herbicide programs that do not include glyphosate. Common postemergence herbicides used in cotton for control of weed species in 2,4-D resistant cotton are glufosinate, 2,4-D, and clethodim.

The objective of this research was to investigate weed control levels provided by glufosinate, 2,4-D, and clethodim as alternatives to glyphosate. The hypothesis of this experiment is that adequate weed control levels will be accomplished with two postemergence applications containing multiple modes of action. This experiment does not include residual herbicides in postemergence herbicide applications, although this is recommended as control is typically increased (Gardner et al., 2006b; Meyer et al., 2015).

Materials and Methods

Field experiments were conducted from 2019 to 2021 at University of Tennessee AgResearch and Education Centers in both Milan, TN (MREC) on a Collins silt loam (coarse-silty, mixed, active, acid, thermic Aquic Udifluvents) and Grand Junction, TN (Ames) on a Collins silt loam and Lexington silt loam (fine-silty, mixed, active, thermic Ultic Hapludalfs) to evaluate postemergence weed control programs in cotton without the use of glyphosate. Experimental units consisted of four, 97 and 102 cm wide rows which were 9 m in length at Ames and MREC, respectively. Treatments were arranged in a randomized complete block design and replicated four times at each location.

The Milan location had a uniform flush of weed species that emerged prior to planting the cotton crop. In 2019, the Ames site required overseeding with weed seed prior to trial establishment to build a weed seed bank which was accomplished with the spreading of seed contaminants from seed cleaners in the area. Contained within the seed contaminants were a greater number of viable soybean seeds than expected which required a blanket paraquat application to terminate the flush of soybeans which likely were glufosinate-resistant. Except for the Ames location in 2019, cotton was seeded into emerged weeds and no burndown or preemergence applications were made. Phytogen 400 W3FE (Corteva Agriscience, Indianapolis, IN) was seeded at a rate of 98,800 seeds ha^{-1} (Table 6). The selected variety was resistant to glyphosate, glufosinate, and 2,4-D. Apart from weed control, cotton was managed based on University of Tennessee Extension agronomic and pest management recommendations (Raper, 2016).

Two postemergence application timings were utilized for experiments including an early-POST and mid-POST. The early-POST application was made approximately three weeks after planting or when cotton reached two to three true leaves (Table 6). The mid-POST application was made 14 d after the early-POST application or when cotton reached four to six true leaves (Table 6). Treatments included single applications early-POST of clethodim (Section® Three Herbicide; WinField United, Arden Hills, MN) at 0.17 kg ai ha^{-1} with a crop oil concentrate at 0.5 percent volume per volume, clethodim + glufosinate (Liberty® 280 SL; BASF Corporation, Research Triangle Park, NC) at 0.66 kg ai ha^{-1}, clethodim + 2,4-D choline salt (Enlist One™ with Colex-D™ Technology; Corteva Agriscience, Indianapolis, IN) at 1.1 kg ae ha^{-1}, glufosinate + 2,4-D, and clethodim + glufosinate + 2,4-D. All treatments were applied at the mid-POST timing following an early-POST application of either glufosinate alone or glufosinate + 2,4-D. A non-treated control was included which provided a total of 16 treatments. Postemergence applications were made with CO_2-powered backpack sprayers calibrated to apply 140 L ha^{-1} at a pressure of 276 kPa. Applications were made with TTI 11002 (TeeJet Technologies, Springfield, IL) nozzles at a walking speed of 4.8 km hr^{-1}.

Estimates of visual weed control were conducted 7, 14, 21, and 28 d after early-POST (DAEP) and 7, 14, 21, and 28 d after mid-POST (DAMP) on a scale of 0 – 100% (0 = no control, 100 = complete control) for each weed species present at the time of application. Broadleaf weed species present across experimental locations included Amaranthus species (*Amaranthus spp*.), morningglory species (*Ipomoea spp*.), prickly sida (*Sida spinosa* L.), and common purslane (*Portulaca oleracea* L.). Annual grasses consisted of goosegrass, johnsongrass, and large crabgrass (*Digitaria sanguinalis,* L.). At 28 DAMP application, aboveground weed biomass samples were collected from a 0.25 m^2 area and dried at 41°C for 72 hours to achieve a constant weight and expressed as percent reduction in biomass relative to the non-treated control.

Data were analyzed in SAS (v. 9.4, SAS Institute Inc., Cary, NC) using the PROC MIXED procedure. Treatments were considered fixed effects. Experimental location and replication were considered random effects to make inferences about herbicide program efficacy across multiple environments (Blouin et al., 2011; Gbur et al., 2012). Analysis of visual weed control estimates did not include the values from the non-treated control.

Data were subjected to analysis of variance and means were separated using Fisher's Protected LSD at the α = 0.05.

Results

Broadleaf Weed Control

Visual broadleaf control was affected by herbicide program across all rating timings and weed species (Table 7). Across all rating timings and broadleaf weeds observed, clethodim only early-POST provided less weed control than all other herbicide treatments (Tables 8 – 13). This is to be expected as clethodim, a graminicide, has no activity on broadleaf weeds. At 7 DAEP, clethodim + 2,4-D provided less Amaranthus species, prickly sida, and common purslane control than other early-POST treatments (Table 8). These results are supported by Merchant et al. (2013) who found that broadleaf control from 2,4-D was often inadequate but control was improved with the addition of glufosinate.

At 14 DAEP, the clethodim + 2,4-D early-POST treatment generally provided less Amaranthus species and prickly sida control and numerically lower common purslane control compared to other combinations of 2,4-D applied early-POST (Table 9). Morningglory species control 14 DAEP provided by clethodim + 2,4-D early-POST resulted in 93% control which was greater than the clethodim + glufosinate + 2,4-D early-POST treatment (80%). While the addition of glufosinate to this treatment did not improve morningglory species control, glufosinate is highly effective in controlling morningglories. Statistical differences observed amongst early-POST treatments of either glufosinate or glufosinate + 2,4-D both 7 and 14 DAEP can be attributed to natural differences in weed population across field sites (Tables 8 and 9). Since weed size and density differed across experimental units, it is also likely that herbicide efficacy was impacted by reduced coverage.

By 21 DAEP and 7 DAMP, all early-POST only treatments (0 – 73%) and glufosinate fb clethodim (65%) provided less prickly sida control than remaining treatments with multiple POST applications (84 – 97%) (Table 10). The same was true for common purslane control except following glufosinate + 2,4-D fb clethodim application which provided control similar to that of early-POST only treatments. Copes et al. (2021) observed variable effectiveness in prickly sida control with only postemergence applications compared to using both PRE and POST herbicides. Amaranthus species control was less following early-POST only treatments (0 – 78%) and glufosinate fb clethodim (72%) than treatments with multiple POST applications (86 – 91%) (Table 10). Glufosinate fb clethodim + 2,4-D (86%) did provide similar levels of control to early-POST only treatments as well. Clethodim + 2,4-D (90%) and glufosinate + 2,4-D (88%) early-POST, provided morningglory species control similar to that of all two-POST programs (86 – 99%).

At both 28 and 35 DAEP, which coincide with 14 and 21 DAMP, respectively, control provided by two POST treatments was generally greater than glufosinate fb clethodim and early-POST only treatments (Tables 11 and 12). Morningglory species control is an exception as only clethodim, clethodim + glufosinate, and clethodim + glufosinate + 2,4-D early-POST provided less control than all other treatments, with clethodim and clethodim + glufosinate + 2,4-D providing the least morningglory control beginning 35 DAEP (Table 12). Gardner et al. (2006b) observed morningglory species control of at least 94% when glufosinate was applied in comparison to preemergence herbicides alone (35-54%). Common purslane control 28 DAEP does not follow the general trend either as all treatments besides glufosinate + 2,4-D fb clethodim (67%) and clethodim only (0%) provided control greater than 91% (Table 11).

At 28 DAMP, the following treatments provided greater than 80% control regardless of broadleaf species: glufosinate or glufosinate + 2,4-D fb clethodim + 2,4-D, glufosinate + 2,4-D, and clethodim + glufosinate + 2,4-D and glufosinate + 2,4-D fb clethodim + glufosinate (Table 13). Control of Amaranthus species was also greater than 80% following applications of glufosinate + 2,4-D fb clethodim. Riar et al. (2011) concluded that to achieve Palmer amaranth control similar to that of PRE fb POST programs, a POST only program required an additional POST application in between the early-POST timing and layby. Morningglory species control was also greater than 80% following applications of glufosinate fb clethodim + glufosinate and clethodim + 2,4-D and glufosinate + 2,4-D early-POST (Table 13). Common purslane control greater than 80% was achieved with applications of clethodim + glufosinate and clethodim + glufosinate + 2,4-D at early-POST. Adequate levels of purslane control observed from early-POST only treatments may better be explained by the suppressive nature of other more upright growing weed species present in plots and crop shading as opposed to treatment effect.

Annual Grass Control

Control of annual grasses was affected by herbicide program 7, 21, and 28 DAEP and 7, 14, 21, and 28 DAMP (Table 7). Annual grass weed control 7 DAEP was less with clethodim alone (47%) and clethodim + 2,4-D (48%) than any other treatment combination (80 – 90%) (Table 14). By 14 DAEP, annual grass control fell below 80% regardless of early-POST application and no differences were observed amongst treatments. Beginning at 21 DAEP and continuing throughout the rating period, annual grass control from a single early-POST application was greater when clethodim was applied (66 – 77%) compared to clethodim + glufosinate (40 – 52%) or clethodim + glufosinate + 2,4-D (38 – 50%). These results agree with Mueller et al. (1989) who observed reduced johnsongrass control when 2,4-D was tank mixed with fenoxaprop, haloxyfop, or sethoxydim.

The addition of glufosinate to graminicides, like clethodim, has been found to cause antagonism with respect to clethodim efficacy on grass weed control (Burke et al., 2005; Gardner et al., 2006a). Chalal and Jhala (2015) observed less control of glyphosate-resistant volunteer corn when ACCase inhibitors were tank-mixed with glufosinate compared to those graminicides applied alone. Harre et al. (2020) observed clethodim antagonism when applied with glyphosate + 2,4-D but combinations of clethodim + 2,4-D did not result in reduced control of glyphosate-resistant corn compared to clethodim alone. When either glufosinate or glufosinate + 2,4-D was applied first, no antagonism was present following mid-POST applications of clethodim or clethodim tank-mixes (Table 14).

Clethodim alone applied early-POST (77%), and all treatments that received two POST applications (68 – 87%) provided greater annual grass control than other early-POST only treatments (42 – 54%) beginning 14 DAMP and continuing throughout the rating period (Table 14). In some cases, a mid-POST application was able to provide greater than 80% annual grass control but by 21 DAMP, no herbicide treatment provided control of annual grass species greater than 80%.

Weed Biomass Reduction

Herbicide program impacted weed biomass reduction relative to the non-treated control 28 DAMP (Table 15). In general, greater biomass reduction was achieved with two postemergence applications in comparison to a single early-POST application (Table 15). However, exceptions were observed. Glufosinate + 2,4-D fb clethodim (74%) resulted in greater biomass reduction than applications of glufosinate fb clethodim (25%) and early-POST applications of clethodim (20%) (Table 15). Glufosinate or glufosinate + 2,4-D fb clethodim + glufosinate (56 – 63%) reduced weed biomass better than clethodim + glufosinate early-POST (21%). Similar weed biomass reduction levels were observed when clethodim + 2,4-D was applied early-POST (53%) and mid-POST following either glufosinate (65%) or glufosinate + 2,4-D (74%). Two applications of glufosinate + 2,4-D (75%) reduced weed biomass more than a single early-POST application of glufosinate + 2,4-D (35%). Glufosinate or glufosinate + 2,4-D fb clethodim + glufosinate + 2,4-D (78%) resulted in greater biomass reduction than clethodim + glufosinate + 2,4-D early-POST (38%).

Discussion

When glyphosate is excluded from in-season weed control programs due to loss of efficacy or other restrictions, there are currently alternative methods for controlling troublesome and problematic weeds. Alternative POST applied herbicides, like glufosinate and 2,4-D, can provide adequate levels of weed control in the absence of glyphosate. Glufosinate, in general, is less efficacious on annual grasses and Amaranthus spp. than other commonly used POST products but control can be improved with the use of residual herbicides and timely applications (Chahal and Jhala, 2015; Gardner et al., 2006b). The addition of 2,4-D to a glufosinate application resulted in either no or minimal differences in control throughout the rating period regardless of weed species. In contrast, Merchant et al. (2013) did observe improvements in weed control when glufosinate and 2,4-D were applied together versus either herbicide alone. Differences in weed size and density at the time of application may have contributed to this difference.

Within this experiment, programs that included two POST applications generally provided adequate broadleaf weed control 28 d after the final application without the use of residuals. Unfortunately, control of annual grass weeds was less than ideal across all environments, but control was generally better with multiple POST applications. However, the addition of preemergence herbicides to the programs could provide greater control as well as reduce selection pressure on the already slim number of POST herbicide modes of actions currently available for use in-season (Gardner et al., 2006b; Riar et al., 2011). There is also the potential that an effective PRE fb POST herbicide program could reduce the chances of needing multiple POST applications (Riar et al., 2011).

Acknowledgements

The authors would like to thank the Research and Education Administration and Staff at Ames and Milan for their assistance and support in completing these trials.

References

Benbrook, C.M. (2016). Trends in glyphosate herbicide use in the United States and globally. *Environmental Sciences Europe, 28,* 1-15. https://doi.org/10.1186/s12302-016-0070-0

Blouin, D.C., Webster, E.P., & Bond, J.A. (2011). On the analysis of combined experiments. *Weed Technology, 25,* 165-169. https://doi.org/10.1614/WT-D-01-00047.1

Burke, I.C., Askew, S.D., Corbett, J.L., & Wilcut, J.W. (2005). Glufosinate antagonizes clethodim control of goosegrass (*Elusine indica*). *Weed Technology, 19,* 664-668. https://doi.org/10.1614/WT-04-214R1.1

Chahal, P.S. & Jhala, A.J. (2015). Herbicide programs for control of glyphosate-resistant volunteer corn in glufosinate-resistant soybean. *Weed Technology, 29,* 431-443. https://doi.org/10.1614/WT-D-15-00001.1

Copes, J.T., Miller, D.K., Godara, R.K., & Griffin, J.L. (2021). Pre-plant and in-crop herbicide programs for prickly sida control in soybean. *Crop, Forage, and Turfgrass Management, 1*. https://doi.org/10.1002/cft2.20100

Culpepper, A.S. (2006). Glyphosate-induced weed shifts. *Weed Technology, 20,* 277-281. https://doi/org/10.1614/WT-04-155R.1

Duke, S.O. (2005). Taking stock of herbicide-resistant crops ten years after introduction. *Pest Management Science, 61,* 211-218. https://doi.org/10.1002/ps.1024

Gardner, A.P., York, A.C., Jordan, D.L., & Monks, D.W. (2006a). Glufosinate antagonizes postemergence graminicides applied to annual grasses and Johnsongrass. *Journal of Cotton Science, 10,* 319-327.

Gardner, A.P., York, A.C., Jordan, D.L., & Monks, D.W. (2006b). Management of annual grasses and *Amaranthus* spp. in glufosinate-resistant cotton. *Journal of Cotton Science, 10,* 328-338.

Gbur, E.E., Stroup, W.W., McCarter, K.S., Durham, S., Young, L.J., Christman, M., West, M., & Kramer, M. (2012). *Analysis of generalized linear mixed models in the agricultural and natural resource sciences*. ASA, CSSA, and SSSA.

Harre, N.T., Young, J.M., & Young, B.G. (2020). Influence of 2,4-D, dicamba, and glyphosate on clethodim efficacy of volunteer glyphosate-resistant corn. *Weed Technology*, *34*, 394-401. https://doi.org/10.1017/wet.2019.124

Heap, I. (2021). The international survey of herbicide resistant weeds. [Online]. Available at www.weedscience.org.

Kruger, G.R., Johnson, W.G., Weller, S.C., Owen, M.D.K., Shaw, D.R., Wilcut, J.W., Jordan, D.L., Wilson, R.G., Bernards, M.L., & Young, B.G. (2009). U.S. grower views on problematic weeds and changes in weed pressure in glyphosate-resistant corn, cotton, and soybean cropping systems. *Weed Technology, 23,* 162-166. https://doi.org/10.1614/WT-08-040.1

Merchant, R.M., Sosnoskie, L.M., Culpepper, A.S., Steckel, L.E., York, A.C., Braxton, L.B., & Ford, J.C. (2013). Weed response to 2,4-D, 2-4-DB, and dicamba applied alone or with glufosinate. *Journal of Cotton Science, 17,* 212-218.

Meyer, C.J., Norsworthy, J.K., Stephenson IV, D.O., Bararpour, M.T., Landry, R.L. & Woolam, B.C. (2015). Control of Johnsongrass in the absence of glyphosate in midsouth cotton production systems. *Weed Technology, 29,* 730-739. https://doi.org/10.1614/WT-D-15-00057.1

Mueller, T.C., Witt, W.W., & Barrett, M. (1989). Antagonism of johnsongreass (*Sorghum halepense*) control with fenoxaprop, haloxyfop, and sethoxydim by 2,4-D. *Weed Technology, 3,* 86-89. https://doi.org/10.1017/S0890037X00031377

Owen, M.D.K. & Zelaya, I.A. (2005). Herbicide-resistant crops and weed resistance to herbicides. *Pest Management Science, 61,* 301-311. https://doi.org/10.1002/ps.1015

Perkins C.M., Mueller, T.C., & Steckel, L.E. (2021). Junglerice control with glyphosate and clethodim as influenced by dicamba and 2,4-D mixtures. *Weed Technology, 35,* 419-425. https://doi.org/10.1017/wet.2021.5

Raper, T.B. (2016). 2016 Tennessee cotton quick facts. UTIA Publication W319.

Riar, D.S., Norsworthy, J.K., & Griffith, G.M. (2011). Herbicide programs for enhanced glyphosate-resistant and glufosinate-resistant cotton (*Gossypium hirsutum*). *Weed Technology, 25,* 526-534. https://doi.org/10.1614/WT-D11-00027.1

Shaner, D.L. (2000). The impact of glyphosate-tolerant crops on the use of other herbicides and on resistance management. *Pest Management Science, 56,* 320-326. https://doi.org/10.1002/(SICI)1526-4998(200004)56:4<320::AID-PS125>3.0.CO;2-B

Sosnoskie, L.M. & Culpepper, A.S. (2014). Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) increases herbicide use, tillage, and hand-weeding in Georgia cotton. *Weed Science, 62,* 393-402. https://doi.org/10.1614/WS-D-13-00077.1

USDA-ARS. (2019). Cotton varieties planted 2019 crop. ARS Cotton and Tobacco Program. Memphis, TN [Online]. Available at: https://apps.ams.usda.gov/Cotton/AnnualCNMarketNewsReports/VarietiesPlanted/2019-VarietiesPlanted.pdf

USDA-ARS. (2020). Cotton varieties planted 2020 crop. ARS Cotton and Tobacco Program. Memphis, TN [Online]. Available at: https://www.ams.usda.gov/mnreports/cnavar.pdf

United States Geological Survey (USGS). (2021). Estimated annual agricultural pesticide use; pesticide use maps – glyphosate. National Water-Quality Assessment Project. [Online]. Available at: https://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=2019&map=GLYPHOSATE&hilo=L&disp=Glyphosate

Van Wychen, L. (2016). 2016 survey of the most common and troublesome weeds in broadleaf crops, fruits, and vegetables in the United States and Canada. WSSA National Weed Survey Dataset. [Online]. Available at: http://wssa.net/wp-content/uploads/2016-Weed-Survey_Broadleaf-crops.xlsx

Van Wychen, L. (2017). 2017 survey of the most common and troublesome weeds in grass crops, pasture, and turf in the United States and Canada. WSSA National Weed Survey Dataset. [Online]. Available at: http://wssa.net/wp-content/uploads/2017-Weed-Survey_Grass-crops.xlsx

Webster, T.M., Scully, B.T., Grey, T.L., & Culpepper, A.S. (2013). Winter cover crops influence *Amaranthus palmeri* establishment. *Crop Protection*, *52,* 130-135. https://doi.org/10.1016/j.cropro.2013.05.015

Appendix

Table 6. Cotton planting dates and herbicide application dates for a study conducted from 2019 to 2021 near Milan and Grand Junction, TN.

	Milan			Grand Junction		
	2019	2020	2021	2019	2020	2021
Cotton Planting Date	23 May	22 May	20 May	29 May	14 May	17 May
Early-POST Date	11 June	19 June	17 June	25 June	16 June	18 June
Mid-POST Date	25 June	30 June	02 July	12 July	29 June	02 July

Table 7. Analysis of variance for the effect of herbicide program combination on percent control of weed species present at 7, 14, 21, and 28 d after early- and mid-POST for experiments conducted in Milan and Grand Junction, TN from 2019 to 2021.

	AMASS[a]	IPOSS	SIDSP	PORTOL	GGGAN
Herbicide Program	-------------------------------		p-value[b]	-------------------------------	
7 DAEP[c]	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
14 DAEP	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.1505
21 DAEP / 7 DAMP[d]	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
28 DAEP / 14 DAMP	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
35 DAEP / 21 DAMP	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
42 DAEP / 28 DAMP	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[a] Abbreviations: Amaranthus spp. (AMASS); Ipomoea spp. (IPOSS); prickly sida (SIDSP); common purslane (POROL); annual grasses (GGGAN)

[b] Data pooled across environment. Non-treated control not included in analysis.

[c] d after early-POST

[d] d after mid-POST

Table 8. Effect of herbicide program combination on weed control 7 d after early-POST for experiments conducted in Milan and Grand Junction, TN from 2019 to 2021.

Herbicide Program		Visual Weed Control Estimates (%)			
Early-POST	Mid-POST	AMASS[a]	IPOSS	SIDSP	POROL
Clethodim	--	0 C[b]	0 D	0 C	0 D
Clethodim + Glufosinate	--	93 A	93 A	89 A	90 A
Clethodim + 2,4-D	--	67 B	54 C	59 B	74 C
Glufosinate + 2,4-D	--	94 A	91 AB	94 A	92 A
Clethodim + Gluf. + 2,4-D	--	91 A	70 BC	89 A	93 A
Glufosinate	Clethodim	94 A	89 AB	93 A	84 ABC
	Clethodim + Glufosinate	93 A	85 AB	92 A	88 AB
	Clethodim + 2,4-D	94 A	93 A	93 A	91 A
	Glufosinate + 2,4-D	90 A	84 AB	92 A	77 BC
	Clethodim + Gluf. + 2,4-D	92 A	78 AB	90 A	89 A
Glufosinate + 2,4-D	Clethodim	94 A	89 AB	93 A	94 A
	Clethodim + Glufosinate	93 A	89 AB	88 A	91 A
	Clethodim + 2,4-D	95 A	91 AB	94 A	91 A
	Glufosinate + 2,4-D	95 A	95 A	95 A	94 A
	Clethodim + Gluf. + 2,4-D	94 A	89 AB	92 A	91 A
Standard Error		2.9	8.3	4.0	5.9

[a] Abbreviations: Amaranthus spp. (AMASS); Ipomoea spp. (IPOSS); prickly sida (SIDSP); common purslane (POROL)

[b] Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 9. Effect of herbicide program combination on weed control 14 d after early-POST for experiments conducted in Milan and Grand Junction, TN from 2019 to 2021.

