ABSTRACT

JOYNER, JOSHUA DANIEL. Integration of HPPD-resistant Cotton (*Gossypium hirsutum*) and Isoxaflutole for Management of Herbicide-resistant Weeds. (Under the direction of Dr. Charles W. Cahoon).

Increasing prevalence of weed biotypes with resistance to one or more mechanisms of action (MOA) poses a significant threat to U.S. cotton production and gives rise to increasing need for additional weed management strategies. Cotton resistant to 4-hydroxyphenylpyrivate dioxygenase (HPPD) inhibiting herbicides is expected to be commercialized in 2023 and will provide an additional tool for managing herbicide-resistant weeds. Experiments were conducted to evaluate this new technology and its integration into North Carolina cotton production.

Herbicide-resistant Palmer amaranth and common ragweed are two of the most troublesome weeds infesting North Carolina cotton. Field experiments were conducted in 2019 and 2020 to evaluate control of these weeds by the HPPD-inhibiting herbicide, isoxaflutole (IFT). Isoxaflutole was compared to standard preemergence (PRE) herbicides acetochlor, diuron, fluometuron, fluridone, fomesafen and pyrithiobac. In addition, IFT was evaluated in combination with the aforementioned herbicides and was compared with grower standard combinations acetochlor + fomesafen and fluridone + fomesafen. An experiment evaluating postemergence (POST) control by IFT was also conducted and compared IFT to glyphosate, glufosinate and pyrithiobac, while also evaluating glyphosate and glufosinate with acetochlor and/or IFT. In the PRE experiment, IFT control of Palmer amaranth varied considerably by year. In 2019, Palmer amaranth was controlled well by IFT alone and IFT combinations, while in 2020 control by IFT containing treatments was in some instances inferior to standard herbicides. Isoxaflutole controlled common ragweed $\geq$ 88% across all locations 4 weeks after application (WAA) and was equal to or better than standard PRE herbicides. Postemergence experiments

demonstrated that IFT is ineffective at controlling emerged weeds but could be an effective POST residual when used alongside other POST herbicides.

A field experiment was conducted in 2019 and 2020 to evaluate the response of HPPD-resistant cotton to PRE, early postemergence (EPOST) and mid-postemergence (MPOST) herbicide treatments. Preemergence treatments included IFT, IFT in combination with acetochlor, diuron, fluometuron, fluridone, fomesafen, pendimethalin or pyrithiobac, or the standard treatment diuron + fomesafen + pendimethalin. In addition, EPOST treatments consisted of IFT, IFT + glyphosate/glufosinate, or dimethenamid-*P* + glufosinate. All treatments, except the nontreated control, received glyphosate + glufosinate MPOST. Cotton injury 9 to 14 days after planting (DAP) was $\leq$ 3% across both years, regardless of treatment. Isoxaflutole EPOST caused minimal injury, while injury from dimethenamid-*P* + glufosinate ranged 3 to 5% and 6 to 9% in 2019 and 2020, respectively and was always greater than injury from IFT or IFT + glyphosate/glufosinate. In both years, IFT caused minimal cotton response, with IFT PRE fb IFT EPOST injuring cotton 0 to 2%. This study demonstrates tolerance to IFT by HPPD-resistant cotton is acceptable in North Carolina.

Integration of HPPD-resistant Cotton (*Gossypium hirsutum*) and Isoxaflutole for Management of Herbicide-resistant Weeds

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Crop Science

Raleigh, North Carolina
2021

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DEDICATION

I dedicate this thesis to my wife, parents, and grandparents. Each of whom at different times and in different ways taught me to believe in myself, to always do my best, and to strive to be a better person each day. Without their love, support, encouragement and numerous life lessons, none of this would have been possible.