Herbicide Program		Visual Weed Control Estimates (%)			
Early-POST	Mid-POST	AMASS[a]	IPOSS	SIDSP	POROL
Clethodim	--	0 E[b]	0 C	0 F	0 E
Clethodim + Glufosinate	--	82 CD	89 AB	80 CDE	81 A-D
Clethodim + 2,4-D	--	77 D	93 A	71 E	74 D
Glufosinate + 2,4-D	--	89 ABC	93 A	88 ABC	86 A-D
Clethodim + Gluf. + 2,4-D	--	86 ABC	80 B	85 BCD	93 AB
Glufosinate	Clethodim	88 ABC	86 AB	84 BCD	83 A-D
	Clethodim + Glufosinate	84 BCD	89 AB	78 DE	80 BCD
	Clethodim + 2,4-D	82 BCD	88 AB	79 DE	93 AB
	Glufosinate + 2,4-D	82 BCD	84 AB	83 BCD	76 CD
	Clethodim + Gluf. + 2,4-D	87 ABC	85 AB	86 A-D	92 AB
Glufosinate + 2,4-D	Clethodim	87 ABC	94 A	90 AB	95 A
	Clethodim + Glufosinate	86 ABC	88 AB	89 AB	91 ABC
	Clethodim + 2,4-D	91 AB	92 A	85 BCD	93 AB
	Glufosinate + 2,4-D	94 A	90 AB	94 A	92 AB
	Clethodim + Gluf. + 2,4-D	94 A	85 AB	92 AB	84 A-D
Standard Error		4.3	3.8	4.8	7.8

[a] Abbreviations: Amaranthus spp. (AMASS); Ipomoea spp. (IPOSS); prickly sida (SIDSP); common purslane (POROL)

[b] Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 10. Effect of herbicide program combination on weed control 21 d after early-POST and 7 d after mid-POST for experiments conducted in Milan and Grand Junction, TN from 2019 to 2021.

Herbicide Program		Visual Weed Control Estimates (%)			
Early-POST	Mid-POST	AMASS[a]	IPOSS	SIDSP	POROL
Clethodim	--	0 F[b]	0 E	0 E	0 C
Clethodim + Glufosinate	--	62 E	78 CD	55 D	60 B
Clethodim + 2,4-D	--	78 CD	90 AB	73 C	63 B
Glufosinate + 2,4-D	--	74 D	88 ABC	68 C	64 B
Clethodim + Gluf. + 2,4-D	--	71 DE	69 D	65 CD	67 B
Glufosinate	Clethodim	72 D	86 BC	65 CD	60 B
	Clethodim + Glufosinate	91 AB	99 A	91 AB	93 A
	Clethodim + 2,4-D	86 BC	95 AB	84 B	89 A
	Glufosinate + 2,4-D	93 AB	99 A	92 AB	95 A
	Clethodim + Gluf. + 2,4-D	96 A	98 AB	95 A	96 A
Glufosinate + 2,4-D	Clethodim	91 AB	94 AB	88 AB	71 B
	Clethodim + Glufosinate	97 A	99 A	97 A	96 A
	Clethodim + 2,4-D	95 AB	98 AB	93 AB	96 A
	Glufosinate + 2,4-D	98 A	99 A	98 A	97 A
	Clethodim + Gluf. + 2,4-D	98 A	99 A	97 A	96 A
Standard Error		6.4	4.2	7.1	15.2

[a] Abbreviations: Amaranthus spp. (AMASS); Ipomoea spp. (IPOSS); prickly sida (SIDSP); common purslane (POROL)

[b] Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 11. Effect of herbicide program combination on weed control 28 d after early-POST and 14 d after mid-POST for experiments conducted in Milan and Grand Junction, TN from 2019 to 2021.

Herbicide Program		Visual Weed Control Estimates (%)			
Early-POST	Mid-POST	AMASS[a]	IPOSS	SIDSP	POROL
Clethodim	--	0 H[b]	0 C	0 G	0 C
Clethodim + Glufosinate	--	55 G	55 B	45 F	93 A
Clethodim + 2,4-D	--	68 EF	88 A	61 DE	96 A
Glufosinate + 2,4-D	--	72 DE	84 A	67 D	94 A
Clethodim + Gluf. + 2,4-D	--	62 FG	40 B	54 EF	93 A
Glufosinate	Clethodim	62 FG	84 A	54 EF	91 A
	Clethodim + Glufosinate	86 BC	98 A	85 BC	96 A
	Clethodim + 2,4-D	86 BC	98 A	83 BC	97 A
	Glufosinate + 2,4-D	91 AB	98 A	90 BC	95 A
	Clethodim + Gluf. + 2,4-D	94 AB	97 A	93 ABC	98 A
Glufosinate + 2,4-D	Clethodim	80 CD	81 A	81 AB	67 B
	Clethodim + Glufosinate	94 AB	96 A	93 C	97 A
	Clethodim + 2,4-D	92 AB	97 A	91 ABC	96 A
	Glufosinate + 2,4-D	96 A	95 A	96 A	95 A
	Clethodim + Gluf. + 2,4-D	96 A	97 A	96 A	96 A
Standard Error		7.1	7.2	7.3	4.5

[a] Abbreviations: Amaranthus spp. (AMASS); Ipomoea spp. (IPOSS); prickly sida (SIDSP); common purslane (POROL)

[b] Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 12. Effect of herbicide program combination on weed control 35 d after early-POST and 21 d after mid-POST for experiments conducted in Milan and Grand Junction, TN from 2019 to 2021.

Herbicide Program		Visual Weed Control Estimates (%)			
Early-POST	Mid-POST	AMASS[a]	IPOSS	SIDSP	POROL
Clethodim	--	0 E[b]	0 D	0 G	0 E
Clethodim + Glufosinate	--	55 D	72 AB	47 F	64 BCD
Clethodim + 2,4-D	--	68 C	90 A	60 E	61 D
Glufosinate + 2,4-D	--	66 C	85 AB	66 DE	63 CD
Clethodim + Gluf. + 2,4-D	--	62 CD	28 C	54 EF	77 ABC
Glufosinate	Clethodim	62 CD	76 AB	57 EF	74 ABCD
	Clethodim + Glufosinate	78 B	96 A	77 BCD	81 A
	Clethodim + 2,4-D	85 AB	96 A	83 ABC	79 A
	Glufosinate + 2,4-D	88 AB	90 A	86 AB	86 A
	Clethodim + Gluf. + 2,4-D	88 AB	96 A	88 AB	83 A
Glufosinate + 2,4-D	Clethodim	78 B	62 B	74 CD	76 ABC
	Clethodim + Glufosinate	88 AB	85 AB	85 ABC	83 A
	Clethodim + 2,4-D	89 A	94 A	86 AB	79 AB
	Glufosinate + 2,4-D	90 A	92 A	91 A	86 A
	Clethodim + Gluf. + 2,4-D	92 A	90 A	90 A	84 A
Standard Error		7.2	10.0	7.5	9.7

[a] Abbreviations: Amaranthus spp. (AMASS); Ipomoea spp. (IPOSS); prickly sida (SIDSP); common purslane (POROL)
[b] Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 13. Effect of herbicide program combination on weed control 42 d after early-POST and 28 d after mid-POST for experiments conducted in Milan and Grand Junction, TN from 2019 to 2021.

Herbicide Program		Visual Weed Control Estimates (%)			
Early-POST	Mid-POST	AMASS[a]	IPOSS	SIDSP	POROL
Clethodim	--	0 G[b]	0 D	0 H	0 E
Clethodim + Glufosinate	--	59 F	72 AB	57 FG	83 ABCD
Clethodim + 2,4-D	--	72 DE	90 A	67 DEF	76 CD
Glufosinate + 2,4-D	--	66 EF	85 AB	64 EFG	73 D
Clethodim + Gluf. + 2,4-D	--	65 EF	28 C	59 FG	88 ABC
Glufosinate	Clethodim	62 EF	76 AB	52 G	77 BCD
	Clethodim + Glufosinate	79 CD	96 A	76 BCD	79 ABCD
	Clethodim + 2,4-D	87 ABC	96 A	86 ABC	88 ABC
	Glufosinate + 2,4-D	86 ABC	90 A	84 ABC	90 AB
	Clethodim + Gluf. + 2,4-D	88 ABC	96 A	87 AB	91 A
Glufosinate + 2,4-D	Clethodim	80 BCD	62 B	74 CDE	78 ABCD
	Clethodim + Glufosinate	89 AB	85 AB	87 AB	89 ABC
	Clethodim + 2,4-D	88 ABC	94 A	86 AB	86 ABC
	Glufosinate + 2,4-D	92 A	92 A	90 A	91 A
	Clethodim + Gluf. + 2,4-D	93 A	90 A	90 A	90 AB
Standard Error		5.9	10.0	6.3	10.0

[a] Abbreviations: Amaranthus spp. (AMASS); Ipomoea spp. (IPOSS); prickly sida (SIDSP); common purslane (POROL)
[b] Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 14. Effect of herbicide program combination on annual grass visual control 7, 14, 21, and 28 d after early-POST and d after mid-POST for experiments conducted in Milan and Grand Junction, TN from 2019 to 2021.

Herbicide Program		Annual grasses Visual Control Estimates					
		DAEP[a]					
		7	14	21	28	35	42
				DAMP[b]			
				7	14	21	28
Early-POST	Mid-POST	%					
Clethodim	--	47 C[c]	70	66 DE	77 BC	72 A	70 A
Clethodim + Glufosinate	--	90 A	79	52 FG	46 DE	40 C	41 C
Clethodim + 2,4-D	--	48 C	65	60 EF	54 D	42 C	42 C
Glufosinate + 2,4-D	--	85 AB	71	45 G	42 E	37 C	32 C
Clethodim + Gluf. + 2,4-D	--	87 AB	76	50 FG	45 DE	38 C	39 C
Glufosinate	Clethodim	80 B	65	73 CD	81 AB	76 A	74 A
	Clethodim + Glufosinate	85 AB	71	90 A	81 AB	71 A	70 A
	Clethodim + 2,4-D	86 AB	66	76 B-D	79 AB	73 A	72 A
	Glufosinate + 2,4-D	81 B	67	85 AB	68 C	59 B	57 B
	Clethodim + Gluf. + 2,4-D	83 AB	66	92 A	79 AB	68 AB	69 A
Glufosinate + 2,4-D	Clethodim	86 AB	69	75 B-D	87 A	76 A	75 A
	Clethodim + Glufosinate	84 AB	67	93 A	82 AB	71 A	67 AB
	Clethodim + 2,4-D	88 A	70	82 A-C	80 AB	73 A	70 A
	Glufosinate + 2,4-D	87 AB	68	89 A	72 BC	66 AB	67 AB
	Clethodim + Gluf. + 2,4-D	87 AB	65	93 A	80 AB	71 A	71 A
Standard Error		5.5	7.6	11.0	10.1	12.2	11.6

[a] d after early-POST
[b] d after mid-POST
[c] Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 15. Effect of herbicide program on percent weed biomass reduction relative to non-treated control at 28 d after mid-POST application for experiments conducted in Milan, TN and Grand Junction, TN from 2019 to 2021.

Herbicide Program		Biomass Reduction
Early-POST	Mid-POST	% of NTC[a]
Clethodim	--	20 D[b]
Clethodim + Glufosinate	--	21 D
Clethodim + 2,4-D	--	53 ABCD
Glufosinate + 2,4-D	--	35 BCD
Clethodim + Gluf. + 2,4-D	--	38 BCD
	Clethodim	25 CD
	Clethodim + Glufosinate	57 ABC
Glufosinate	Clethodim + 2,4-D	65 AB
	Glufosinate + 2,4-D	65 AB
	Clethodim + Gluf. + 2,4-D	78 A
	Clethodim	74 A
	Clethodim + Glufosinate	63 AB
Glufosinate + 2,4-D	Clethodim + 2,4-D	74 A
	Glufosinate + 2,4-D	75 A
	Clethodim + Gluf. + 2,4-D	78 A
Standard Error		20.3
p-value		0.0002

[a] Non-Treated Control

[b] Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

CHAPTER III: SYNTHETIC AUXIN INJURY ON SUSCEPTIBLE COTTON, PART I: RELATIONSHIPS BETWEEN AERIAL REFELCTANCE DATA, CROP INJURY, AND YIELD

Abstract

Synthetic auxin drift onto sensitive cotton (*Gossypium hirsutum* L.) cultivars has impacted many producers across the U.S. Cotton Belt. Currently, the spatial scope and severity of auxin damage in-season is most often estimated by an agronomist. The use of remote sensing technology has the potential to objectively quantify the spatial scope and severity of drift damage. Experiments were conducted in 2019, 2020, and 2021in Grand Junction, TN to determine: 1) the effect of reflectance data collection timing; 2) the effect of auxin exposure timing; 3) the value of near infrared (NIR) and red-edge (RE) reflectance versus reflectance within the visible spectrum data, and 4) if/how visual injury relates to aerial reflectance data. Applications of 2,4-D or dicamba were made to susceptible cotton cultivars at 1X, 1/4X, 1/16X, 1/64X, 1/256X and 1/1024X rates at either matchhead square (MHS) or two weeks after first bloom (FB+2WK). Non-treated controls were included for each application timing as well. Aerial reflectance data was collected 7, 14, 21, and 28 d after application. Unsupervised classification of images into vegetative and non-vegetative pixels did not increase correlations between vegetative indices (VIs) and application rate. Overall, the VIs which generated the strongest correlations with application rate, visual injury, and relative lint yield were RE based but similar correlations were also noted with VIs calculated from reflectance in the visible spectrum. Correlations were greater when auxin injury occurred at MHS than FB+2WK. Results suggest reflectance measured within the visible spectrum can quantify the scope and severity of auxin injury if the injury occurs early during the growing season.

Introduction

Herbicide-resistant (HR) Palmer amaranth (*Amaranthus palmeri* S. Wats) has plagued row crop producers in the United States. The over-reliance on single herbicides for control of weed species has proven to not be a sustainable weed control practice (Cahoon et al., 2015). Herbicide-resistant weed species have developed and weed species shifts have occurred making weed control practices more difficult (Cahoon et al., 2015; Culpepper, 2006). Integrated weed control practices for control of troublesome weed species are necessary for extending the lifespan of current herbicides. Unfortunately, there are still escapes of troublesome weed species that can cause issues later into the season and there are limited effective postemergence herbicide options in cotton (*Gossypium hirsutum* L.). Synthetic auxin-resistant cotton is the latest HR crop released to help combat HR weed species.

In 2020, 73 percent of U.S. cotton hectarage planted carried the XtendFlex™ trait which provides resistance to dicamba, glyphosate, and glufosinate and 17 percent of U.S cotton hectarage carried the Enlist™ trait which provides resistance to 2,4-D, glyphosate, and glufosinate (USDA, 2020). This widespread adoption of synthetic auxin-resistant cotton cultivars can be attributed to combatting HR weed species and to protecting against certain auxin drift from neighboring fields (Buol et al., 2019; Cahoon et al., 2015). Both 2,4-D and dicamba are volatile compounds; therefore, complaints of off-target movement and damage have accompanied applications of auxin-like herbicides since their release (Egan et al., 2014; Wax et al., 1969). Off-target applications of dicamba and 2,4-D occur due to particle drift, vapor drift, or sprayer contamination (Buol et al., 2019; Cundiff et al., 2017; Egan et al., 2014).

Injury symptoms observed in plants are unique to synthetic auxins and include: epinastic growth, leaf strapping or cupping, twisting, chlorosis, stunting, loss of apical dominance, and delayed maturity (Buol et al., 2019; Byrd et al., 2016). Some injury symptoms can be observed shortly after exposure, even at low application rates. Low-dose exposures do not always impact yield, but mid- and end-of-season management decisions must often be altered to account for delays in maturity. Cotton growth stage impacts the severity of visual injury and yield effects; while cotton exposed to synthetic auxins during vegetative growth stages exhibit greater injury symptoms than cotton exposed to synthetic auxins after reproductive growth has begun, greater visual injury levels do not always translate to greater yield penalties (Byrd et al., 2016; Egan et al., 2014; Everitt & Keeling, 2009).

Visual ratings of synthetic-auxin damage are commonly used to assess spatial scope and severity of injury (Sciumbato et al., 2004a). Other assessment methods include height measurements, biomass measurements, or visual differences in coloration. There is strong bias associated with visual injury ratings due to their subjective nature (Ali et al., 2013). It is hypothesized that remote sensing could potentially provide a more consistent and objective measurement of crop injury from synthetic auxin drift. Remote sensing has been used for decades to determine nutrient status, estimate crop injury or yield, and to locate and identify weed species in a field, along with many other applications (Ali et al., 2013; Atzberger, 2013; Henry et al., 2004; Tucker et al., 1980).

To date, little data has been published pertaining to the use of aerial remote sensing technologies for detection of synthetic-auxin injury in cotton. Cotton reflectance as related to 2,4-D injury has been investigated using handheld sensors (Suarez et al., 2016; Suarez et al., 2017). Suarez et al (2017) observed strong relationships between the green wavelength and the NIR range and yield. Work regarding the relationship of auxin damage and reflectance data in soybean (*Glycine max* (L.) Merr.) has recently been published (Abrantes et al., 2021; Oseland et al., 2021). Previously published work has focused on the detection of glyphosate injury in row crops with remote sensing technologies (Ali et al., 2013; Everman et al., 2008; Henry et al., 2004). Ali et al. (2013) concluded that analyzing images for reflectance values in the visible range was a viable option for herbicides which directly affect chlorophyll levels. Henry and colleagues (2004) reported that regrowth of corn after paraquat applications decreased the accuracy of distinguishing rate responses from the untreated control using various indices including the normalized difference vegetation index (NDVI) (Rouse et al., 1973; Tucker, 1979).

The NDVI index assesses plant health since healthy plants absorb red light and reflect near-infrared (NIR) light. In unhealthy plants the absorption and reflectance of these wavelengths and NDVI values decrease. While NDVI is widely used in remote sensing applications, there are flaws due to the tendency of the signal to become saturated at high vegetation densities making NDVI less precise in detecting differences in crop health later in the growing season when vegetation density increases (Boegh et al., 2002).

Additional vegetative indices (VIs) have been developed with higher sensitivities to biomass changes, especially later in the growing season. These include the green normalized difference vegetation index (GNDVI) and the normalized difference red edge (NDRE) (Gitelson and Merzlyak, 1994; Gitelson et al., 1996). The simplified canopy chlorophyl content index (SCCCI) combines NDVI and NDRE (Barnes et al., 2000). Various ratios between wavelengths have also been found to correlate with plant stressors (Gitelson and Merzlyak, 1997). Vegetative indices that only use wavelengths from the visible spectrum have also been identified that correlate with plant growth (Bendig et al., 2015; Gitelson et al., 2002; Meyer and Neto, 2008). The ability to utilize Red/Green/ Blue (RGB)-based indices would greatly reduce the investment and time required to remotely analyze crop status when compared to the cost of NIR and red-edge (RE) cameras.

The ability to remotely collect reflectance data to accurately assess auxin damage would benefit the agricultural industry. When auxin damage is incurred by a producer, civil cases and insurance claims are common methods for receiving damages for lost yield. Damages are based on the extent of yield loss which currently requires waiting for harvest to ensure an objective measurement of yield loss. Currently, visual observations of auxin injury in-season cannot adequately or objectively predict yield loss. Aside from the potential yield damages from auxin-drift, the use of remotely sensed reflectance data might provide more accurate information about the state of the injured crop leading to more informed management decisions (Henry et al., 2004).

The overall objective of this research was to determine if the severity of auxin injury can be measured with aerial remote sensing. Secondary objectives included determining the effect of reflectance data collection timing and the effect of auxin exposure timing on the ability to remotely measure auxin injury, if NIR and RE reflectance is needed to measure injury remotely or if data from the RGB spectrum would suffice and defining how visual injury relates to aerial reflectance data. The hypotheses are that (1) collecting reflectance data approximately 14 d after auxin exposure is appropriate for capturing auxin injury, (2) remotely collected reflectance data will better relate to auxin injury and yield effects when exposure occurs during vegetative growth rather than reproductive, and (3) visible spectrum reflectance data will perform similarly to NIR and RE reflectance data.

Materials and Methods

Field experiments were conducted from 2019 to 2021 at the University of Tennessee Ames AgResearch and Education Center located near Grand Junction, TN on a Memphis silt loam (fine-silty, mixed, active, thermic Typic Hapludalfs) to evaluate the relationship between reflectance data of auxin-injured cotton collected from an unmanned aerial system (UAS), visual injury ratings of auxin-injured cotton, and yield. Two experiments were conducted at this location: 2,4-D tolerant cotton sprayed with dicamba, and dicamba-tolerant cotton sprayed with 2,4-D. Experimental units were 5.8 m by 9 m and arranged in a randomized complete block design with four replications.

Cotton was seeded at 98,800 seeds ha^{-1} during May of each year (Table 16). Varieties selected for these experiments were PHY 400 W3FE (Corteva Agriscience, Indianapolis, IN), a 2,4-D tolerant variety, and DP 1725 B2XF (Bayer CropScience, Research Triangle Park, NC), a dicamba-tolerant variety. Less than 1% of TN cotton acreage is planted without an auxin-tolerant trait; therefore, using a cultivar with sensitivity to both auxins would not have been representative of TN cotton production systems (USDA, 2020). Cotton was managed based on University of Tennessee Extension agronomic and pest management recommendations (Raper, 2016). Maintenance herbicide applications did not include 2,4-D or dicamba.

Treatments consisted of 2,4-D choline salt (Enlist One™ with Colex-D™ Technology, Dow AgroSciences, Indianapolis, IN) at the following rates: 1,064 (1X), 266 (1/4X), 67 (1/16X), 17 (1/64X), 4 (1/256X), and 1 (1/1024X) g ae ha^{-1} and dicamba (Xtendimax® with VaporGrip® Technology, Bayer Crop Science, St. Louis, MO) at the following rates: 558 (1X), 139 (1/4X), 35 (1/16X), 9 (1/64X), 2 (1/256X), and 0.5 (1/1024X) g ae ha^{-1}. Applications were made at match-head square (MHS) and two weeks after first bloom (FB+2WK) (Table 16). Non-treated plots were included for each application timing as well. The center four rows of each experimental unit were treated, leaving a two-row border in between treated areas to minimize drift effects. Applications were made with a MudMaster Multi-Purpose Sprayer (Bowman Manufacturing, Newport, AR) operating at a pressure of 276 kPa with XR 11002 (TeeJet Technologies, Springfield, IL) nozzles and an application volume of 140 L ha^{-1} at a ground speed of 6.4 km hr^{-1}.

A DJI Inspire drone (DJI, Shenzhen, China) equipped with a hyperspectral double 4K sensor (Sentera, Inc., Minneapolis, MN) mapped the experimental location 7, 14, 21, and 28 d after each application (Table 17). Flight plans were made using the Field Agent application (Sentera, Inc., Minneapolis, MN). Flights were conducted at an altitude of 61 m with an overlap of 80% resulting in a spatial resolution of 1.8 cm. The collected images and reflectance data were stitched in Pix4D (Pix4D Inc., Pilly, Switzerland) and then uploaded into ArcMap 10.7 (Environmental Systems Research Institute, Inc., Redlands, CA) for analysis (Fig. 1). Plot boundaries were drawn and added to the map. The Sentera Double 4K sensor has two cameras, one that captures red, green, and blue bands and one that captures RE and near-infrared (NIR) bands. To calculate indices that use bands from both cameras, bands were normalized to one another, and a correction factor was applied. Normalization of bands was accomplished using equations provided by the sensor manufacturer (Table 17).

Once bands were corrected, NDVI was calculated using the equation found in Table 17 (Fig. 1). The NDVI raster was clipped to the plot boundaries and the image was classified using the Iso Cluster Unsupervised Classification available in ArcMap 10.7 to classify each pixel as vegetation, shadow, or soil (Fig. 1). The pixels classified as shadow and soil were removed from the image to isolate the crop (Fig. 1). Reflectance values for the five bands were determined for both the unclassified and classified image and spatial statistics was performed using the zonal statistics tool resulting in a single value for each band for each experimental unit. These data were exported into Excel where VIs were calculated. The VIs of interest and their respective calculations are reported in Table 18.

Visual crop injury ratings were recorded at each application as well as 7, 14, 21, and 28 d after each application timing. Cotton was harvested from the center two rows of each experimental unit using a spindle picker modified for small-plot research.

Data were analyzed using the multivariate method in JMP Pro (v 16, SAS Institute Inc., Cary, NC) by both auxin technology and application timing as both factors influence overall response. Application rate was transformed using the following equation to capture a linear response against application rate: $Log(Application\ Rate) = log(application\ rate\ +\ 0.0045)$. A constant was included in the log transformation to maintain the untreated plots in the analysis (Bellégo et al., 2021). Pearson correlations were first examined between VI calculated with both unclassified and classified data, % pixel retained, and the log transformed application rate for data collected 7, 14, 21, and 28 d after each application during the 2019 growing season to determine the effect timing of reflectance data collection has on response. Pearson correlations were also examined between VI calculated from unclassified data, % pixels retained, the log transformed application rate, injury ratings, and relative lint yield data collected 14 d after each application across all years of the experiment. Relative lint yield (RLY) was calculated using the following equation to normalize yield across years: $RLY = (observed\ lint\ yield\ /\ maximum\ yearly\ plot\ yield)\ *\ 100$.

Results and Discussion

Unclassified vs. Classified Images for VI Calculations

Analysis of unclassified images resulted in greater Pearson Correlation values between VI and application rate than analysis of classified images 21 d after a MHS application of either 2,4-D or dicamba and 14 d after a FB+2WK application of 2,4-D (Tables 19 and 21). Utilizing classified images for GNDVI analysis never improved correlation values between VI and application rate at any application timing or timing of reflectance data collection for either auxin (Tables 19 and 21).

Over all herbicides and application timings, when comparing VI calculated from unclassified and classified images, Pearson correlation values were greater when analyzing unclassified images 54 percent of the time (Tables 19 and 21). Fig. 2 illustrates the difference in VI values calculated from unclassified and classified images as application rate increases; the difference is more pronounces following exposure of 2,4-D at MHS compared to exposure at FB+2WK.

When the non-vegetative pixels in an image were removed from analysis and the actively growing regions of the cotton plant were retained, the ability to distinguish between VI based on application rate was diminished. The information that can be used from the classification of images comes from the percent of pixels that are retained through the classification process (Fig. 2). The limited increase in correlation provided by the relatively time consuming and computationally demanding classification procedure will likely result in practitioners' use of unclassified data. Therefore, the remainder of the analysis will focus on VIs derived from unclassified images and the % pixel retained.

Timing of Reflectance Data Collection

Pearson correlation (r) values were greatest 28 d after MHS application and 21 d after FB+2WK application for both 2,4-D on dicamba-tolerant cotton and dicamba on 2,4-D tolerant cotton (Tables 19 – 22). Marple et al. (2008) observed greatest visual cotton injury 28 d after application of either 2,4-D or dicamba regardless of growth stage at the time of application. Sosnoskie et al. (2015) observed peak cotton injury from 2,4-D formulations 28 d after application when applied to 5- to 7- leaf cotton. However, the relationship between soybean injury and application rate of dicamba and 2,4-D was described as a quadratic response by Abrantes et al. (2019). For soybeans exposed to dicamba, greater r^2 values were observed 8 d after treatment whereas the relationship between injury and application rate with soybean exposure to 2,4-D formulations were greatest 15 d after treatment.

Further analysis of the relationship between aerial reflectance data and visual injury from auxin herbicides focused on reflectance data collected 14 d after each application. Based on previous research, this timeframe for data collection falls between injury symptom development and the potential for regrowth in experimental units which receive sub-lethal rates of auxin herbicide. In a similar experiment in soybeans, Oseland et al. (2021) observed inconsistent response in reflectance data collected both 7 and 28 d after application; therefore, reflectance data from 14 d after application was presented. Furthermore, Sciumbato et al. (2004b) did not observe consistent cotton injury response to synthetic auxin exposure at 4- to 6-leaf growth stage until 14 d after treatment. Additionally, Smith et al. (2017) observed visual injury from 2,4-D exposure on sensitive cotton to increase or remain the same up to 28 d after treatment regardless of application timing and cotton injury following dicamba exposure began to decrease 21 d after application. Waiting to remotely assess cotton response to auxin herbicides, especially when dealing with low to ultra-low rates of auxins, can allow that crop to begin putting on new growth which could mask some effects.

Visible Spectrum vs. NIR and RE Data

Overall, Modified Green-Red Vegetation Index (MGRVI) and Visual Atmospheric Reflectance Index (VARI) were two of the best VIs based on correlation values (Tables 23 and 24). Of the three RGB VIs of interest, MGRVI and VARI ranked in the top five VIs evaluated for overall performance for both dicamba and 2,4-D applied at either MHS or FB+2WK based on Pearson correlation values (Table 25). For both dicamba and 2,4-D applied at FB+2WK, Excess Green (ExG) also ranked in the top five VIs evaluated based on Pearson correlation values but did not rank well following auxin exposure at MHS. Jay et al. (2019) observed strong correlations between VARI and NDVI when assessing reflectance in sugar beets (*Beta vulgaris* L.). Abrantes et al. (2019) found three RGB VIs (MGRVI, Modified Photochemical Reflectance Index (MPRI), and (ExG) were superior in relating reflectance with soybean injury and yield following exposure to dicamba and two formulations of 2,4-D.

Based on these data, remotely assessing injury from auxin herbicides with visible spectrum data is a viable option. The ability to use a VI that only requires wavelengths from the visible spectrum would greatly reduce the cost and time investment for the end-user while still providing an objective method for assessing auxin injury (Oseland et al., 2021).

Timing of Auxin Exposure

In general, Pearson Correlation values for the relationships between VIs 14 d after application and application rate, visual injury 14 d after application, and RLY were greater than those relationships made 14 d after a FB+2WK application of both 2,4-D and dicamba (Tables 23 and 24). The severity of visual injury symptoms following auxin exposure during reproductive growth stages in cotton was less than when applications are made during vegetative growth stages (Buol et al., 2019; Marple et al., 2008). The difference in cotton response based on growth stage translates to analysis of VI as well. Based on these results, a general idea of cotton growth stage at time of auxin exposure is needed to relate aerial reflectance data with visual injury, application rate and RLY.

Relationships between VI, Application Rate, Visual Injury, and Relative Lint Yield

Correlation values between VIs, percent of pixel retained, application rate, visual injury, and RLY from MHS applications of 2,4-D or dicamba on sensitive cotton all exceeded an absolute value of 0.60, except for SCCCI correlated with rate, injury, and RLY ($r \leq 0.58$) (Tables 23 and 24). The correlation values between VIs and percent pixel retained with application rate and RLY from FB+2WK applications of 2,4-D on sensitive cotton ranged from -0.42 to 0.41. Visual injury had a stronger correlation with VI when 2,4-D was applied to sensitive cotton at FB+2WK with all VI but SCCCI, GNDVI, and

RE/Green exceeding -0.63 (Table 23). When dicamba was applied to sensitive cotton at FB+2WK, correlation values between VIs and percent pixel retained ranged from -0.18 to 0.22 (Table 24).

The correlations between application rate, visual injury, and RLY when either dicamba or 2,4-D were applied were generally stronger than correlations between VIs and parameters of interest for both application timings, although differences between correlation values following MHS applications were minimal (Tables 23 and 24). Previous research has reported conflicting conclusions between visual injury and lint yield regarding relationship strength. Sciumbato et al. (2004b) observed strong linear relationships between visual injury and lint yield in two out of three years of the experiment; adverse weather conditions during one growing season were cited for the weaker relationship. Conversely, Johnson et al. (2012) reported much lower correlations between yield and visual injury 7 and 14 d after auxin applications to 20 – 30 cm cotton compared to these data and cited indeterminate growth and the ability of cotton to compensate for stress as reasons for poor correlation.

The VIs which correlate most strongly with application rate, visual injury, and RLY across both herbicide active ingredient and application timing were NDRE, RE/Red, MGRVI, and VARI (Table 25). Even though NDVI did not rank highly for auxin exposure at both application timings, performance of NDVI was further evaluated with the top performing VIs due to the widespread use and popularity of the index. In order to normalize responses and define sensitivities, the sensitivity equivalent (SEq) was calculated as described by Solari et al. (2008) for the five selected VIs after which the root mean square error (RMSE), slope, and SEq were compared (Tables 26 and 27). For application rate, visual injury, and RLY, of 2,4-D and dicamba on sensitive cotton for MHS and FB+2WK applications, NDRE and RE/Red were generally most sensitive, although differences between VI for most correlations were minimal. Oseland et al. (2021) similarly observed NDRE to perform better than other commonly used VI when dicamba was applied to sensitive soybeans, and various researchers have reported high sensitivity to changes in chlorophyll content with the RE region in various cropping systems (Schlemmer et al., 2005).

Based on Pearson correlation values between VI and application rate, visual injury, and RLY, the best performing RGB VI was MGRVI and the best performing NIR or RE VI was NDRE. The relationships between these two indices and application rate, visual injury, and RLY are depicted for applications of 2,4-D and dicamba in Figs. 3 and 4, respectively. MGRVI and NDRE captured 14 d after MHS application of 2,4-D and dicamba on sensitive cotton strongly correlate with application rate, visual injury, and RLY with r^2 values ranging from 0.64 to 0.87 (Figs. 3 and 4). The greatest r^2 value between VI and application rate and VI and visual injury was with NDRE after sensitive cotton was exposed to 2,4-D at MHS (r^2 = 0.87 and 0.84, respectively). The greatest r^2 value between VI and RLY was with NDRE after exposure to dicamba at MHS (r^2 = 0.74). Oseland et al. (2021) reported NDRE as the most consistent VI for predicting yield loss in soybean following auxin exposure. MGRVI and NDRE captured 14 d after FB+2WK application of 2,4-D on sensitive cotton both had r^2 values of 0.41 when related to visual injury (Fig. 3). MGRVI and NDRE captured 14 d after FB+2WK applications of dicamba on sensitive cotton poorly correlated with application rate, visual injury, and RLY with r^2 values all less than 0.05 (Fig. 4).

Perceived Hurdles to Adoption

These results suggest remotely acquired reflectance data may be used to assess injury and yield effects of auxin exposure if exposure to auxin herbicides occurs during the MHS growth stage. Exposure at FB+2WK resulted in considerably lower correlations between reflectance and parameters of interest. Based on these data, the best predictor of RLY when cotton is exposed to auxin herbicides at FB+2WK is visual injury ($r \geq -0.74$) (Tables 23 and 24). Marple et al. (2008) reported improved correlation values between visual injury and lint yield when injury ratings were taken later in the growing season. While correlation values seemed to improve over time between VIs and application rate, remote assessment of auxin damage should be completed after initial injury symptoms appear but before regrowth occurs.

Overall, the two best VI in terms of correlations between VI, application rate, visual injury, and RLY were both RE based, followed by MGRVI and VARI and then NDVI. The inclusion of the RE wavelength in VI calculations improved correlation values which agrees with previous research (Schlemmer et al., 2005). However, the performance of MGRVI and VARI in this experiment suggest that RGB reflectance data would suffice when assessing auxin damage remotely. While NDVI was superior to some of the other VIs investigated in this experiment, the usefulness of NDVI becomes limited due to the tendency for saturation when the crop canopy closes (Hatfield et al., 2019). Hatfield et al. (2019) suggested to use caution when NDVI values exceed 0.75.

In this experiment, just over 50% of NDVI readings in experimental units were greater than 0.75. However, ground collected reflectance values reported by Suarez et al. (2017) pointed to stronger correlations with the green wavelength and the NIR region.

It should be noted that environmental conditions, particularly water deficits, play a critical role in a crop's response to auxin applications (Johnson et al., 2012; Oseland et al., 2021). The unpredictability of the frequency and amount of rainfall during a growing season make it difficult to confidently predict yield effects. While more in-depth investigations related to this topic could improve models, assessing auxin damage remotely certainly has the potential to provide a more objective method for predicting yield loss when applications are made at MHS under normal environmental conditions. To remotely detect auxin injury from exposure at FB+2WK, additional research will be required. It is likely that pattern recognition or some other artificial intelligence procedures to quantify the parameters captured in visual ratings will be required at FB+2WK, as opposed to the more simplistic calculations of raw reflectance data required at MHS.

Acknowledgements

The authors would like to thank the Ames AgResearch and Education Center for their support in getting these trials conducted. The authors would also like to thank Dr. Lori Duncan for her assistance with processing reflectance data.

References

Abrantes, T.C., Silva Queiroz, A.R., Lucio, F.R., Mendes Jr., C.W., Kuplich, T.M., Bredemeier, C., & Merotto Jr., A. (2021). Assessing the effects of dicamba and 2,4 Dichlorophenoxyacetic acid (2,4D) on soybean through vegetation indices derived from Unmanned Aerial Vehicle (UAV) based RGB imagery. *International Journal of Remote Sensing, 42*, 2740-2758. https://doi.org/10.1080/01431161.2020.1832283

Ali, A., Streibig, J.C., Duus, J., & Andreasen, C. (2013). Use of image analysis to assess color response on plants caused by herbicide application. *Weed Technology, 27,* 604-611. https://doi.org/10.1614/WT-D-12-00136.1

Atzberger, C. (2013). Advances in remote sensing of agriculture: context description, existing operational monitoring systems and major information needs. *Remote Sensing, 5,* 949-981. https://doi.org/10.3390/rs5020949

Barnes, E.M., Clark, T.R., Richards, S.E., Colaizzi, P.D., Haberland, J., Kostrzewski, M., Waller, P., Choi, C., Riley, E., Thompson, T., Lascano, R.J., Li, H., & Moran, M.S. (2000). Coincident detection of crop water stress, nitrogen status, and canopy density using ground-based multispectral data. In P.C. Robert et al. (Eds.), *Proceedings of the Fifth International Conference on Precision Agriculture, 16-19 July, 2000, Bloomington, MN, USA* (pp. 1 – 15). American Society of Agronomy.

Bellégo, C., Benatia, D., & Pate, L. (2021). Delating with logs and zeros in regression models. *CREST – Série des Documents de Travail n° 2019-13*. https://doi.org/10.2139.ssrn.3444996

Bendig, J., Kang, Y., Aasen, H., Bolten, A., Bennertz, S., Janis Broscheit, M.L.G., & Bareth, G. (2015). Combining UAV-based plant height from crop surface models, visible, and near infrared vegetation indices for biomass monitoring in barley. *International Journal of Applied Earth Observation and Geoinformation, 39,* 79–87. https://doi.org/10.1016/j. jag.2015.02.012

Boegh, E., Soegaard, H., Broge, N., Hasager, C.B., Jensen, N.O., Schelde, K., & Thomsen, A. (2002). Airborne multispectral data for quantifying leaf area index, nitrogen concentration, and photosynthetic efficiency in agriculture. *Remote Sensing of Environment, 81,* 179–193. https://doi.org/10.1016/S0034-4257(01)00342-X

Buol, J.T., Reynolds, D.B., Dodds, D.M., Mills, J.A., Nichols, R.L., Bond, J.A., Jenkins, J.N., & DuBien, J.L. (2019). Effect of growth stage on cotton response to a sublethal concentration of dicamba. *Weed Technology, 33,* 1-8. https://doi.org/10.1017/wet.2018.95

Byrd, S.A., Collins, G.D., Culpepper, A.S., Dodds, D.M., Edmisten, K.L., Wright, D.L., Morgan, G.D., Baumann, P.A., Dotray, P.A., Manuchehri, M.A., Jones, A., Grey, T.L., Webster, T.M., Davis, J.W., Whitaker, J.R., Roberts, P.M., Snider, J.L., & Porter, W.M. (2016). Cotton stage of growth determines sensitivity to 2,4-D. *Weed Technology, 30,* 601-610. https://doi.org/10.1614/WT-D-15-00191.1

Cahoon, C.W., York, A.C., Jordan, D.L., Everman, W.J., Seagroves, R.W., Culpepper, A.S., & Eure, P.M. (2015). Palmer amaranth (*Amanranthus palmeri*) management in dicamba-resistant cotton. *Weed Technology, 29,* 758-770. https://doi.org/10.1614/WT-D-15-00041.1

Culpepper, A.S. (2006). Glyphosate-induced weed shifts. *Weed Technology, 20,* 277-281. https://doi.org/10.1614/WT-06-199.1

Cundiff, G.T., Reynolds, D.B., & Mueller, T.C. (2017). Evaluation of dicamba persistence among various agricultural hose types and cleanout procedures using soybean (*Glycine max*) as a bio-indicator. *Weed Science, 65,* 305-316. https://doi.org/10.1017/wsc.2019.29

Egan, J.T., Barlow, K.M., & Mortensen, D.A. (2014). A meta-analysis on the effects of 2,4-D and dicamba drift on soybean and cotton. *Weed Science, 62,* 193-206. https://doi.org/10.1614/WS-D-13-00025.1

Everitt, J.D., & Keeling, J.W. (2009). Cotton Growth and Yield Response to Simulated 2,4-D and Dicamba Drift. *Weed Technology, 23,* 503–506. https://doi.org/10.1614/WT-08-061.1

Everman, W.J., Medlin, C.R., Dirks, R.D., Bauman, T.T., & Biehl, L. (2008). The effect of postemergence herbicides on the spectral reflectance of corn. *Weed Technology, 22,* 514-522. https://doi.org/10.1614/WT-07-021.1

Gitelson, A.A., & Merzlyak, M.N. (1994). Quantitative estimation of chlorophyll using reflectance spectra: Experiments with autumn chestnut and maple leaves. *Journal of Photochemistry and Photobiology, 22,* 247–252. https://doi.org/10.1016/1011-1344(93)06963-4

Gitelson, A.A., & Merzlyak, M.N. (1997). Remote estimation of chlorophyll content in higher plant leaves. *International Journal of Remote Sensing, 18,* 2691-2697. https://doi.org/10.1080/014311697217558

Gitelson, A.A., Kaufman, Y.J., & Merzlyak, M.N. (1996). Use of a green channel in remote sensing of global vegetation from EOS-MODIS. *Remote Sensing of Environment, 58,* 289-298. https://doi.org/10.1016/S0034-4257(96)00072-7

Gitelson, A.A., Kaufman, Y.J., Stark, R., & Rundquist, D. (2002). Novel algorithms for remote estimation of vegetation fraction. *Remote Sensing of Environment, 80,* 76-87. https://doi.org/10.1016/S0034-4257(01)00289-9

Hatfield, J.L., Prueger, J.H., Sauer, T.J., Dold, C., O'Brien, P., & Wacha, K. (2019). Applications of vegetative indices from remote sensing to agriculture: past and future. *Inventions, 4*. https://doi.org/10.3390/inventions4040071

Henry, W.B., Shaw, D.R., Reddy, K.R., Bruce, L.M., & Tamhankar, H.D. (2004). Remote sensing to detect herbicide drift on crops. Weed Technology, 18, 358-368. https://doi.org/10.1614/WT-03-098

Jay, S., Baret, F., Dutarte, D., Malatesta, G., Héno, S., Comar, A., Weiss, M., & Maupas, F. (2019). Exploiting the centimeter resolution of UAV multispectral imagery to improve remote-sensing estimates of canopy structure and biochemistry in sugar beet crops. *Remote Sensing of Environment, 231*. https://doi.org/10.1016/j.rse.2018.09.011

Johnson, V.A., Fisher, L.R., Jordan, D.L., Edmisten, K.L., Stewart, A.M., & York, A.C. (2012). Cotton, peanut, and soybean response to sublethal rates of dicamba, glufosinate, and 2,4-D. *Weed Technology, 26,* 195-206. https://doi.org/10/1614/WT-D-11-00054.1

Marple, M.E., Al-Khatib, K., & Peterson, D.E. (2008). Cotton injury and yield as affected by simulated drift of 2,4-D and dicamba. *Weed Technology, 22,* 609-614. https://doi.org/10.1614/WT-07-095.1

Meyer, G.E., & Neto, J.C. (2008). Verification of color vegetation indices for automated crop imaging applications. *Computers and Electronics in Agriculture, 63,* 282–293. https://doi.org/10.1016/j.compag.2008.03.009

Oseland, E., Shannon, K., Zhou, J., Fritschi, F., Bish, M.D., & Bradley, K.W. (2021). Evaluating the spectral response and yield of soybean following exposure to sublethal rates of 2,4-D and dicamba at vegetative and reproductive growth stages. *Remote Sensing, 13*. https://doi.org/10.3390/rs13183682

Raper, T.B. (2016). 2016 Tennessee cotton quick facts. UTIA Publication W319.

Rouse, J.W., Haas, R.H., Schell, J.A., & Deering, D.W. (1973). Monitoring vegetation systems in the Great Plains with ERTS. *3rd ERTS Symposium, NASA SP-351, Greenbelt, Maryland, USA*. (pp. 309 – 317).

Schlemmer, M.R., Francis, D.D., Shanahan, J.F., & Schepers, J.S. (2005). Remotely measuring chlorophyll content in corn leaves with differing nitrogen levels and relative water content. Agronomy Journal, 97, 106–112. https://doi.org/10.2134/agronj2005.0106

Sciumbato, A.S., Chandler, J.M., Senseman, S.A., Bovey, R.W., & Smith, K.L. (2004a). Determining exposure to auxin-like herbicides. I. Quantifying injury to cotton and soybean. *Weed Technology, 18,* 1125-1134. https://doi.org/10.1614/WT-03-105R1

Sciumbato, A.S., Chandler, J.M., Senseman, S.A., Bovey, R.W., & Smith, K.L. (2004b). Determining exposure to auxin-like herbicides. II. Practical application to quantify volatility. *Weed Technology, 18,* 1135-1142. https://doi.org/10.1614/WT-03-229R

Smith, H.C., Ferrell, J.A., Webster, T.M., & Fernandez, J.V. (2017). Cotton response to simulated auxin herbicide drift using standard and ultra-low carrier volumes. *Weed Technology, 31,* 1-9. https://doi.org/10.1614/WT-D-16-00101.1

Suarez, L.A., Apan, A., & Werth, J. (2016). Hyperspectral sensing to detect the impact of herbicide drift on cotton growth and yield. *ISPRS Journal of Photogrammetry and Remote Sensing, 120,* 65-76. https://doi.org/10.1016/j.isprsjprs.2016.08.004

Suarez, L.A., Apan, A., & Werth, J. (2017). Detection of phenoxy herbicide dosage in cotton crops through the analysis of hyperspectral data. *International Journal of Remote Sensing, 38,* 6528-6553. https://doi.org/10.1080/01431161.2017.1362128

Solari, F., Shanahan, J., Ferguson, R., Schepers, J., & Gitelson, A. (2008). Active sensor reflectance measurements of corn nitrogen status and yield potential. *Agronomy Journal, 100,* 571–579. https://doi.org/10.2134/agronj2007.0244

Sosnoskie, L.M., Culpepper, A.S., Braxton, L.B., & Richburg, J.S. (2015). Evaluating the volatility of three formulations of 2,4-D when applied in the field. *Weed Technology, 29,* 177-184. https://doi.org/10.1614./WT-D-14-00128.1

Tucker, C.J., Holben, B.N., Elgin Jr., J.H., McMurtrey III, J.E. (1980). Relationship of spectral data to grain yield variation. *Photogrammetric Engineering and Remote Sensing, 46,* 657-666.

USDA-ARS. (2020). *Cotton varieties planted 2020 crop*. ARS Cotton and Tobacco Program. Memphis, TN. https://www.ams.usda.gov/mnreports/cnavar.pdf

Wax, L.M., Knuth, L.A., & Slife, F.W. (1969). Response of soybeans to 2,4-D, dicamba, and picloram. *Weed Science, 17,* 388-393. https://doi.org/10.1017/S004317450005431X

Appendix

Table 16. Cotton planting dates, herbicide application dates, and cotton harvest dates for 2,4-D and dicamba experiments located in Grand Junction, TN from 2019 to 2021.

Event	2019	2020	2021
Cotton Planting	06 May	14 May	17 May
Matchhead Square Application	25 June	29 June	06 July
First Bloom + 2 wk Application	18 July	29 July	10 Aug
Cotton Harvest	19 Nov	17 Nov	01 Nov

Table 17. Sentera Double 4K (Sentera, Inc., Minneapolis, MN) multispectral sensor specifications and equations for band corrections to remove cross talk between bands.

Band	Center Wavelength	Bandwidth	Equation
Blue	446 (nm)	60 (nm)	$1.377 * Blue - 0.182 * Green - 0.061 * Red$
Green	548	45	$-0.199 * Blue + 1.420 * Green - 0.329 * Red$
Red	650	70	$-0.034 * Blue - 0.110 * Green + 1.150 * Red$
Red Edge	720	40	$-0.956 * NIR + 1.000 * Red\ Edge$
Near-Infrared	840	20	$2.426 * NIR - 0.341 * Red\ Edge$

Table 18. Vegetative indices calculated from spectral reflectance collected by the Sentera Double4K (Sentera, Inc., Minneapolis, MN) multispectral sensor.

Acronym	Name	Vegetative Index	Reference
VARI	Visual Atmospheric Reflectance Index	$\frac{Green - Red}{Green + Red - Blue}$	Gitelson et al., 2002
ExG	Excess Green	$2 * Green - Red - Blue$	Meyer and Neto, 2008
MGRVI	Modified Green-Red Vegetation Index	$\frac{Green^2 - Red^2}{Green^2 + Red^2}$	Bendig et al., 2015
NDVI	Normalized Difference Vegetative Index	$\frac{2.7^a * NIR - Red}{2.7 * NIR + Red}$	Rouse et al., 1973
NDRE	Normalized Difference Red Edge Index	$\frac{NIR - Red\ Edge}{NIR + Red\ Edge}$	Gitelson and Merzlyak, 1994
SCCCI	Simplified Canopy Chlorophyll Content Index	$\frac{NDRE}{NDVI}$	Barnes et al., 2000
RE/Green	Red Edge / Green	$\frac{2.7 * Red\ Edge}{Green}$	Gitelson and Merzlyak, 1997
RE/Red	Red Edge / Red	$\frac{2.7 * Red\ Edge}{Red}$	Gitelson and Merzlyak, 1997
GNDVI	Green NDVI	$\frac{2.7 * NIR - Green}{2.7 * NIR + Green}$	Gitelson et al., 1996

[a] A correction factor of 2.7 is applied to the NIR or RE band in equations that use bands from both cameras on the Sentera Double 4K multispectral sensor. Correction factor provided by Sentera.

Table 19. Pearson correlation (r) between vegetative indices calculated from unclassified and classified images and % pixel retained with 2,4-D application rate from 7, 14, 21, and 28 d after either the matchhead square or 2 weeks after first bloom application of 2,4-D on sensitive cotton from the 2019 growing season.

		Matchhead Square Application				Two Weeks After First Bloom Application			
		7 [a]	14	21	28	7	14	21	28
Index		Pearson Correlation (r)							
VARI	Unclassified	-0.5333	-0.5154	-0.5115	-0.9017	-0.9017	-0.9257	-0.9050	-0.9227
VARI	Classified	**-0.5444** [b]	**-0.5371**	-0.3958	**-0.9347**	**-0.9347**	-0.8252	-0.8523	-0.8881
ExG	Unclassified	-0.5498	-0.5321	-0.4427	-0.8842	-0.8842	-0.9437	-0.8029	-0.9173
ExG	Classified	**-0.5762**	**-0.5605**	-0.3480	**-0.8845**	**-0.8845**	-0.7728	-0.6039	-0.7642
MGVRI	Unclassified	-0.5377	-0.5272	-0.5262	-0.8932	-0.8932	-0.9152	-0.9030	-0.9164
MGVRI	Classified	**-0.5546**	**-0.5274**	-0.3464	**-0.9220**	**-0.9220**	-0.7522	-0.8023	-0.8530
NDVI	Unclassified	-0.5189	-0.5329	-0.5458	-0.7623	-0.7623	-0.7670	-0.8189	-0.7964
NDVI	Classified	-0.4894	-0.4797	-0.3211	**-0.8621**	**-0.8621**	-0.3960	**-0.8954**	**-0.8728**
NDRE	Unclassified	-0.5171	-0.5304	-0.5358	-0.7912	-0.7912	-0.7993	-0.8542	-0.8312
NRDE	Classified	-0.4642	-0.3944	-0.3076	**-0.8886**	**-0.8886**	-0.5657	**-0.9092**	**-0.8906**
CCCI	Unclassified	-0.3623	-0.5448	-0.5459	-0.7835	-0.7835	-0.7241	-0.8323	-0.7773
CCCI	Classified	0.2513	0.3659	-0.1959	-0.7333	-0.7333	-0.4254	**-0.8338**	**-0.8541**
GNDVI	Unclassified	-0.4509	-0.5077	-0.5540	-0.6015	-0.6015	-0.5412	-0.6930	-0.6206
GNDVI	Classified	0.3750	0.0172	-0.3306	-0.3331	-0.3331	-0.0445	-0.4482	-0.0680
RE/Green	Unclassified	-0.3885	-0.4769	-0.5139	-0.6242	-0.6242	-0.5643	-0.7459	-0.6645
RE/Green	Classified	0.1164	**0.5026**	-0.2239	-0.2677	-0.2677	-0.0514	-0.4913	-0.1296
RE/Red	Unclassified	-0.4815	-0.4681	-0.4610	-0.8411	-0.8411	-0.8549	-0.8818	-0.8728
RE/Red	Classified	-0.3904	-0.3820	-0.3730	**-0.8863**	**-0.8863**	-0.7530	**-0.8996**	**-0.8774**
% Pixel Retained		-0.4840	-0.4444	-0.4753	-0.7243	-0.7243	-0.8886	-0.8030	-0.8695

[a] Represents reflectance data collection timing: 7, 14, 21, or 28 d after application.

[b] Bold values represent an increase in Pearson correlation values when classified images were used for vegetative indices compared to unclassified images.

Table 20. Performance ranking of vegetative indices based on Pearson correlation values between vegetative indices calculated from unclassified and classified images and % pixel retained with 2,4-D application rate from 7, 14, 21, and 28 d after either the matchhead square or 2 weeks after first bloom application of 2,4-D on sensitive cotton from the 2019 growing season.

		Matchhead Square Application				Two Weeks After First Bloom Application			
		7	14	21	28	7	14	21	28
Index		------------	------------	------------	------------ Rank	------------	------------	------------	------------
VARI	Unclassified	2 [a]	3	4	1	4	1	3	2
VARI	Classified	2	3	4	1	1	4	3	2
ExG	Unclassified	2	3	4	1	3	1	4	2
ExG	Classified	2	3	4	1	1	2	4	3
MGVRI	Unclassified	2	3	4	1	4	2	3	1
MGVRI	Classified	2	3	4	1	1	4	3	2
NDVI	Unclassified	4	3	2	1	4	3	1	2
NDVI	Classified	2	3	4	1	3	4	1	2
NDRE	Unclassified	4	3	2	1	4	2	1	2
NRDE	Classified	2	3	4	1	3	4	1	2
CCCI	Unclassified	4	3	2	1	2	4	1	3
CCCI	Classified	3	2	4	1	3	4	2	1
GNDVI	Unclassified	4	3	2	1	3	4	1	2
GNDVI	Classified	1	4	3	2	2	4	1	3
RE/Green	Unclassified	4	3	2	1	3	4	1	2
RE/Green	Classified	4	1	3	2	2	4	1	3
RE/Red	Unclassified	2	3	4	1	4	3	1	2
RE/Red	Classified	2	3	4	1	2	4	1	3
Overall		**2**	**3**	**4**	**1**	**3**	**4**	**1**	**2**
% Pixel Retained		2	4	3	1	4	1	3	2

[a] Reflectance data collection timing ranked from 1 – 4 (1 = highest correlation value, 4 = lowest correlation value) within each VI for each application timing based on Pearson correlation values between VI and application rate. Overall ranking represents the ranking of data collection timings over all VIs calculated with unclassified and classified images

Table 21. Pearson correlation (r) between vegetative indices calculated from unclassified and classified images and % pixel retained with dicamba application rate from 7, 14, 21, and 28 d after either the matchhead square or 2 weeks after first bloom application of dicamba on sensitive cotton from the 2019 growing season.

		Matchhead Square Application				Two Weeks After First Bloom Application			
		7 [a]	14	21	28	7	14	21	28
Index		Pearson Correlation (r)							
VARI	Unclassified	-0.4744	-0.5632	-0.5685	-0.7247	-0.7247	-0.7244	-0.7315	-0.7326
VARI	Classified	**-0.5180** [b]	**-0.5778**	-0.5531	**-0.8010**	**-0.8010**	**-0.8047**	**-0.7812**	**-0.8200**
ExG	Unclassified	-0.5098	-0.4879	-0.5218	-0.8286	-0.8286	-0.8128	-0.8315	-0.8552
ExG	Classified	-0.4912	-0.4781	-0.4638	**-0.8350**	**-0.8350**	-0.7947	-0.7555	-0.8212
MGVRI	Unclassified	-0.4921	-0.5649	-0.5660	-0.7096	-0.7096	-0.7011	-0.7214	-0.7146
MGVRI	Classified	**-0.5203**	**-0.5728**	-0.5424	**-0.8026**	**-0.8026**	**-0.7886**	**-0.7917**	**-0.8184**
NDVI	Unclassified	-0.4787	-0.5542	-0.5617	-0.6149	-0.6149	-0.6112	-0.6256	-0.6194
NDVI	Classified	-0.4658	-0.5060	-0.5447	**-0.6880**	**-0.6880**	**-0.6585**	**-0.6823**	**-0.6864**
NDRE	Unclassified	-0.4792	-0.5583	-0.5657	-0.6315	-0.6315	-0.6220	-0.6439	-0.6352
NRDE	Classified	-0.4666	**-0.5642**	-0.5412	**-0.7091**	**-0.7091**	**-0.6602**	**-0.7240**	**-0.7092**
CCCI	Unclassified	-0.4374	-0.5498	-0.5500	-0.5889	-0.5889	-0.5787	-0.5930	-0.5884
CCCI	Classified	0.2170	0.2148	-0.4582	**-0.7122**	**-0.7122**	**-0.6360**	**-0.7158**	**-0.6966**
GNDVI	Unclassified	-0.4312	-0.5159	-0.5041	-0.4475	-0.4475	-0.4335	-0.5019	-0.4737
GNDVI	Classified	0.1655	0.0965	0.2839	0.3730	0.3730	0.3938	-0.0789	0.2236
RE/Green	Unclassified	-0.4090	-0.5169	-0.5344	-0.4267	-0.4267	-0.4065	-0.4914	-0.4558
RE/Green	Classified	**0.4112**	0.4047	0.3348	0.1695	0.1695	0.2362	-0.1825	0.0631
RE/Red	Unclassified	-0.4287	-0.5459	-0.5652	-0.6928	-0.6928	-0.6967	-0.7105	-0.7085
RE/Red	Classified	**-0.4497**	**-0.5538**	-0.5350	**-0.7319**	**-0.7319**	-0.6766	**-0.7380**	**-0.7269**
% Pixel Retained		-0.4662	-0.5344	-0.5624	-0.5950	-0.5950	-0.6792	-0.6004	-0.6511

[a] Represents reflectance data collection timing: 7, 14, 21, or 28 d after application.

[b] Bold values represent an increase in Pearson correlation values when classified images were used for vegetative indices compared to unclassified images.

Table 22. Performance ranking of vegetative indices based on Pearson correlation values between vegetative indices calculated from unclassified and classified images and % pixel retained with dicamba application rate from 7, 14, 21, and 28 d after either the matchhead square or 2 weeks after first bloom application of dicamba on sensitive cotton from the 2019 growing season.

		Matchhead Square Application				Two Weeks After First Bloom Application			
		7	14	21	28	7	14	21	28
Index		------------------------ Rank ------------------------							
VARI	Unclassified	4 [a]	3	2	1	3	4	2	1
VARI	Classified	4	2	3	1	3	2	4	1
ExG	Unclassified	3	4	2	1	3	4	2	1
ExG	Classified	2	3	4	1	1	3	4	2
MGVRI	Unclassified	4	3	2	1	3	4	1	2
MGVRI	Classified	4	2	3	1	2	4	3	1
NDVI	Unclassified	4	3	2	1	3	4	1	2
NDVI	Classified	4	3	2	1	1	4	3	2
NDRE	Unclassified	4	3	2	1	3	4	1	2
NRDE	Classified	4	2	3	1	3	4	1	2
CCCI	Unclassified	4	3	2	1	2	4	1	3
CCCI	Classified	3	4	2	1	2	4	1	3
GNDVI	Unclassified	4	1	2	3	3	4	1	2
GNDVI	Classified	2	1	3	4	2	1	4	3
RE/Green	Unclassified	4	2	1	3	3	4	1	2
RE/Green	Classified	4	3	2	1	3	1	2	4
RE/Red	Unclassified	4	3	2	1	4	3	1	2
RE/Red	Classified	4	2	3	1	2	4	1	3
Overall		**4**	**3**	**2**	**1**	**3**	**4**	**1**	**2**
% Pixel Retained		4	3	2	1	4	1	3	2

[a] Reflectance data collection timing ranked from 1 – 4 (1 = highest correlation value, 4 = lowest correlation value) within each VI for each application timing based on Pearson correlation values between VI and application rate. Overall ranking represents the ranking of data collection timings over all VIs calculated with unclassified and classified images

Table 23. Pearson correlation (r) between vegetative indices computed from unclassified images, % pixel retained, 2,4-D application rate, visual injury, and relative lint yield and parameters of interest calculated from 14 d after either the matchhead square or 2 weeks after first bloom application of 2,4-D on sensitive cotton from 2019 to 2021.

	Matchhead Square Application			Two Weeks After First Bloom Application		
	Rate	Injury	RLY [a]	Rate	Injury	RLY
	---------- Pearson Correlation (r) ----------					
VARI	-0.8785	-0.8352	0.8196	-0.4056	-0.6406	0.3477
ExG	-0.7600	-0.7834	0.7629	-0.4222	-0.6697	0.3671
MGRVI	-0.8905	-0.8506	0.8136	-0.4114	-0.6430	0.3536
NDVI	-0.9127	-0.9017	0.7821	-0.3436	-0.6264	0.3128
NDRE	-0.9338	-0.9160	0.8021	-0.4124	-0.6422	0.4073
SCCCI	-0.5819	-0.5409	0.4543	-0.2968	-0.5454	0.3199
GNDVI	-0.8581	-0.9003	0.7088	0.0200	-0.1420	0.0131
RE/Green	-0.8184	-0.8213	0.6898	-0.0251	-0.1649	0.1431
RE/Red	-0.9321	-0.9032	0.8831	-0.3668	-0.6378	0.3711
% Pixel Retained	-0.8751	-0.8812	0.7561	-0.3654	-0.5829	0.3998
Rate	-	0.9680	-0.8912	-	0.7887	-0.9086
Injury	0.9680	-	-0.9150	0.7887	-	-0.8147
Relative Lint Yield	-0.8912	-0.9150	-	-0.9086	-0.8147	-

[a] Relative Lint Yield

Table 24. Pearson correlation (r) between vegetative indices computed from unclassified images, % pixel retained, dicamba application rate, visual injury, and relative lint yield and parameters of interest calculated from 14 d after either the matchhead square or 2 weeks after first bloom application of dicamba on sensitive cotton from 2019 to 2021.

	Matchhead Square Application			Two Weeks After First Bloom Application		
	Rate	Injury	RLY [a]	Rate	Injury	RLY
	---------- Pearson Correlation (r) ----------					
VARI	-0.7955	-0.8239	0.8221	-0.1463	0.0369	0.1419
ExG	-0.6630	-0.7148	0.6454	-0.1611	-0.0255	0.1249
MGRVI	-0.8112	-0.8376	0.8407	-0.1398	0.0463	0.1487
NDVI	-0.8057	-0.8321	0.8566	-0.1137	-0.0041	0.1825
NDRE	-0.8129	-0.8557	0.8585	-0.1477	-0.0767	0.2215
SCCCI	-0.5571	-0.5403	0.5826	-0.0799	-0.1195	0.1210
GNDVI	-0.6754	-0.7167	0.7492	-0.0105	-0.0612	0.1150
RE/Green	-0.5989	-0.6018	0.6570	-0.0153	-0.1644	0.1117
RE/Red	-0.8444	-0.8547	0.8747	-0.1603	-0.1507	0.1843
% Pixel Retained	-0.7591	-0.8233	0.7996	-0.1391	-0.1760	0.2087
Rate	-	0.9559	-0.8428	-	0.6808	-0.8183
Injury	0.9559	-	-0.9108	0.6808	-	-0.7389
Relative Lint Yield	-0.8428	-0.9108	-	-0.8183	-0.7389	-

[a] Relative Lint Yield

Table 25. Ranking of vegetative indices based on Pearson correlation values between unclassified images and auxin application rate, visual injury, and relative lint yield calculated from 14 d after either the matchhead square or 2 weeks after first bloom application on sensitive cotton from 2019 to 2021.

	Matchhead Square Application				Two Weeks After First Bloom Application			
	Rate	Injury	RLY [a]	**Overall**	Rate	Injury	RLY	**Overall**
Index	Rank							
2,4-D on XtendFlex								
VARI	5 [b]	6	2	**5**	4	4	5	**5**
ExG	8	8	6	**7**	1	1	3	**1**
MGRVI	4	5	3	**4**	3	2	4	**3**
NDVI	3	3	5	**3**	6	6	7	**6**
NDRE	1	1	4	**2**	2	3	1	**2**
SCCCI	9	9	9	**9**	7	7	6	**7**
GNDVI	6	4	7	**6**	9	9	9	**9**
RE/Green	7	7	8	**7**	8	8	8	**8**
RE/Red	2	2	1	**1**	5	5	2	**4**
Dicamba on Enlist Cotton								
VARI	5	5	5	**5**	4	7	5	**5**
ExG	7	7	8	**7**	1	8	6	**3**
MGRVI	3	3	4	**3**	5	6	4	**3**
NDVI	4	4	3	**4**	6	9	3	**7**
NDRE	2	1	2	**2**	3	4	1	**2**
SCCCI	9	9	9	**9**	7	3	7	**6**
GNDVI	6	6	6	**6**	9	5	8	**9**
RE/Green	8	8	7	**8**	8	1	9	**7**
RE/Red	1	2	1	**1**	2	2	2	**1**

[a] Relative Lint Yield

[b] Vegetative indices (VI) ranked from 1 – 9 (1 = highest correlation value, 9 = lowest correlation value) within each relationship between VI and parameter of interest (application rate, visual injury, and RLY). Overall ranking represents the ranking of VIs combined over all parameters of interest for each auxin and application timing combination.

Table 26. Root mean square errors, slopes, and sensitivity equivalents for selected vegetative indices computed from unclassified images 14 d after 2,4-D exposure and 2,4-D application rate, visual injury 14 d after 2,4-D exposure, and relative lint yield from 2019 to 2021.

	Matchhead Square Application			Two Weeks After First Bloom App.		
	Rate	Injury	RLY [a]	Rate	Injury	RLY
	---------------------------- Root Mean Square Error -----------------------------					
VARI	0.0900	0.1000	0.1100	0.1500	0.1300	0.1500
MGRVI	0.1100	0.1200	0.1400	0.1700	0.1500	0.1700
NDVI	0.0800	0.0700	0.1200	0.0700	0.0600	0.0700
NDRE	0.0700	0.0700	0.1200	0.0800	0.0800	0.0800
RE/Red	0.5500	0.6100	0.7100	1.0700	0.9700	1.0700
	--------------------------------------- Slope ---------------------------------------					
VARI	-0.1482	-0.0046	0.0043	-0.0583	-0.0048	0.0016
MGRVI	-0.1853	-0.0060	0.0053	-0.0676	-0.0055	0.0019
NDVI	-0.1497	-0.0045	0.0040	-0.0230	-0.0021	0.0007
NDRE	-0.1648	-0.0047	0.0044	-0.0326	-0.0029	0.0011
RE/Red	-1.2490	-0.0387	0.0369	-0.3739	-0.0343	0.0124
	-------------------------- Sensitivity Equivalent (SEq) --------------------------					
VARI	-1.6467	-0.0464	0.0391	-0.3888	-0.0369	0.0109
MGRVI	-1.6845	-0.0498	0.0377	-0.3976	-0.0367	0.0112
NDVI	-1.8713	-0.0648	0.0333	-0.3286	-0.0342	0.0098
NDRE	-2.3543	-0.0673	0.0368	-0.4074	-0.0362	0.0132
RE/Red	-2.2709	-0.0634	0.0519	-0.3494	-0.0353	0.0116

[a] Relative Lint Yield

Table 27. Root mean square errors, slopes, and sensitivity equivalents for selected vegetative indices computed from unclassified images 14 d after dicamba exposure and dicamba application rate, visual injury 14 d after dicamba exposure, and relative lint yield from 2019 to 2021.

	Matchhead Square Application			Two Weeks After First Bloom App.		
	Rate	Injury	RLY [a]	Rate	Injury	RLY
	---------------------------- Root Mean Square Error ------------------------------					
VARI	0.1100	0.1000	0.1000	0.1300	0.1300	0.1300
MGRVI	0.1300	0.1100	0.1200	0.1400	0.1400	0.1400
NDVI	0.0900	0.0700	0.0700	0.0700	0.0600	0.0700
NDRE	0.1000	0.0700	0.0900	0.0700	0.0700	0.0700
RE/Red	0.8600	0.8000	0.7800	1.0300	1.0100	1.0300
	--------------------------------------- Slope --					
VARI	-0.1294	-0.0055	0.0040	-0.0169	-0.0004	0.0006
MGRVI	-0.1546	-0.0067	0.0048	-0.0177	-0.0005	0.0007
NDVI	-0.1035	-0.0042	0.0033	-0.0072	-0.0000	0.0004
NDRE	-0.1246	-0.0045	0.0040	-0.0092	-0.0004	0.0005
RE/Red	-1.1990	-0.0505	0.0375	-0.1484	-0.0119	0.0061
	-------------------------- Sensitivity Equivalent (SEq) ---------------------------					
VARI	-1.1764	-0.0551	0.0403	-0.1299	-0.0028	0.0045
MGRVI	-1.1892	-0.0612	0.0402	-0.1264	-0.0035	0.0048
NDVI	-1.1500	-0.0593	0.0474	-0.1028	-0.0003	0.0059
NDRE	-1.2460	-0.0646	0.0441	-0.1317	-0.0064	0.0071
RE/Red	-1.3942	-0.0632	0.0480	-0.1441	-0.0118	0.0059

[a] Relative Lint Yield

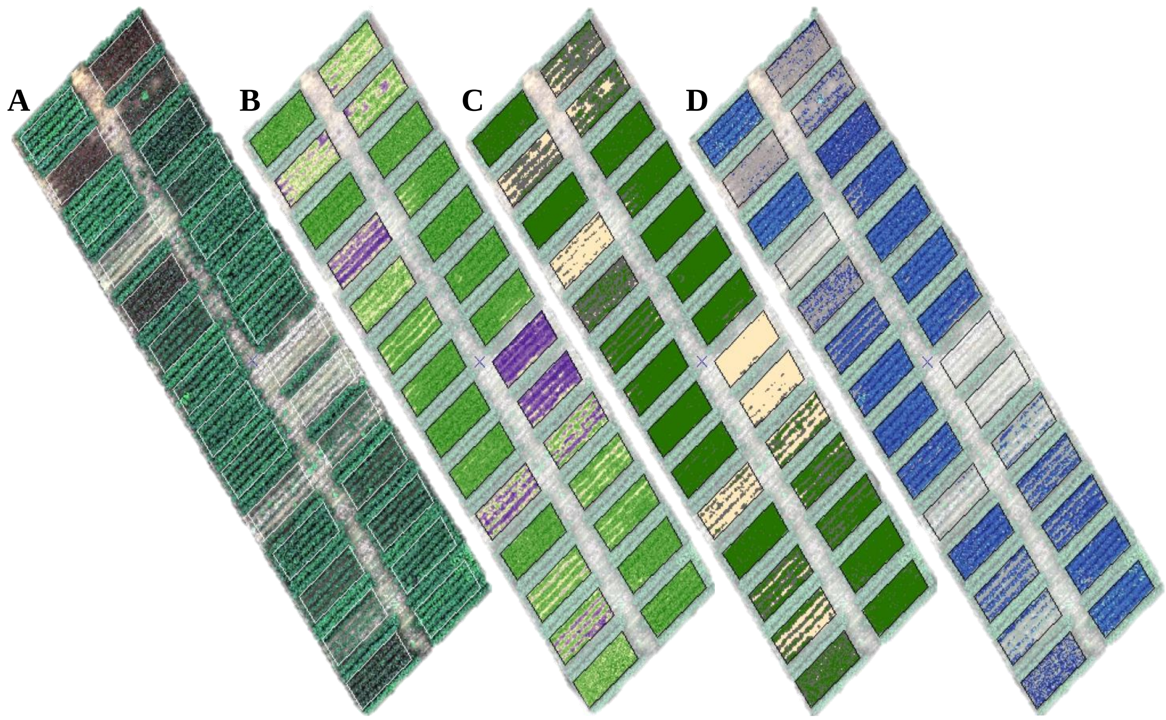

Figure 1. Research trial plots at Ames AgResearch and Education Center taken 14 d after first bloom + 2-week application of 2,4-D on susceptible cotton in 2019. Polygons represent experimental units (A). Calculated NDVI layer over orthomosaic image (green = 1; purple = 0) (B). Iso Cluster Unsupervised Classification of NDVI resulted in 3 classes: vegetation (green), canopy (grey) and soil (tan) (C). Image was reclassified to only carry forward vegetative growth (D).

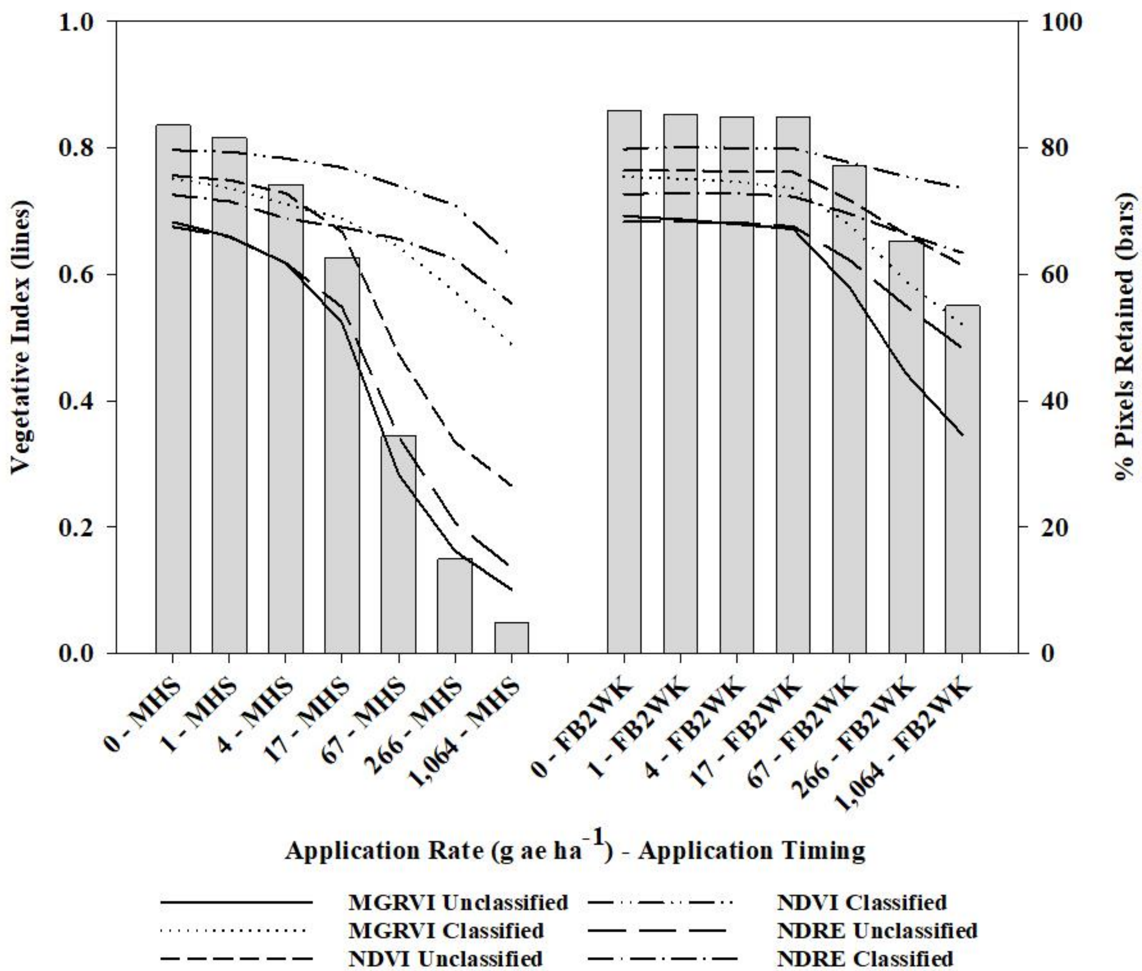

Figure 2. Vegetative index values calculated from both unclassified and classified images (lines) collected 14 d after application and the percent of pixels retained from the classification procedure (bars) for applications of 2,4-D at various rates at both matchhead square (MHS) and two weeks after first bloom (FB+2WK).

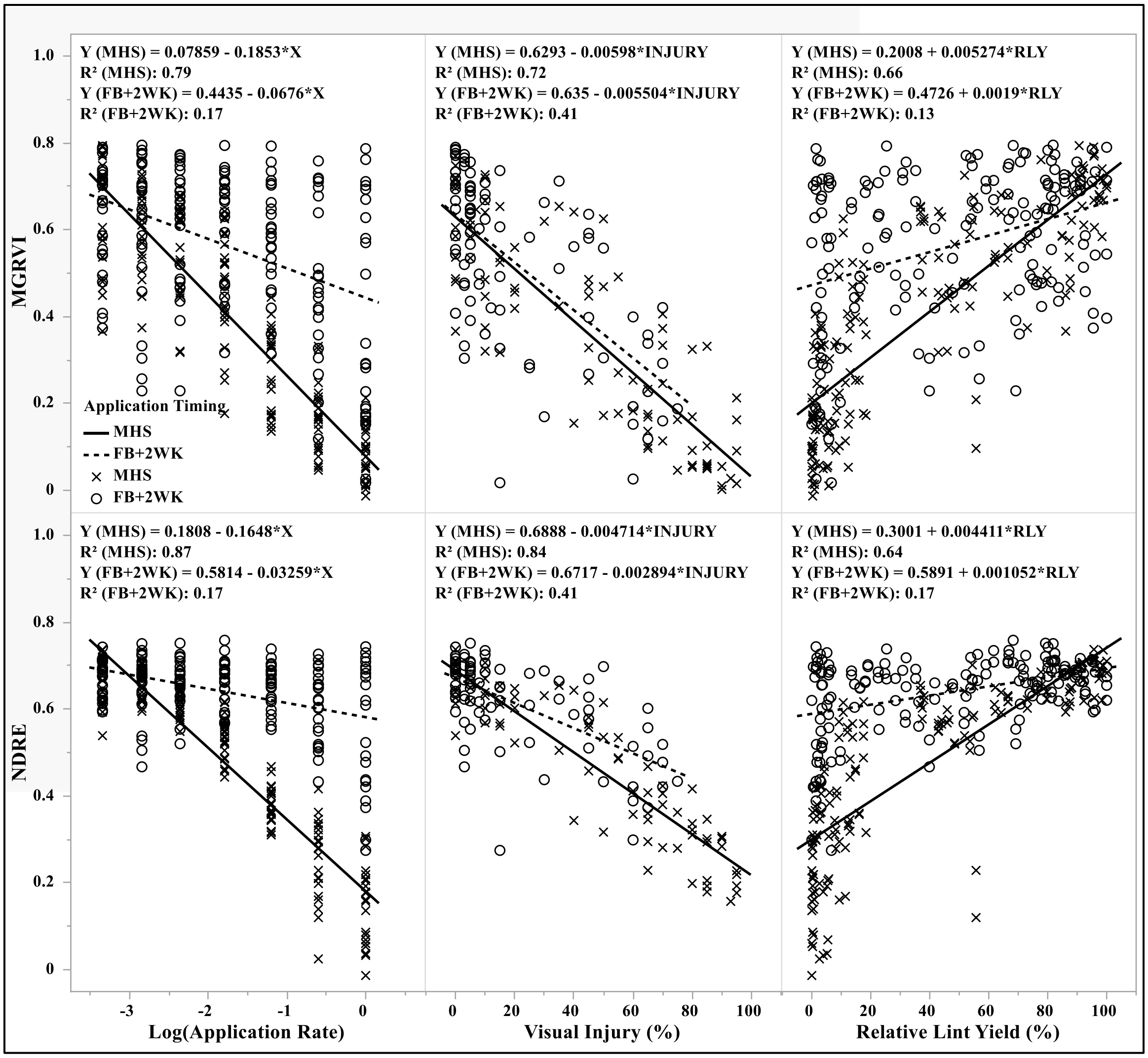

Figure 3. Relationships between Modified Green-Red Vegetation Index (MGRVI) and Normalized Difference Red-Edge (NDRE) 14 d after 2,4-D application to sensitive cotton with log transformed application rate, visual injury observed 14 d after application, and relative lint yield from experiments located in Grand Junction, TN from 2019 to 2021.

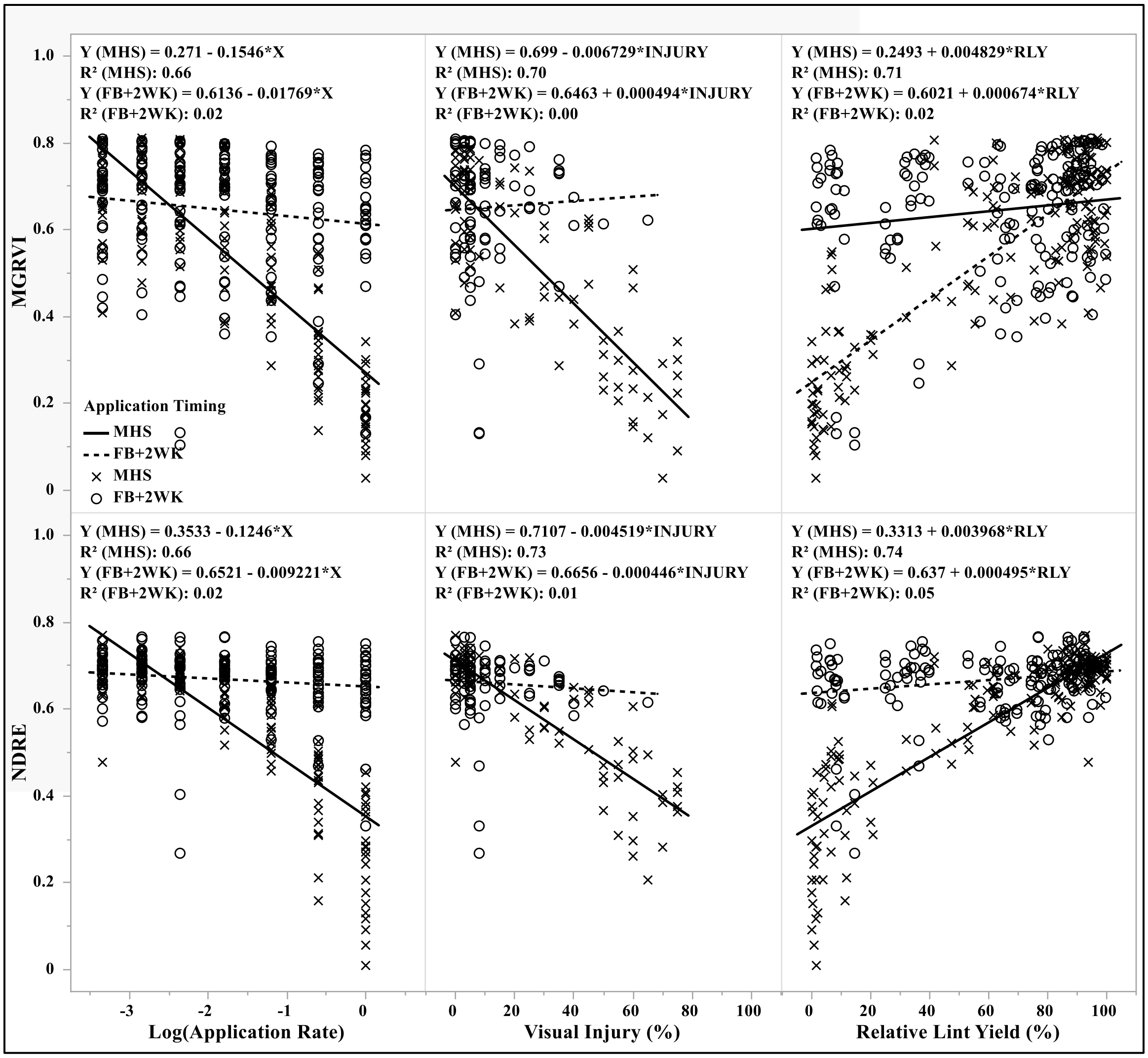

Figure 4. Relationships between Modified Green-Red Vegetation Index (MGRVI) and Normalized Difference Red-Edge (NDRE) 14 d after dicamba application to sensitive cotton with log transformed application rate, visual injury observed 14 d after application, and relative lint yield from experiments located in Grand Junction, TN from 2019 to 2021.

CHAPTER IV: SYNTHETIC AUXIN INJURY ON SUSCEPTIBLE COTTON, PART II: EFFECTS ON YIELD COMPONENTS

Abstract

Auxin-tolerant cotton (*Gossypium hirsutum* L.) cultivars are the latest tools producers use to combat herbicide-resistant weed species during the growing season. The widespread implementation of auxin-tolerant crops has led to an increase in in-season applications of auxins. Auxin drift has subsequently become a more prominent issue in the agricultural industry and causes producers to shift management tactics. Yield partitioning research based on auxin application timing has been conducted but more information is needed concerning application rate and the interaction between application rate and timing. Experiments were conducted from 2019 to 2021 in Grand Junction, TN to determine the effects of synthetic auxin exposure on boll positioning, development, and production. Applications of 2,4-D or dicamba were made to cotton cultivars of the opposite technology at either matchhead square or two weeks after first bloom. Non-treated experimental units were also included. More severe impacts on overall lint yield, yield partitioning, and yield components were observed following exposure to 2,4-D than dicamba. Application rate and timing also impacted yield components and partitioning. Exposure to 2,4-D during vegetative growth caused increased partitioning to vegetative and aborted fruiting positions but decreased partitioning to position 1, zone 2 (nodes 9 through 12), and zone 3 (nodes 13 and above) as application rate increased. Exposure to investigated 2,4-D rates at FB+2WK and dicamba rates at MHS and FB+2WK did not impact percent yield partitioning. Environmental conditions following applications of 2,4-D or dicamba plan an important role in the recovery and growth of cotton and subsequent yield penalties.

Introduction

Integrated weed control practices for control of troublesome weed species are necessary for extending the lifespan of currently available herbicides. Weed control practices include but are not limited to crop and herbicide mode of action (MOA) rotation, the use of residual herbicides, or the implementation of cover crops (Culpepper, 2006). Unfortunately, there may be escapes of troublesome weed species that can cause issues later into the growing season and the next year. To combat herbicide-resistant broadleaf weed species, seed companies have developed and released corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and soybean (*Glycine max* (L.) Merr.) cultivars with resistance to synthetic auxin herbicides.

The auxin-resistant traits allow for an additional broadcast postemergence weed control options. These traits are particularly important in cotton, as there are limited postemergence herbicide options effective on glyphosate-resistant *Amaranthus palmeri*. Corteva's Enlist™ system (Corteva Agriscience, Indianapolis, IN) allows PhytoGen™ cultivars that include the Enlist™ trait to be treated with 2,4-D in-season. Bayer's XtendFlex™ system (Bayer Crop Science, St. Louis, MO) allows cultivars which include the XtendFlex™ trait to be treated with dicamba in-season. Both systems (Enlist and XtendFlex) also provide tolerance to post applications of glufosinate and glyphosate.

In 2020, 73 percent of U.S. cotton acreage planted carried the XtendFlex™ trait followed by 17 percent which carried the Enlist™ trait (USDA, 2020). This widespread adoption of synthetic auxin-resistant cotton cultivars can be attributed to combatting herbicide-resistant weed species and to protecting against auxin drift from neighboring fields (Buol et al., 2019a; Cahoon et al., 2015).

Synthetic auxin herbicides were some of the first developed herbicides for broadleaf weed control (Egan et al., 2014). They are widely used compounds because of their versatility that allows them to be used in numerous applications. However, some synthetic auxins are volatile compounds that may cause distinct visual symptomology on sensitive plant species (Egan et al., 2014; Wax et al., 1969). Complaints of off-target movement of auxin herbicides have occurred since their release. Off-target applications of dicamba and 2,4-D can occur due to particle drift, vapor drift, or sprayer contamination (Buol et al., 2019a; Cundiff et al., 2017; Egan et al., 2014).

In susceptible plant species, synthetic-auxin herbicides mimic the function of indole-3-acetic acid (IAA), a naturally occurring plant growth hormone (Song, 2014). Susceptible cotton is generally more sensitive to 2,4-D whereas susceptible soybeans are more sensitive to dicamba applications – meaning that lower concentrations of the active ingredient are required to produce visual injury symptoms and yield effects (Egan et al., 2014; Johnson et al., 2012). Injury symptoms observed in cotton plants are unique to synthetic auxins and include epinastic growth, leaf strapping or cupping, twisting, chlorosis, stunting, and loss of apical dominance (Byrd et al., 2016; Sciumbato et al., 2004). Exposure to auxin-like herbicides also delays maturity of the cotton crop (Buol et al., 2019a). Low-dose exposures do not always impact yield but end of season management may require adjustments to account for the delayed maturity in cotton.

Cotton growth stage at time of synthetic auxin exposure impacts the severity of the injury and yield effects (Buol et al., 2019a; Buol et al., 2019b; Egan et al., 2014). Visual injury symptoms observed in season can oftentimes only serve as a guideline to yield effects (Egan et al., 2014). In the meta-analysis conducted by Egan et al. (2014), severe visual injury due to dicamba exposure often did not translate to yield loss while severe visual injury due to 2,4-D exposure resulted in yield reduction. Byrd et al. (2016) observed yield effects correlated with boll population rather than visual injury ratings. Conversely, Egan et al. (2014) concluded that yield effects were reduced when cotton was exposed to 2,4-D during reproductive stages.

Buol et al. (2019a; 2019b) observed that while yield may not be impacted when sensitive cotton is exposed to dicamba or 2,4-D during early reproductive stages (PHS, MHS, and early flowering), fruiting structures will partition more to upper nodes and outer positions which may impact maturity and fiber quality. Suarez et al. (2017) determined that yield response to dosage levels in cotton exposed to 2,4-D with four to eight true leaves was difficult to distinguish but if cotton with 11 to 12 nodes was sprayed, dosage responses were distinguishable at harvest. The severity of auxin injury and recovery is dependent on environment, water deficits will likely cause more severe injury and yield impacts (Buol et al., 2019b; Byrd et al., 2015; Sciumbato et al., 2014).

While previous research has investigated timing effects of synthetic auxin exposure, the objective of this research was to determine effects of auxin application rate and timing on boll development and partitioning, seed production, and fiber quality. The hypotheses of this research are that (1) more drastic impacts will be observed due to 2,4-

D exposure compared to dicamba, (2) auxin exposure during vegetative growth will have a greater impact on yield partitioning in that bolls will be more prevalent on upper and outer positions, but (3) greater yield penalties will accompany auxin exposure during reproductive stages.

Materials and Methods

Field experiments were conducted from 2019 to 2021 at the University of Tennessee Ames AgResearch and Education Center located near Grand Junction, TN on a Memphis silt loam (fine-silty, mixed, active, thermic Typic Hapludalfs) to evaluate synthetic auxin effects on the yield components of sensitive cotton cultivars. Two experiments were conducted at this location: 2,4-D-tolerant cotton sprayed with dicamba and dicamba-tolerant cotton sprayed with 2,4-D. Less than 1% of TN cotton acreage is planted without an auxin-tolerant trait; therefore, using a cultivar with sensitivity to both auxins would not have been representative of TN cotton production systems (USDA, 2020). Experimental units consisted of six, 97 cm spaced rows that were 9 m in length and arranged in a randomized complete block design with four replications for each experiment.

Cotton was seeded on at 98,800 seeds ha^{-1} during May of each year (Table 28). Varieties selected for these experiments were PHY 400 W3FE (Corteva Agriscience, Indianapolis, IN), a 2,4-D tolerant variety, and DP 1725 B2XF (Bayer CropScience, Research Triangle Park, NC), a dicamba-tolerant variety. Cotton was managed based on University of Tennessee Extension agronomic and pest management recommendations (Raper, 2016). Maintenance herbicide applications did not include 2,4-D or dicamba.

Dicamba and 2,4-D application timings were triggered at matchhead square (MHS) and two weeks following first bloom (FB+2WK) (Table 28). Treatments consisted of 2,4-D choline salt (Enlist One™ with Colex-D™ Technology, Dow AgroSciences, Indianapolis, IN) at the following rates: 1,064 (1X), 266 (1/4X), 67 (1/16X), 17 (1/64X), 4 (1/256X), and 1 (1/1024X) g ae ha^{-1} and dicamba (Xtendimax® with VaporGrip® Technology, Bayer Crop Science, St. Louis, MO) at the following rates: 558 (1X), 139 (1/4X), 35 (1/16X), 9 (1/64X), 2 (1/256X), and 0.5 (1/1024X) g ae ha^{-1}. Non-treated control plots were included for each application timing. The center four rows of each experimental unit were treated, leaving a two-row border between treated areas to minimize drift effects. Applications were made with a MudMaster Multi-Purpose Sprayer (Bowman Manufacturing, Newport, AR) operating at a pressure of 276 kPa with XR 11002 (TeeJet Technologies, Springfield, IL) nozzles and an application volume of 140 L ha^{-1} at a ground speed of 6.4 km hr^{-1}.

Plant height and total node count were collected from six plants within each experimental unit 14 and 28 days after each application. Yield components were determined after collecting and processing a 25-boll seed cotton sample from each experimental unit. Boll size (g) was determined by dividing the total seed cotton weight by 25. Boll samples were ginned and separated into the seed and fiber. Lint percentage was determined by dividing the lint weight (g) by the total seed cotton weight (g) and multiplying by 100. The weights of two samples of 50 fuzzy seed were also recorded. The seed index was determined by averaging the two, 50-seed weights and then multiplying by 2. Lint index was calculated by multiplying the lint percentage by the seed index and dividing that value by the difference of 100 minus the lint percentage. The number of seeds per boll was calculated by multiplying boll size by the difference of 100 minus lint percentage and then dividing that value by the seed index. The lint from the ginned boll sample was sent for HVI analysis at the USDA Fiber Classing Office in Memphis, TN.

Cotton plants in one meter of row were collected from nontreated experimental units, and those treated with 1/64X, 1/256X, and 1/1024X rates of dicamba or 2,4-D. Yield partitioning data was focused on the three lowest rates of each auxin herbicide and the nontreated control to determine differences in yield partitioning even if mechanical yield was not impacted. Collected plants were transported back to a storage facility and box mapped according to procedures described by Jenkins et al. (1990). Cotton plants were broken down into three vertical zones: zone one containing fruit from sympodial branches up to the eighth node; zone two containing fruiting positions from nodes nine through 12; and zone three containing bolls from nodes 13 and above. Horizontal positioning was determined by distance from the main stem, meaning the bolls harvested from the fruiting position closest to the main stem were classified as position one, the next fruiting position was classified as position two, and any remaining fruiting positions were classified as position three.

Bolls harvested from monopodial branches were classified as vegetative and any bolls harvested from fruiting positions that occurred after the plant had lost apical dominance were classified as aborted. Vegetative and aborted fruiting sites were treated as independent from both horizontal and vertical partitioning. Both boll counts and weights were recorded for each fruiting site. Yield partitioning data for each zone and position are presented as the percentage of total seed cotton weight. The number of bolls collected from 1 m of row was recorded and converted to the number of bolls per hectare based on box mapping data. Boll counts generated from box mapping data include bolls that would not be mechanically harvested due to them being hard-locked, malformed, or too small; therefore, the number of bolls per hectare was also calculated using mechanical harvest data. Seed cotton yields were converted to g ha^{-1} and then divided by the boll size generated from boll sample data. Mechanical yields were collected by harvesting the center two rows of each experimental unit with a spindle picker modified for small-plot research.

Data were analyzed in SAS (v 9.4, SAS Institute Inc., Cary, NC) using the PROC MIXED procedure. Analysis was separated by experiment. Application rate and timing were fixed effects whereas year and replication were considered as random effects for each analysis. Data were analyzed with year as a random effect to make inferences regarding synthetic auxin effects over a range of environments (Blouin et al., 2011; Gbur et al., 2012). Means were subjected to analysis of variance and separated using Fischer's Protected LSD at $\alpha = 0.05$. Figures were developed to illustrate yield partitioning using SigmaPlot (v. 14.5, Systat Software Inc., Chicago, IL).

Results and Discussion

2,4-D Applied to Dicamba-Tolerant Cotton

Cotton plant height and total nodes were affected by application rate 14 and 28 d after MHS application and 28 d after FB+2WK application (Table 29; $p \leq 0.0060$). In general, plant height and total nodes were reduced as 2,4-D rate increased. Reduction in plant height and total nodes occurred both 14 and 28 d after MHS application when 17 g ae ha^{-1} or greater was applied at MHS. No differences in plant height or total nodes were observed amongst application rates 14 d after plots treated at FB+2WK; heights ranged from 98 to 106 cm and total nodes ranged from 16 to 18. By 28 d after FB+2WK application, reductions in plant height and total nodes were observed when 266 and 1,064 g ae ha^{-1} were applied. In contrast, Buol et al. (2019b) observed cotton height increased at the end of the season in plots that had been exposed to 8 g ae ha^{-1} at squaring and early flowering. Sciumbato et al. (2014) observed height reduction compared to a nontreated control following applications of 2,4-D greater than 0.01 times a 0.53 kg ai ha^{-1} use rate of 2,4-D at 4- to 6-leaf cotton in two out of three years but differences in total node counts were minimal across all rates investigated. Slight differences in data collection timings from year to year and the environment after application likely contributed to differences in reported plant height responses.

Mechanical lint yield was affected by an interaction between 2,4-D application rate and timing (Table 30; $p < 0.0001$). Amongst applications made at MHS, the untreated and applications of 1 g ae ha^{-1} (1,536 and 1,468 kg ha^{-1}, respectively) outyielded applications of 4 g ae ha^{-1} and greater (35 – 856 kg ha^{-1}). Amongst FB+2WK applications, the untreated and applications of 1 g ae ha^{-1} (1,521 and 1,323 kg ha^{-1}, respectively) outyielded applications of 17 g ae ha^{-1} and greater (41 – 848 kg ha^{-1}). Results agree with those of Buol et al. (2019b), who recorded an 18 – 21% reduction in yield relative to the untreated control when cotton was exposed to 8.3 g ae ha^{-1} 2,4-D at 7-leaf stage, pinhead square (PHS), and MHS. These results also agree with Everitt and Keeling (2009) who observed a decrease in cotton lint yield as 2,4-D application rate increased when applied from vegetative growth stages to first bloom. Lint percentage was also affected by an interaction between application rate and timing, but differences were slight; percentages only ranged from 44-47% (Table 30; $p < 0.0001$).

The percent of total seed cotton partitioned to position 1 was impacted by the interaction between application rate and timing (Table 31; $p < 0.0001$; Fig. 5). For MHS applications, each increase in 2,4-D rate resulted in a decrease in the percentage of total seed cotton weight partitioned to position 1. No differences were observed between application rate for FB+2WK application (54 – 62%). Application rate and timing each effected the percentage of total seed cotton partitioned to position 2 (Table 31; $p \leq 0.0120$; Fig. 5). The untreated experimental units and plots which received 1 g ae ha^{-1} had 19 percent of the total seed cotton weight partitioned to position 2, which was greater than plots treated with 4 and 17 g ae ha^{-1} (15 and 12%, respectively) regardless of application timing. Applications made at FB+2WK (18%) resulted in a greater percentage of total seed cotton weight partitioned to position 2 than applications made at MHS (15%), regardless of application rate. Application rate impacted partitioning to position 3 (Table 31; $p = 0.0004$; Fig. 5); cotton treated with 17 g ae ha^{-1} (9%) had a greater percentage of total seed cotton weight partitioned to position 3 than the untreated and cotton treated with 4 and 1 g ae ha^{-1} (3 – 4%).

Partitioning within zone 1 (nodes 5 – 8) was not impacted by application rate or timing (Table 31; $p \geq 0.2842$; Fig. 5). Vertical partitioning was impacted by the 2,4-D application rate and timing interaction in zone 2 (nodes 9 – 12) and 3 (nodes 13 and above) (Table 31; $p \leq 0.0316$; Fig. 5). Within zone 2, the untreated and applications of 1 g ae ha^{-1} applied at MHS (40 and 33%, respectively) had a greater percentage of total seed cotton weight than 4 and 17 g ae ha^{-1} applied at MHS (15 and 11%, respectively). No differences were observed within zone 2 between application rate for FB+2WK applications (36 – 44%). Within zone 3, applications of 1 g ae ha^{-1} at MHS (23%) resulted in a greater percentage of total seed cotton than applications of 4 and 17 g ae ha^{-1} at MHS (16 and 5%, respectively). Untreated experimental units for the FB+2WK application timing (24%) resulted in a greater percentage of total seed cotton in zone 3 than applications of 4 and 17 g ae ha^{-1} at FB+2WK (17 and 15%, respectively).

Boll partitioning to both vegetative (fruiting positions on sympodial branches) and aborted (fruiting positions after loss of apical dominance) fruiting positions were impacted by the interaction of application rate and timing (Table 31; $p \leq 0.0230$; Fig. 5). Applications of 2,4-D at 4 and 17 g ae ha^{-1} at MHS (42 and 38%, respectively) resulted in a greater percentage of total seed cotton weight in vegetative fruiting positions than the untreated and applications of 1 g ae ha^{-1} at MHS (13 and 20%, respectively). Applications of 17 g ae ha^{-1} at MHS (24%) had a greater percentage of total seed cotton weight on aborted fruiting positions than the untreated and applications of 1 and 4 g ae ha^{-1} at MHS (1, 6, and 12%, respectively). No differences were observed between application rates of the FB+2WK application for either vegetative or aborted fruiting positions. Similar results were reported by Buol et al. (2019b), who also observed a general decrease in percent total seed cotton weight to position 1, position 2, zone 1, and zone 2 when 8.3 g ae ha^{-1} 2,4-D was applied from early vegetative to MHS stage. Similarly, the boll distribution in these scenarios was shifted to vegetative and aborted fruiting positions.

The number of bolls present per hectare was affected by the interaction of application rate and timing when data was generated from yield partitioning data and from mechanical yield data (Table 32; p = 0.0170 and 0.0479, respectively). From the data generated from yield portioning data, there was no difference amongst application timing when the untreated and 2,4-D rates of 1 and 4 g ae ha^{-1} (848,000 – 911,000, 871,000 – 949,000, and 701,000 – 805,000 bolls ha^{-1}, respectively) were applied. More bolls were produced per hectare when 17 g ae ha^{-1} was applied at FB+2WK (684,000 bolls ha^{-1}) compared to 17 g ae ha^{-1} at MHS (380,000 bolls ha^{-1}). When boll counts per hectare were generated using mechanical yield data, more accurately representing harvestable boll counts, there was no difference amongst application timing when the untreated and 2,4-D rates of 1, 4, 67, 266, and 1,064 g ae ha^{-1} (746,000 – 805,000, 660,000 – 823,000, 512,000 – 618,000, 98,200 – 294,000, 105,000 – 218,000, and 32,800 – 73,100 bolls ha^{-1}, respectively) were applied. More bolls were produced per hectare when 17 g ae ha^{-1} was applied at FB+2WK (531,000 bolls ha^{-1}) compared to 17 g ae ha^{-1} at MHS (307,000 bolls ha^{-1}).

Seed index was not impacted by 2,4-D application rate or timing (Table 32; $p \geq 0.0726$). Seed index ranged from 8.3 to 9.6 g per 100 seed. Lint index was affected by application rate (Table 32; $p = 0.0074$). Across application timing, a greater lint index was observed with the untreated and when applications of 1 g ae ha^{-1} (8.0 and 8.0, respectively) compared to applications of 67, 266, and 1,064 g ae ha^{-1} (7.5, 7.2, and 7.3, respectively).

The number of seeds per boll was impacted by both application rate and application timing (Table 32; $p \leq 0.0105$). The greatest number of seed per boll was produced with the untreated and applications of 2,4-D at 1 g ae ha^{-1} with 30 and 29 seed per boll, respectively. As application rate increased, the number of seeds per boll decreased. Applications of 2,4-D at FB+2WK produced bolls with an average of 27 seed per boll which was greater than bolls produced when 2,4-D applications were made at MHS (25 seed $boll^{-1}$).

Cotton boll size was affected by the interaction of application rate and timing (Table 32; $p = 0.0013$). No differences in boll size between application timing were observed for the untreated and the following 2,4-D rates: 1, 266, and 1,064 g ae ha^{-1} (5.2 – 5.3, 5.0 – 5.2, 3.6 – 3.8, and 3.2 – 3.6 g, respectively). Applications of 4, 17, and 67 g ae ha^{-1} made at FB+2WK (5.2, 4.7, and 4.3 g, respectively) produced a larger boll size than those same application rates at MHS (4.3, 4.1, and 3.8, respectively). In contrast, Sciumbato et al. (2014) did not observe differences in boll weights when low-rates of 2,4-D were applied to cotton at 4- to 6-leaf stage. It is likely that the earlier timing of exposure in the Sciumbato et al. (2014) experiments could have limited the boll size impacts compared to the MHS and FB+2WK exposures tested within these experiments.

Fiber micronaire was impacted by application rate of 2,4-D (Table 33; $p < 0.0001$). The untreated and treatments that received 1 and 4 g ae ha^{-1} (4.7, 4.7, and 4.5, respectively) across application timings had greater micronaire than treatments that received 67, 266, and 1,064 g ae ha^{-1} (4.2, 4.2, and 4.2, respectively). Similar reductions in micronaire were captured by Buol et al. (2019b) when cotton was exposed to 8.3 g ae ha^{-1} 2,4-D during PHS and MHS.

Fiber length was affected by both application rate and timing (Table 33; $p \leq 0.0163$). Longer fibers were produced with the untreated and applications of 1, 4, and 17 g ae ha^{-1} (1.17, 1.17, 1.18, and 1.17 in, respectively) than applications of 266 g ae ha^{-1} (1.12 in). Applications made at MHS (1.18 in) produced longer fibers than applications made at FB+2WK (1.13 in). Buol et al. (2019b) observed a reduction in fiber length when applications of 8.3 g ae ha^{-1} were made during flowering.

Fiber strength was impacted by the interaction between application rate and timing (Table 33, $p = 0.0167$). Amongst 2,4-D applications made at MHS, greater fiber strength was accomplished with 17 g ae ha^{-1} (31.2 g tex^{-1}) compared to the untreated (30.0 g tex^{-1}). Among applications made at FB+2WK, greater fiber strength with the untreated (30.1 g tex^{-1}) compared to applications of 266 g ae ha^{-1} (28.5 g tex^{-1}) which also produced greater fiber strength than applications of 1,064 g ae ha^{-1} (25.1 g tex^{-1}).

Uniformity was impacted by the interaction of application rate and timing (Table 33; $p = 0.0004$). There were no differences in fiber length uniformity between application rates at MHS; uniformity ranged from 82.5 to 83.7%. The untreated and applications of 2,4-D at 1 and 4 g ae ha^{-1} at FB+2WK (83.5, 82.9, and 82.5%, respectively) produced more uniform fibers than applications of 67, 266, and 1,064 g ae ha^{-1} at FB+2WK (81.1, 79.5, and 78.5%, respectively). Buol et al. (2019b) observed reduction in fiber uniformity when 8.3 g ae ha^{-1} was applied to sensitive cotton at MHS and first bloom. While differences in fiber quality were observed due to 2,4-D exposure, most values did not fall within a range to cause discounted market values. The exception to this statement was with fiber strength and uniformity when a full rate of 2,4-D was applied at FB+2WK.

Dicamba Applied to 2,4-D-Tolerant Cotton

Cotton plant height and total nodes were affected by dicamba rate 14 and 28 d after MHS application and 28 d after FB+2WK application (Table 34; $p \leq 0.0311$). Reductions in plant height and total nodes occurred both 14 and 28 d after MHS application when 35 g ae ha^{-1} or greater was applied. No differences in plant height or total nodes were observed amongst dicamba rates 14 d after plots were treated at FB+2WK. At 28 d after FB+2WK application, plants were shorter in plots that received 558 and 139 g ae ha^{-1} than the untreated and plots that were treated with 0.5 g ae ha^{-1}. Cotton plants in plots that received 558 and 139 g ae ha^{-1} at FB+2WK had fewer total nodes 28 d after FB+2WK application than the untreated and plots that received 0.5 or 2 g ae ha^{-1}.

Mechanical lint yield was affected by an interaction between dicamba application rate and timing (Table 35; $p = 0.0017$). No differences in lint yield between application timings were observed between the untreated experimental units and plots treated with 0.5, 2, 9, and 35 g ae ha^{-1} (1,294 – 1,465, 1,478 – 1,513, 1,316 – 1,515, 1,283 – 1,435, and 912 – 962 kg ha^{-1}, respectively). These data agree with previous findings which observed minimal to no yield reductions following low-rate exposure to dicamba (Egan et al., 2014; Everitt and Keeling, 2009). Buol et al. (2019a) observed a decrease in yield relative to the non-treated control when 35 g ae ha^{-1} dicamba was applied seven weeks after emergence which corresponded with MHS. Plots treated with 139 and 558 g ae ha^{-1} at FB+2WK (493 and 250 kg ha^{-1}, respectively) outyielded plots treated with those same rates at MHS (181 and 26 kg ha^{-1}, respectively).

Lint percentage was impacted by the interaction between application rate and timing (Table 35; $p < 0.0001$). No differences were observed in lint percentage between application timings for the untreated and the following dicamba rates: 0.5, 2, and 9 g ae ha^{-1} (46 – 47%). However, lint percentage for plots treated with 35, 139, and 558 g ae ha^{-1} at FB+2WK (46, 43, and 41%, respectively) was greater than those same rates at MHS (47, 46, and 47%, respectively). In contrast, Buol et al. (2019a) did not observe a timing effect on lint percentage when 35 g ae ha^{-1} was applied.

The percent of total seed cotton partitioned to position 1 was impacted by the interaction between dicamba application rate and timing (Table 36; $p = 0.0206$; Fig. 6). For MHS applications, a greater percentage of total seed cotton weight was partitioned to position 1 when untreated (71%) than when 9 g ae ha^{-1} (63%) was applied. The opposite was true for application rates applied at FB+2WK where a greater percentage of total seed cotton weight was partitioned to position 1 when 9 g ae ha^{-1} (71%) was applied than the untreated (62%). Partitioning within position 2 was not impacted by application rate or timing (Table 36; $p \geq 0.2288$; Fig. 6). The percentage of total seed cotton weight partitioned to position 2 ranged from 13 – 16%. Partitioning to position 3 was impacted by the interaction between application rate and timing (Table 36; $p = 0.0162$; Fig. 6). For MHS applications, a greater percentage of total seed cotton weight was partitioned to position 2 for the untreated and when 9 g ae ha^{-1} (2 and 3%) was applied than when 2 g ae ha^{-1} (0%) was applied. For dicamba rates applied at FB+2WK, a greater percentage of total seed cotton weight was partitioned to position 2 for the untreated (3%) than when 0.5 and 9 g ae ha^{-1} (2 and 2%) was applied.

Partitioning within zone 1 (nodes 5 – 8) was impacted by application timing (Table 36; $p = 0.0379$; Fig. 6). When cotton was exposed to dicamba at FB+2WK (25%), a greater percentage of seed cotton was partitioned to zone 1 than when cotton was exposed to dicamba at MHS (22%). Vertical partitioning was not impacted by dicamba application rate or timing in zone 2 (nodes 9 – 12) and 3 (nodes 13 and above) (Table 36; $p \geq 0.2238$; Fig. 6). Partitioning to zone 2 ranged from 39 – 45% of total seed cotton weight and partitioning in zone 3 ranged from 17 – 21% of total seed cotton weight.

Boll partitioning to vegetative (fruiting positions on sympodial branches) fruiting positions was impacted by the interaction of application rate and timing (Table 36; $p = 0.0381$; Fig. 6). No differences were observed amongst dicamba rates applied at MHS; the percentage of total seed cotton weight partitioned to vegetative positions ranged from 13 – 18%. The untreated plots for the FB+2WK application timing yielded 19% of fruit partitioned to vegetative positions compared to 12% when 9 g ae ha^{-1} was applied at

FB+2WK. No differences were observed in terms of partitioning to aborted fruiting positions (fruiting positions following loss of apical dominance) (Table 36; $p \geq 0.7875$; Fig. 6). The percentage of total seed cotton weight partitioned to aborted fruiting positions ranged from 1 – 2%. In contrast, Buol et al. (2019a) observed pre-flowering exposure of 35 g ae ha^{-1} dicamba caused less partitioning to position 1, position 2, zone 1, and zone 2 and more partitioning to vegetative and aborted fruiting positions compared to the non-treated control.

The number of bolls present per hectare was affected by application rate and timing of dicamba when data were calculated based on yield partitioning data (Table 37; $p \leq 0.0336$). Across application timings, 891,000 bolls ha^{-1} were produced when 9 g ae ha^{-1} was applied which was greater than the untreated and when 2 g ae ha^{-1} (814,000 and 788,000 bolls ha^{-1}, respectively) was applied. Following dicamba applications at MHS, 870,000 bolls ha^{-1} were produced compared to 809,000 bolls ha^{-1} produced following applications at FB+2WK. Boll counts per hectare were also calculated using mechanical yield data to represent harvestable boll counts more accurately; the number of bolls ha^{-1} calculated in this manner was impacted by the interaction between application rate and timing (Table 37; $p = 0.0007$). The three lowest dicamba application rates at both application timings and the untreated for the FB+2WK application timing had more bolls ha^{-1} (673,000 – 751,000 bolls ha^{-1}) than applications of dicamba at 35, 139, and 558 g ae ha^{-1} at both MHS and FB+2WK (50,700 – 522,000 bolls ha^{-1}). Foster and Griffin (2019) evaluated soybean yield components following exposure to dicamba of 1/2 to 1/1000 of the labeled rate at vegetative and reproductive growth stages; reduction in the number of main stem pods per plant occurred beginning with 0.6 g ae ha^{-1} but plants were able to compensate by producing more pods on lateral branches. Dicamba applications made during reproductive stages impacted main stem pod production, but the plants were not able to compensate and produce lateral branch pods that late in the growing season. While the response of sensitive cotton to 2,4-D was similar to the response Foster and Griffin (2019) observed, the response of sensitive cotton to dicamba was notably different; fruiting shifts were slight and typically resulted in a simple reduction in overall fruiting bodies.

Seed index was impacted by dicamba application rate (Table 37; $p < 0.0001$). Seed index was reduced to 8.4 when 558 g ae ha^{-1} was applied compared to all other application rates in which seed index ranged from 9.4 to 9.6. Lint index was impacted by the interaction of application rate and timing (Table 37; $p < 0.0001$). For the untreated and applications of dicamba 0.5, 2, and 9 g ae ha^{-1} (8.2 – 8.3), no differences in lint index were observed between application timings. For applications of 35, 139, and 558 g ae ha^{-1}, lint index was greater following FB+2WK (8.5, 8.2, and 7.7, respectively) applications than MHS (8.0, 7.4, and 5.5, respectively) applications.

The number of seeds per boll was impacted by the interaction between application rate and timing (Table 37; $p = 0.0005$). For the untreated and applications of dicamba at 0.5, 2, 9, and 35 g ae ha^{-1} (27 – 31 seed $boll^{-1}$), no differences in the number of seed per boll were observed between application timings. For applications of 139 and 558 g ae ha^{-1}, the number of seeds per boll was greater following FB+2WK (29 and 31 seed $boll^{-1}$, respectively) applications than MHS (26 and 26 seed $boll^{-1}$, respectively) applications.

Cotton boll size was affected by the interaction of application rate and timing (Table 37; $p = 0.0129$). The untreated and applications of dicamba at 0.5, 2, and 9 g ae ha^{-1} (5.4 – 5.6 g) at MHS produced larger bolls than applications of 139 and 558 g ae ha^{-1} (3.8 – 4.6 g) at MHS. No differences in boll size occurred between application rates applied at FB+2WK; boll size ranged from 5.0 to 5.4 g.

Fiber micronaire, length, and strength were each impacted by the interaction between dicamba application rate and timing (Table 38; $p \leq 0.0013$). For the untreated and applications of 0.5, 2, 9, and 35 g ae ha^{-1}, no differences in micronaire or length were observed between application timings; micronaire ranged from 4.5 to 4.8 and fiber length ranged from 1.15 to 1.17 in. Applications of dicamba at 139 and 558 g ae ha^{-1} at FB+2WK (4.7 and 4.7, respectively) resulted in greater micronaire than those same rates applied at MHS (3.1 and 4.1, respectively). Applications of 139 and 558 g ae ha^{-1} at FB+2WK (1.14 and 1.13 in, respectively) produced cotton with shorter fibers than cotton exposed to those same rates applied at MHS (1.21 and 1.22 in, respectively). For the untreated and applications of dicamba at 0.5, 2, and 9 g ae ha^{-1}, no differences in fiber strength were observed between application timings; fiber strength ranged from 31.6 to 32.4 g tex^{-1}. Fiber strength following an application of 35 g ae ha^{-1} at FB+2WK (32.4 g tex^{-1}) was greater than that same rate applied at MHS (31.0 g tex^{-1}). Applications of 139 and 558 g ae ha^{-1} at FB+2WK (31.7 and 29.9 g tex^{-1}, respectively) produced fiber with less strength than those same application rates at MHS (34.0 and 32.9 g tex^{-1}, respectively). Uniformity was impacted by dicamba application rate (Table 38; $p = 0.0140$). Fiber produced after exposure to 2 g ae ha^{-1} (84.2%) was more uniform than fiber produced following exposure to 35, 139, and 558 g ae ha^{-1} (83.1, 83.5, and 83.2%, respectively). While differences in fiber quality were observed due to dicamba exposure, no values fell within a range to cause discounted market values.

Impacts of Weather

Cotton's response to auxin injury during the growing season is dependent on environment, specifically water availability. Periods of drought or water stress following exposure to 2,4-D can exacerbate injury symptomology and yield effects. Sciumbato et al. (2014) reported the least yield impacts from 2,4-D exposure from the growing season that had the most consistent rainfall following 2,4-D application at 4- to 6-leaf cotton. In this research, during the growing season with the least amount of rainfall following 2,4-D application, up to 84% yield reductions were recorded. In general, the severity of yield effects due to auxin exposure is more severe during vegetative growth but given an amicable environment following exposure, cotton can compensate.

Conclusions

Cotton exposed to 2,4-D was characterized by lint yield reductions at rates ≥ 4 g ae ha^{-1}, whereas cotton exposed to dicamba was characterized by lint yield reductions at rates ≥ 35 g ae ha^{-1}. While 2,4-D applied to sensitive cotton at the MHS stage caused reductions of fruit partitioned at the 1^{st} horizontal position as rate increased, strong shifts in fruit partitioned at the 1^{st} horizontal position were not generated by dicamba or 2,4-D applied at MHS or 2,4-D applied at FB+2WK (Figs. 5 and 6). Furthermore, the application of dicamba at FB+2WK had minor impacts on boll size, but applications of dicamba and 2,4-D at MHS and the application of 2,4-D at FB+2WK resulted in substantial decreases in boll size as rate increased. The number of seeds per boll decreased as application rate of 2,4-D increased and when exposure occurred during vegetative growth compared to reproductive growth. The timing of exposure only affected the number of seeds per boll when cotton was exposed to dicamba at rates of 139 and 558 g ae ha^{-1}. Cotton is clearly more sensitive to low-rates of 2,4-D than dicamba, but low rates of both auxin herbicides impacted yield components. While light rates of auxin injury to susceptible cotton will likely not be detected in fruit partitioning, injury will likely appear in reductions in seed number and boll size.

Acknowledgements

The authors would like to thank the Ames AgResearch and Education Center for their support in getting these trials conducted.

References

Blouin, D.C., Webster, E.P., & Bond, J.A. (2011). On the analysis of combined experiments. *Weed Technology, 25,* 165-169. https://doi.org/10.1614/WT-D-01-00047.1

Buol, J.T., Reynolds, D.B., Dodds, D.M., Mills, J.A., Nichols, R.L., Bond, J.A., Jenkins, J.N., & DuBien, J.L. (2019a). Effect of growth stage on cotton response to a sublethal concentration of dicamba. *Weed Technology, 33,* 1-8. https://doi.org/10.1017/wet.2018.95

Buol, J.T., Reynolds, D.B., Dodds, D.M., Mills, J.A., Nichols, R.L., Bond, J.A., Jenkins, J.N., & DuBien, J.L. (2019b). The effect of cotton growth stage on response to a sublethal concentration of 2,4-D. *Weed Technology, 33*, 321.328. https://doi.org/10.1017/wet.2019.9

Byrd, S.A., Collins, G.D., Culpepper, A.S., Dodds, D.M., Edmisten, K.L., Wright, D.L., Morgan, G.D., Baumann, P.A., Dotray, P.A., Manuchehri, M.R., Jones, A., Grey, T.L., Webster, T.M., Davis, J.W., Whitaker, J.R., Roberts, P.M., Snider, J.L., & Porter, W.M. (2016). Cotton stage of growth determines sensitivity to 2,4-D. *Weed Technology, 30,* 601-610. https://doi.org/10.1614/WT-D-15-00191.1

Cahoon, C.W., York, A.C., Jordan, D.L., Everman, W.J., Seagroves, R.W., Culpepper, A.S., & Eure, P.M. (2015). Palmer amaranth (*Amanranthus palmeri*) management in dicamba-resistant cotton. *Weed Technology, 29,* 758-770. https://doi.org/10.1614/WT-D-15-00041.1

Culpepper, A.S. (2006). Glyphosate-induced weed shifts. *Weed Technology, 20,* 277-281. https://doi.org/10.1614/WT-04-155R.1

Cundiff, G.T., Reynolds, D.B., & Mueller, T.C. (2017). Evaluation of dicamba persistence among various agricultural hose types and cleanout procedures using soybean (*Glycine max*) as a bio-indicator. *Weed Science, 65,* 305-316. https://doi.org/10.1017/wsc.2016.29

Egan, J.T., Barlow, K.M., & Mortensen, D.A. (2014). A meta-analysis on the effects of 2,4-D and dicamba drift on soybean and cotton. *Weed Science, 62,* 193-206. https://doi.org/10.1614/WS-D-13-00025.1

Foster, M.R. & Griffin, J.L. (2019). Changes in soybean yield components in response to dicamba. *Agrosystems, Geosciences and Environment, 2.* https://doi.org/10.2134/age2019.04.0026

Gbur, E.E., Stroup, W.W., McCarter, K.S., Durham, S., Young, L.J., Christman, M., West, M., & Kramer, M. (2012). *Analysis of generalized linear mixed models in the agricultural and natural resource sciences*. ASA, CSSA, and SSSA.

Johnson, V.A., Fisher, L.R., Jordan, D.L., Edmisten, K.L., Stewart, A.M., & York, A.C. (2012). Cotton, peanut, and soybean response to sublethal rates of dicamba, glufosinate, and 2,4-D. *Weed Technology, 26,* 195-206. https://doi.org/10.1614/WT-D-11-00054.1

Jenkins, J.N., McCarty Jr., J.C., & Parrott, W.L. (1990). Effectiveness of fruiting sites in cotton: yield. *Crop Science, 30,* 365-369.

Raper, T.B. (2016). 2016 Tennessee cotton quick facts. UTIA Publication W319.

Sciumbato, A.S., Chandler, J.M., Senseman, S.A., Bovey, R.W., & Smith, K.L. (2004). Determining exposure to auxin-like herbicides. I. Quantifying injury to cotton and soybean. *Weed Technology, 18,* 1125-1134. https://doi.org/10.1614/WT-03-105R1

Sciumbato, A.S., Senseman, S.A., Steele, G.L., Chandler, J.M., Cothren, J.T., & Kirk, I.W. (2014). The effect of 2,4-D drift rates on cotton (*Gossypium hirsutum* L.) growth and yield. *Plant Health Progress*, *15*, 67-73. https://doi.org/10.1094.PHP-RS-13-0108

Song, Y. (2014). Insight into the mode of action of 2,4-dichlorophenoxyacetic acid (2,4-D) as an herbicide. *Journal of Integrative Plant Biology, 56,* 106-113. https://doi.org/10.1111/jipb.12131

Suarez, L.A., Apan, A., & Werth, J. (2017). Detection of phenoxy herbicide dosage in cotton crops through the analysis of hyperspectral data. *International Journal of Remote Sensing*, *38,* 6528-6553. https://doi.org/10.1080/01431161.2017.1362128

USDA-ARS. (2020). *Cotton varieties planted 2020 crop*. ARS Cotton and Tobacco Program. Memphis, TN. https://www.ams.usda.gov/mnreports/cnavar.pdf

Wax, L.M., Knuth, L.A., & Slife, F.W. (1969). Response of soybeans to 2,4-D, dicamba, and picloram. Weed Science, 17, 388-393. https://doi.org/10.1017/S004317450005431X

Appendix

Table 28. Cotton planting dates, herbicide application dates, and cotton harvest dates for 2,4-D and dicamba experiments located in Grand Junction, TN from 2019 to 2021.

Event	2019	2020	2021
Cotton Planting	06 May	14 May	17 May
Matchhead Square Application	25 June	29 June	06 July
First Bloom + 2 wk Application	18 July	29 July	10 Aug
Cotton Harvest	19 Nov	17 Nov	01 Nov

Table 29. Effect of 2,4-D application rate on cotton plant height and total node count 14 and 28 d after MHS and FB+2WK (A and B, respectively) applications for experiments conducted in Grand Junction, TN from 2019 to 2021.

Rate (g ae ha^{-1})	14 DA-A	28 DA-A	14 DA-B	28 DA-B
	------------------------------- Plant Height (cm) -------------------------------			
1,064	35 D[a]	35 E	100	89 B
266	37 D	41 E	98	90 B
67	42 C	53 D	102	95 AB
17	59 B	84 C	103	100 A
4	80 A	103 B	106	104 A
1	78 A	110 A	104	102 A
0	78 A	107 AB	105	105 A
p-value	< 0.0001	< 0.0001	0.3060	0.0060
Standard Error	2.2	3.0	9.4	14.7
	------------------------------------ Total Nodes ------------------------------------			
1,064	10 C	9 D	16	15 B
266	10 C	10 D	16	15 B
67	10 C	12 C	16	17 A
17	12 B	15 B	18	18 A
4	13 A	16 AB	16	17 A
1	13 A	16 A	16	17 A
0	14 A	16 AB	17	17 A
p-value	< 0.0001	< 0.0001	0.4298	0.0005
Standard Error	0.7	0.6	0.8	0.7

[a] Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 30. Effect of 2,4-D application rate, application timing, and their interaction on cotton lint yield and lint percentage for experiments conducted in Grand Junction, TN from 2019 to 2021.

Effect	Lint Yield	Lint Percentage
Rate (g ae ha^{-1})	kg ha^{-1}	%
1,064	38	46
266	146	47
67	306	46
17	647	46
4	1,041	46
1	1,396	46
0	1,528	47
p-value; Standard Error	< 0.0001; 53.9	0.0684; 0.3
Timing		
MHS	661	45
FB+2WK	797	46
p-value; Standard Error	0.0002; 35.7	< 0.0001; 0.3
Rate – Timing		
1,064 – MHS	35 F[a]	45 C-G
1,064 – FB+2WK	41 F	46 A-E
266 – MHS	156 F	44 F-G
266 – FB+2WK	136 F	46 C-F
67 – MHS	129 F	44 G
67 – FB+2WK	483 E	47 A
17 – MHS	447 E	45 G
17 – FB+2WK	848 D	47 AB
4 – MHS	856 D	45 F
4 – FB+2WK	1,226 C	46 D
1 – MHS	1,468 AB	46 CD
1 – FB+2WK	1,323 BC	46 D-F
0 – MHS	1,536 A	46 B-D
0 – FB+2WK	1,521 A	47 A-C
p-value; Standard Error	< 0.0001; 71.9	< 0.0001; 0.5

[a] Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 31. Effect of 2,4-D application rate, application timing, and their interaction on yield partitioning for experiments conducted in Grand Junction, TN from 2019 to 2021.

	---------- Horizontal Position ---------			-------------- Vertical Zone ------------				
Effect	1	2	3	1	2	3	Vegetative	Aborted
Rate (g ae ha^{-1})	------------	------------	------------	% Total	------------	------------	------------	------------
17	36	12 B[a]	9 A	22	24	10	29	14
4	45	15 B	4 B	18	29	16	29	7
1	57	19 A	3 B	21	36	22	17	3
0	61	19 A	3 B	22	38	23	16	1
p-value	< 0.0001	0.0003	0.0004	0.4480	< 0.0001	< 0.0001	< 0.0001	0.0012
Standard Error	2.4	3.1	1.8	6.2	1.9	1.9	4.6	2.3
Timing								
MHS	41	15 B	5	20	25	16	28	11
FB+2WK	58	18 A	4	22	39	19	17	2
p-value	< 0.0001	0.0120	0.3967	0.4096	< 0.0001	0.0602	< 0.0001	0.0004
Standard Error	1.7	3.0	1.6	6.1	1.4	1.5	4.3	1.7
Rate – Timing								
17 – MHS	17 D	10	10	22	11 C	5 D	38 A	24 A
17 – FB+2WK	54 B	15	8	23	38 AB	15 C	20 B	4 BC
4 – MHS	29 C	11	5	15	15 C	16 C	42 A	12 B
4 – FB+2WK	60 AB	18	3	21	44 A	17 BC	17 B	2 C
1 – MHS	53 B	19	3	19	33 B	23 AB	20 B	6 BC
1 – FB+2WK	62 AB	19	3	24	40 AB	21 ABC	15 B	1 C
0 – MHS	65 A	19	2	24	40 AB	21 ABC	13 B	1 C
0 – FB+2WK	58 AB	20	3	20	36 AB	24 A	18 B	1 C
p-value	< 0.0001	0.1554	0.5129	0.2842	< 0.0001	0.0316	< 0.0001	0.0230
Standard Error	3.5	3.4	2.1	6.6	2.6	2.4	5.1	3.3

[a] Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 32. Effect of 2,4-D application rate, application timing, and their interaction on number of bolls ha^{-1} calculated with yield partitioning data and mechanical yield data, seed index, lint index, number of seed boll^{-1}, and boll size for experiments conducted in Grand Junction, TN from 2019 to 2021.

Effect	Bolls ha^{-1} (SE[a])		Seed Index	Lint Index	Seed Boll^{-1}	Boll Size (g)
Rate (g ae ha^{-1})	Yield Partitioning	Mechanical Yield				
1,064	--	53,000 (87 K)	8.6 (0.33)	7.3 (0.33) BC	22 (1.4) E	3.4 (0.22)
266	--	161,000 (51 K)	8.8 (0.22)	7.2 (0.23) C	24 (1.0) DE	3.7 (0.16)
67	--	196,000 (55 K)	8.9 (0.18)	7.5 (0.19) BC	25 (0.9) CD	4.1 (0.14)
17	532,000 (71 K)	419,000 (44 K)	9.2 (0.16)	7.9 (0.17) AB	26 (0.8) C	4.4 (0.13)
4	753,000 (71 K)	565,000 (41 K)	9.3 (0.16)	7.9 (0.17) AB	27 (0.8) B	4.7 (0.13)
1	910,000 (71 K)	742,000 (50 K)	9.4 (0.16)	8.0 (0.17) A	29 (0.8) A	5.1 (0.13)
0	879,000 (71 K)	776,000 (45 K)	9.2 (0.16)	8.0 (0.17) A	30 (0.8) A	5.2 (0.13)
p-value	< 0.0001	< 0.0001	0.0726	0.0074	< 0.0001	< 0.0001
Timing						
MHS	719,000 (65 K)	391,000 (30 K)	9.2 (0.10)	7.6 (0.13)	25 (0.7) B	4.3 (0.11)
FB+2WK	818,000 (65 K)	441,000 (33 K)	9.0 (0.11)	7.8 (0.14)	27 (0.7) A	4.5 (0.11)
p-value	0.0202	0.2342	0.1197	0.2479	0.0105	0.0102
Rate – Timing						
1,064 – MHS	--	32,800 (100 K) F	9.0 (0.38)	7.4 (0.38)	22 (1.5)	3.6 (0.25) EF
1,064 – FB+2WK	--	73,100 (141 K) DEF	8.3 (0.54)	7.1 (0.53)	21 (2.1)	3.2 (0.34) F
266 – MHS	--	218,000 (72 K) DEF	9.3 (0.31)	7.4 (0.31)	23 (1.3)	3.8 (0.21) EF
266 – FB+2WK	--	105,000 (71 K) EF	8.3 (0.54)	6.9 (0.31)	24 (1.3)	3.6 (0.21) EF
67 – MHS	--	98,200 (71 K) EF	9.2 (0.27)	7.2 (0.27)	23 (1.2)	3.8 (0.19) EF
67 – FB+2WK	--	294,000 (82 K) DE	8.6 (0.23)	7.8 (0.24)	26 (1.0)	4.3 (0.16) CD
17 – MHS	380,000 (83 K) C[b]	307,000 (64 K) D	9.3 (0.22)	7.6 (0.23)	24 (1.0)	4.1 (0.16) DE
17 – FB+2WK	684,000 (83 K) B	531,000 (59 K) C	9.1 (0.22)	8.1 (0.23)	27 (1.0)	4.7 (0.16) BC
4 – MHS	701,000 (83 K) B	512,000 (54 K) C	9.1 (0.22)	7.6 (0.23)	26 (1.0)	4.3 (0.16) D
4 – FB+2WK	805,000 (83 K) AB	618,000 (59 K) BC	9.5 (0.22)	8.3 (0.23)	29 (1.0)	5.2 (0.16) A
1 – MHS	949,000 (83 K) A	823,000 (82 K) A	9.3 (0.22)	8.0 (0.23)	29 (1.0)	5.0 (0.16) AB

Table 32. Continued

Effect	Bolls ha^{-1} (SE[a])		Seed Index	Lint Index	Seed $Boll^{-1}$	Boll Size (g)
Rate – Timing	Yield Partitioning	Mechanical Yield				
1 – FB+2WK	871,000 (83 K) A	660,000 (54 K) ABC	9.6 (0.22)	8.0 (0.23)	30 (1.0)	5.2 (0.16) A
0 – MHS	848,000 (83 K) AB	746,000 (54 K) AB	9.1 (0.22)	7.9 (0.23)	31 (1.0)	5.3 (0.16) A
0 – FB+2WK	911,000 (83 K) A	805,000 (71 K) A	9.3 (0.22)	8.1 (0.23)	30 (1.0)	5.2 (0.16) A
p-value	0.0170	0.0479	0.0829	0.2834	0.0930	0.0013

[a] Standard Error

[b] Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 33. Effect of 2,4-D application rate, application timing, and their interaction on fiber micronaire, length, strength, and uniformity for experiments conducted in Grand Junction, TN from 2019 to 2021.

Effect	Micronaire	Length	Strength	Uniformity
Rate (g ae ha^{-1})	(SE[a])	in	g tex^{-1}	%
1,064	4.2 (0.20) CD[b]	1.13 (0.025) BC	27.6 (0.69)	81.0 (0.87)
266	4.2 (0.14) CD	1.12 (0.019) C	29.6 (0.50)	81.0 (0.76)
67	4.2 (0.12) D	1.14 (0.017) BC	29.8 (0.44)	82.1 (0.73)
17	4.3 (0.11) CD	1.17 (0.017) AB	30.6 (0.41)	82.7 (0.71)
4	4.5 (0.11) BC	1.18 (0.017) A	29.9 (0.41)	82.8 (0.71)
1	4.7 (0.11) A	1.17 (0.017) AB	30.1 (0.41)	83.2 (0.72)
0	4.7 (0.11) AB	1.17 (0.017) AB	30.0 (0.41)	83.6 (0.71)
p-value	< 0.0001	0.0163	0.0059	< 0.0001
Timing				
MHS	4.4 (0.09)	1.18 (0.015) A	30.6 (0.34)	83.3 (0.68)
FB+2WK	4.4 (0.09)	1.13 (0.015) B	28.8 (0.36)	81.4 (0.69)
p-value	0.9985	< 0.0001	< 0.0001	< 0.0001
Rate – Timing				
1,064 – MHS	4.2 (0.23)	1.17 (0.028)	30.1 (0.78) A-D	83.4 (0.93) AB
1,064 – FB+2WK	4.2 (0.32)	1.08 (0.038)	*25.1*[c] (1.06) E	*78.5* (1.13) E
266 – MHS	4.3 (0.19)	1.16 (0.024)	30.7 (0.66) AB	82.5 (0.85) ABC
266 – FB+2WK	4.1 (0.19)	1.09 (0.024)	28.5 (0.66) D	*79.5 (0.85) E*
67 – MHS	4.2 (0.17)	1.19 (0.022)	30.7 (0.58) AB	83.2 (0.80) AB
67 – FB+2WK	4.3 (0.15)	1.10 (0.020)	29.0 (0.52) CD	81.1 (0.77) D
17 – MHS	4.3 (0.14)	1.18 (0.019)	31.2 (0.50) A	83.7 (0.76) A
17 – FB+2WK	4.2 (0.14)	1.16 (0.019)	29.9 (0.50) BCD	81.8 (0.76) CD
4 – MHS	4.4 (0.14)	1.20 (0.019)	30.7 (0.50) AB	83.0 (0.76) AB
4 – FB+2WK	4.6 (0.14)	1.15 (0.019)	29.2 (0.50) CD	82.5 (0.76) BC
1 – MHS	4.7 (0.14)	1.19 (0.019)	30.5 (0.50) AB	83.5 (0.76) AB
1 – FB+2WK	4.8 (0.14)	1.15 (0.019)	29.8 (0.50) BCD	82.9 (0.76) AB

Table 33 Continued.

Effect	Micronaire	Length	Strength	Uniformity
Rate – Timing	(SE[a])	in	g tex^{-1}	%
0 – MHS	4.7 (0.14)	1.17 (0.019)	30.0 (0.50) BC	83.7 (0.76) A
0 – FB+2WK	4.7 (0.14)	1.17 (0.019)	30.1 (0.50) BC	83.5 (0.76) AB
p-value	0.9159	0.0748	0.0167	0.0004

[a] Standard Error

[b] Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

[c] Italicized values represent fiber quality grades which call for discounted market value.

Table 34. Effect of dicamba application rate on cotton plant height and total node count 14 and 28 d after MHS and FB+2WK (A and B, respectively) applications for experiments conducted in Grand Junction, TN from 2019 to 2021.

Rate (g ae ha^{-1})	14 DA-A	28 DA-A	14 DA-B	28 DA-B
	------------------------------ Plant Height (cm) ------------------------------			
558	39 D[a]	38 D	94	89 CD
139	44 D	45 C	95	87 D
35	64 C	73 B	100	91 BCD
9	74 B	98 A	100	94 ABCD
2	78 AB	103 A	99	96 ABC
0.5	78 AB	103 A	104	100 A
0	80 A	103 A	100	98 AB
p-value	< 0.0001	< 0.0001	0.1490	0.0311
Standard Error	3.7	5.2	8.4	13.0
	--------------------------------- Total Nodes --------------------------------			
558	10 C	9 C	15	15 CD
139	11 C	9 C	16	15 D
35	13 B	14 B	16	15 BCD
9	13 AB	16 A	16	16 ABC
2	14 A	15 A	16	17 AB
0.5	14 A	15 A	16	17 A
0	14 A	16 A	16	17 AB
p-value	< 0.0001	< 0.0001	0.2395	0.0171
Standard Error	0.6	0.5	0.7	0.5

[a] Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 35. Effect of dicamba application rate, application timing, and their interaction on cotton lint yield and lint percentage for experiments conducted in Grand Junction, TN from 2019 to 2021.

Effect	Lint Yield	Lint Percentage
Rate (g ae ha^{-1})	kg ha^{-1}	%
558	138	44
139	337	45
35	937	47
9	1,359	46
2	1,415	46
0.5	1,496	46
0	1,379	46
p-value; Standard Error	< 0.0001; 77.0	< 0.0001; 0.5
Timing		
MHS	989	45
FB+2WK	1,028	47
p-value; Standard Error	0.3143; 63.8	< 0.0001; 0.4
Rate – Timing		
558 – MHS	26 F[a]	41 D
558 – FB+2WK	250 E	47 AB
139 – MHS	181 EF	43 C
139 – FB+2WK	493 D	46 B
35 – MHS	962 C	46 B
35 – FB+2WK	912 C	47 A
9 – MHS	1,435 AB	46 AB
9 – FB+2WK	1,283 B	46 AB
2 – MHS	1,515 A	46 B
2 – FB+2WK	1,316 AB	47 AB
0.5 – MHS	1,513 A	46 B
0.5 – FB+2WK	1,478 AB	46 B
0 – MHS	1,294 B	46 AB
0 – FB+2WK	1,465 AB	46 B
p-value; Standard Error	0.0017; 92.3	< 0.0001; 0.5

[a] Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 36. Effect of dicamba application rate, application timing, and their interaction on yield partitioning for experiments conducted in Grand Junction, TN from 2019 to 2021.

	----------- Horizontal Position ----------			-------------- Vertical Zone -------------				
Effect	1	2	3	1	2	3	Vegetative	Aborted
Rate (g ae ha^{-1})	-- % Total	--						
9	67	15	2	22	41	20	15	1
2	68	14	1	22	42	19	15	2
0.5	66	14	2	24	40	18	17	1
0	67	14	3	24	42	17	16	1
p-value	0.9456	0.9095	0.0176	0.6005	0.8000	0.3887	0.8238	0.7884
Standard Error	2.9	4.5	1.5	7.8	1.5	5.0	3.8	0.8
Timing								
MHS	67	14	2	22 B	42	19	15	1
FB+2WK	66	15	2	25 A	41	18	16	1
p-value	0.5148	0.4641	0.3777	0.0379	0.2238	0.2670	0.7136	0.7875
Standard Error	2.6	4.4	1.5	7.7	1.1	4.9	3.6	0.6
Rate – Timing								
9 – MHS	63 BC[a]	14	3 AB	18	41	21	18 AB	2
9 – FB+2WK	71 A	15	2 BCD	27	42	19	12 C	1
2 – MHS	70 AB	13	0 D	21	42	20	15 ABC	2
2 – FB+2WK	66 ABC	15	2 ABC	23	42	17	15 ABC	2
0.5 – MHS	66 ABC	16	2 BCD	24	42	18	16 ABC	1
0.5 – FB+2WK	66 ABC	13	2 CD	24	39	18	18 ABC	1
0 – MHS	71 A	13	2 ABC	24	45	17	13 BC	1
0 – FB+2WK	62 C	15	3 A	24	39	17	19 A	1
p-value	0.0206	0.2288	0.0162	0.1220	0.3470	0.8702	0.0381	0.8102
Standard Error	3.6	4.6	1.6	7.9	2.1	5.2	4.1	1.2

[a] Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 37. Effect of dicamba application rate, application timing, and their interaction on number of bolls ha^{-1} calculated with yield partitioning data and mechanical yield data, seed index, lint index, number of seed boll^{-1}, and boll size for experiments conducted in Grand Junction, TN from 2019 to 2021.

Effect	Bolls ha^{-1} (SE[a])		Seed Index	Lint Index	Seed Boll^{-1}	Boll Size (g)
Rate (g ae ha^{-1})	Yield Partitioning	Mechanical Yield				
558	--	114,000 (71 K)	8.4 (0.36) B	6.6 (0.33)	29 (1.5)	4.5 (0.47)
139	--	225,000 (64 K)	9.6 (0.33) A	7.8 (0.31)	27 (1.4)	4.9 (0.44)
35	--	496,000 (62 K)	9.4 (0.32) A	8.3 (0.30)	27 (1.3)	5.2 (0.42)
9	891,000 (84 K) A[b]	695,000 (62 K)	9.6 (0.32) A	8.3 (0.30)	28 (1.3)	5.2 (0.42)
2	788,000 (84 K) C	712,000 (62 K)	9.5 (0.32) A	8.2 (0.30)	29 (1.3)	5.3 (0.42)
0.5	866,000 (84 K) AB	737,000 (62 K)	9.5 (0.32) A	8.2 (0.30)	29 (1.3)	5.4 (0.42)
0	814,000 (84 K) BC	671,000 (62 K)	9.5 (0.32) A	8.2 (0.30)	30 (1.3)	5.5 (0.42)
p-value	0.0336	< 0.0001	< 0.0001	< 0.0001	0.0003	0.0283
Timing						
MHS	870,000 (82 K) A	499,000 (58 K)	9.3 (0.31)	7.7 (0.28)	28 (1.3)	5.1 (0.41)
FB+2WK	809,000 (82 K) B	543,000 (58 K)	9.4 (0.30)	8.2 (0.28)	29 (1.3)	5.2 (0.41)
p-value	0.0276	0.0516	0.2037	< 0.0001	0.1222	0.1405
Rate – Timing						
558 – MHS	--	50,700 (86 K) F	8.1 (0.42)	5.5 (0.38) E	26 (1.7) EF	3.8 (0.55) D
558 – FB+2WK	--	177,000 (80 K) F	8.7 (0.39)	7.7 (0.36) CD	31 (1.6) AB	5.3 (0.52) A-C
139 – MHS	--	101,000 (72 K) F	9.7 (0.36)	7.4 (0.33) D	26 (1.5) F	4.6 (0.48) CD
139 – FB+2WK	--	348,000 (72 K) E	9.6 (0.36)	8.2 (0.33) A-C	29 (1.5) B-D	5.2 (0.48) A-C
35 – MHS	--	522,000 (67 K) CD	9.2 (0.34)	8.0 (0.31) BC	27 (1.4) D-F	4.9 (0.45) BC
35 – FB+2WK	--	469,000 (67 K) D	9.6 (0.34)	8.5 (0.31) A	28 (1.4) D-F	5.4 (0.45) AB
9 – MHS	921,000 (88 K)	714,000 (67 K) A	9.6 (0.34)	8.3 (0.31) AB	28 (1.4) C-E	5.4 (0.45) AB
9 – FB+2WK	861,000 (88 K)	676,000 (67 K) AB	9.6 (0.34)	8.3 (0.31) AB	28 (1.4) C-E	5.0 (0.45) BC
2 – MHS	849,000 (88 K)	751,000 (67 K) A	9.6 (0.34)	8.2 (0.31) A-C	30 (1.4) A-C	5.4 (0.45) AB
2 – FB+2WK	727,000 (88 K)	673,000 (67 K) AB	9.4 (0.34)	8.2 (0.31) A-C	28 (1.4) C-E	5.1 (0.45) A-C
0.5 – MHS	905,000 (88 K)	745,000 (67 K) A	9.5 (0.34)	8.2 (0.31) A-C	29 (1.4) A-C	5.5 (0.45) AB

Table 37. Continued

Effect	Bolls ha^{-1} (SE[a])		Seed Index	Lint Index	Seed $Boll^{-1}$	Boll Size (g)
Rate – Timing	Yield Partitioning	Mechanical Yield				
0.5 – FB+2WK	826,000 (88 K)	730,000 (67 K) A	9.6 (0.34)	8.2 (0.31) A-C	29 (1.4) B-D	5.4 (0.45) AB
0 – MHS	805,000 (88 K)	610,000 (67 K) BC	9.4 (0.34)	8.2 (0.31) A-C	31 (1.4) A	5.6 (0.45) A
0 – FB+2WK	823,000 (88 K)	732,000 (67 K) A	9.5 (0.34)	8.2 (0.31) A-C	29 (1.4) A-C	5.3 (0.45) AB
p-value	0.3267	0.0007	0.6449	< 0.0001	0.0005	0.0129

[a] Standard Error

[b] Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

Table 38. Effect of dicamba application rate, application timing, and their interaction on fiber micronaire, length, strength, and uniformity for experiments conducted in Grand Junction, TN from 2019 to 2021.

Effect	Micronaire	Length	Strength	Uniformity
Rate (g ae ha^{-1})	(SE[a])	in	g tex^{-1}	%
558	3.9 (0.09)	1.18 (0.018)	31.4 (0.63)	83.2 (0.69) BC
139	4.4 (0.07)	1.18 (0.017)	32.9 (0.53)	83.5 (0.64) ABC
35	4.5 (0.06)	1.15 (0.016)	31.7 (0.48)	83.1 (0.62) C
9	4.7 (0.06)	1.17 (0.016)	32.2 (0.47)	84.0 (0.61) AB
2	4.7 (0.06)	1.17 (0.016)	32.3 (0.47)	84.2 (0.61) A
0.5	4.6 (0.06)	1.16 (0.016)	32.3 (0.47)	84.1 (0.61) AB
0	4.6 (0.06)	1.16 (0.016)	31.9 (0.48)	84.0 (0.62) AB
p-value	< 0.0001	0.3063	0.2024	0.0140
Timing				
MHS	4.3 (0.04)	1.17 (0.015)	32.3 (0.41)	83.9 (0.58)
FB+2WK	4.6 (0.04)	1.15 (0.015)	31.9 (0.40)	83.6 (0.58)
p-value	< 0.0001	0.0008	0.1164	0.1439
Rate – Timing				
558 – MHS	3.1 (0.15) D[b]	1.22 (0.022) A	32.9 (0.87) AB	84.2 (0.83)
558 – FB+2WK	4.7 (0.12) AB	1.13 (0.020) CD	29.9 (0.73) D	82.1 (0.75)
139 – MHS	4.1 (0.10) C	1.21 (0.019) A	34.0 (0.66) A	83.7 (0.71)
139 – FB+2WK	4.7 (0.10) AB	1.14 (0.018) D	31.7 (0.65) BC	83.4 (0.71)
35 – MHS	4.6 (0.09) AB	1.15 (0.017) BCD	31.0 (0.59) CD	83.4 (0.67)
35 – FB+2WK	4.5 (0.08) B	1.15 (0.017) BCD	32.4 (0.57) B	82.8 (0.66)
9 – MHS	4.7 (0.08) AB	1.16 (0.017) BCD	32.1 (0.57) BC	83.9 (0.66)
9 – FB+2WK	4.7 (0.08) AB	1.17 (0.017) B	32.3 (0.57) BC	84.2 (0.66)
2 – MHS	4.7 (0.08) AB	1.17 (0.017) B	32.2 (0.57) BC	84.4 (0.66)
2 – FB+2WK	4.8 (0.08) A	1.16 (0.017) BCD	32.3 (0.57) B	84.0 (0.66)
0.5 – MHS	4.6 (0.08) AB	1.17 (0.017) BC	32.4 (0.57) B	84.1 (0.66)
0.5 – FB+2WK	4.6 (0.08) AB	1.16 (0.017) BCD	32.3 (0.57) BC	84.1 (0.66)

Table 38 Continued.

Effect	Micronaire	Length	Strength	Uniformity
Rate – Timing	(SE[a])	in	g tex^{-1}	%
0 – MHS	4.6 (0.09) AB	1.15 (0.017) BCD	31.6 (0.59) BC	83.6 (0.67)
0 – FB+2WK	4.6 (0.08) AB	1.17 (0.017) B	32.2 (0.57) BC	84.5 (0.66)
p-value	< 0.0001	< 0.0001	0.0013	0.0623

[a] Standard Error

[b] Means within a column followed by the same letter are not significantly different at $p \leq 0.05$.

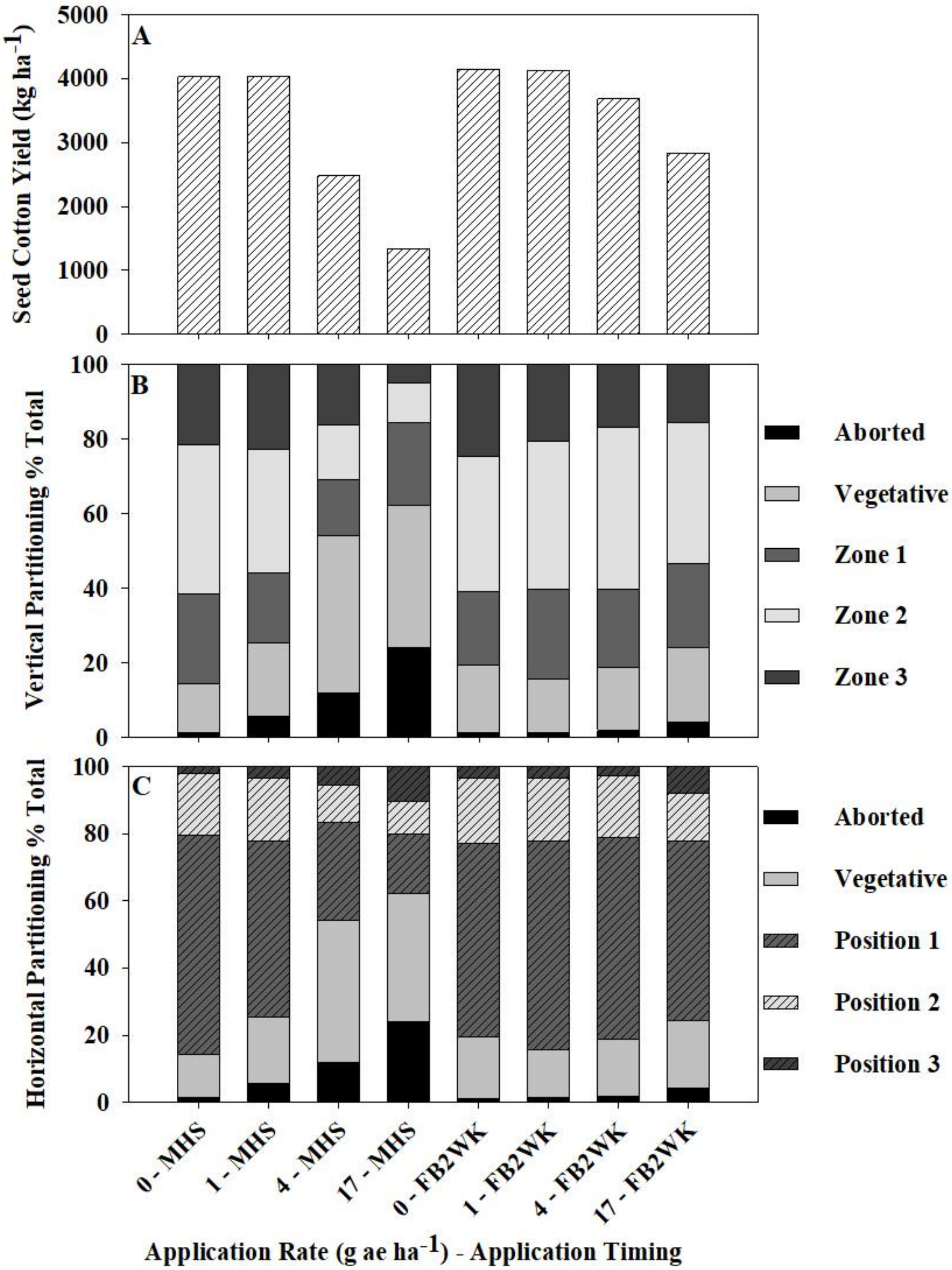

Figure 5. Seed cotton yield calculated from yield partitioning data (A) and vertical (B) and horizontal (C) yield partitioning data presented as percent total seed cotton yield as affected by 2,4-D application rate at matchhead square (MHS) and two weeks after first bloom (FB+2WK).

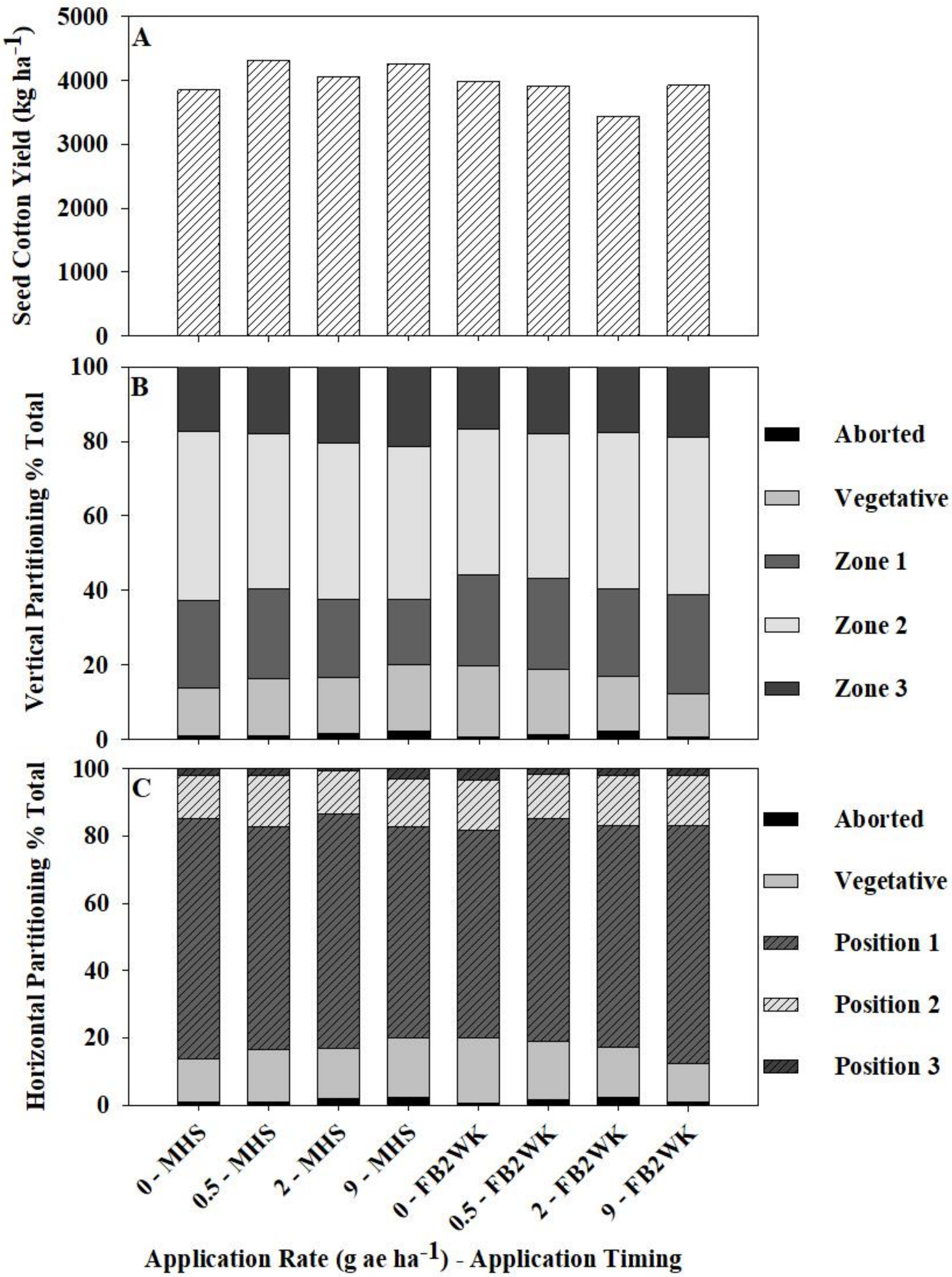

Figure 6. Seed cotton yield calculated from yield partitioning data (A) and vertical (B) and horizontal (C) yield partitioning data presented as percent total seed cotton yield as affected by dicamba application rate at matchhead square (MHS) and two weeks after first bloom (FB+2WK).

VITA

Savana Davis Denton was born in Fort Myers, FL in 1996 and moved to the Eastern Shore of Virginia in 2007. She is the daughter of Richard and Kim Davis of Starkville, MS and has two older sisters, Linsey and Ashton, who are both in the medical field. She attended Broadwater Academy and graduated in 2014. The summer prior to starting college she worked for her father at Lipman Produce and got her first hands-on experience in the agricultural world.

She was raised a Mississippi State Bulldog fan by her parents and obtained a B.S. degree in Agronomy with a concentration in Integrated Crop Management from Mississippi State University in 2017. While completing her undergraduate degree she worked for Dr. Darrin Dodds, the Extension Cotton Specialist, as a student worker. She was able to participate in an Undergraduate Research Program under the direction of Dr. Dodds and gained some experience with cotton agronomic research. She was then afforded the opportunity to pursue a M.S. degree under the direction of Drs. Dodds, Jason Krutz, and Jeffrey Gore at Mississippi State University. Savana completed her M.S. degree in Agronomy with a minor in Entomology in 2019. Her thesis research evaluated cotton agronomic practices including irrigation, fertility, and cover crops.

Savana met her husband, Drew Denton, while attending Mississippi State University. They married in March of 2019 and reside in West TN, where Drew was born and raised. In August 2020, they welcomed a baby boy, Beau, to their family. She is currently working towards completion of a PhD under the direction of Dr. Tyson Raper at the University of Tennessee. Throughout her academic career, Savana has presented her research at numerous local, regional, and national professional meetings in both oral and poster competitions. She is currently an author or co-author of 3 refereed journal articles, 2 extension publications, and 48 professional society abstracts. Upon completion of her doctorate degree, Savana will begin a career with the National Cotton Council in Cordova, TN as a Senior Agronomic Scientist.