BIOGRAPHY

Joshua Joyner grew up in the town of Four Oaks, North Carolina. As a child he spent time around his family's farm, but music was his first childhood love. In 2011, Joshua graduated from South Johnston High School and attended Appalachian State University to pursue a degree in music. In 2013, Joshua realized he missed the family farm, and returned home to work over summer breaks. Despite the rekindling of his love for farming, he completed his education at Appalachian State, graduating in 2015 with a degree in Music Performance. He immediately enrolled at NC State and began a second undergraduate degree in Agronomy. Starting in 2016, Joshua started interning at a BASF research farm that focuses on controlling herbicide resistant weeds and realized his passion for field research and weed science. Upon graduating from North Carolina State University in 2018, Joshua worked as an agronomist for BASF at the same research farm where his appreciation for research began. In 2019, he returned to NC State and commenced his graduate research under Dr. Charlie Cahoon, focusing primarily on cotton weed management. Joshua has recently started his own crop consulting and research company, Insight Agronomics L.L.C., which he hopes will serve the needs of North Carolina farmers. Joshua is choir director and an active member at Antioch United Methodist Church in Four Oaks, NC. In his spare time, Joshua enjoys working on his family's farm, practicing/performing music, watching sports, and spending time with his wife, Caroline.

ACKNOWLEDGMENTS

I thank Dr. Charlie Cahoon for the countless hours of mentorship, encouragement, and kindness you have shown me throughout my time in graduate school. You have been a great example for what it means to serve North Carolina farmers, and that is a lesson I will carry with me long after graduate school. Thank you for persuading me to return to school; it was worth it.

Thanks to Drs. Wes Everman and Guy Collins for serving on my committee and dedicating their time, knowledge, and experience throughout this process.

I thank Zack Taylor for always being willing to show me the ropes and pushing me to be the best researcher I could be. You have been a great friend and mentor throughout my time in graduate school. It has been a privilege to learn from you.

Special thanks to Andrew Blythe for being a great friend/classmate, and for all the assistance in the field. This would not have been possible without your hard work.

Thanks to Eric Jones. You have taken countless hours to mentor and encourage me. You molded me into a better student and researcher along the way and for that I am appreciative.

I thank my parents, Danny and Angela Joyner. You have supported me every step of the way. Thank you for teaching me what it means to work hard and to always put forth my best effort. Your love has always been unwavering.

Lastly, thanks to my wife, Caroline. Thank you for always believing in me, being willing to walk alongside me every step of the way. You challenge me to be a better version of myself, every single day. I love you.

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CHAPTER I

Literature Review

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INTRODUCTION

Weed interference has been shown to reduce cotton (*Gossypium Hirsutum* L.) growth and lint yield (Ma et al. 2015) with global estimates of 9% annual losses (Oerke 2006). Prior to the commercialization of glyphosate-resistant (GR) cotton, cotton weed management depended largely on residual herbicides, due to limited safe and efficacious postemergence (POST) herbicide options (Braswell et al. 2016; Wilcut et al. 1995). Following the discovery of GR Palmer amaranth in 2005 and its subsequent spread to every cotton producing state in the U.S. (Heap 2020; USDA-NASS 2020a), there is now a great need for effective preemergence (PRE), POST, POST residual, and POST-directed (POST-DIR) herbicides to maintain weed control (Dilpreet et al. 2011).

Soil-applied residual herbicides are once again a major component of successful weed management in cotton and are encouraged by extension weed scientists. Soil-residual herbicides routinely used in cotton include PPO-inhibitors applied preplant and/or PRE and very long chain fatty acid (VLCFA) inhibitors applied PRE and/or early POST (Cahoon and York 2020; Culpepper 2019). Future management of herbicide-resistant weeds, most notably Palmer amaranth, is complicated by evolution of PPO-resistance (Copeland et al. 2018; Copeland et al. 2019; Varanasi et al. 2019; Ward et al. 2013) and recent discoveries of metabolic resistance to VLCFA-inhibiting herbicides (Brabham et al. 2019). Despite resistance concerns with the aforementioned herbicides, additional soil-residual herbicides are available. Diuron, fluometuron, pendimethalin, and fluridone are also utilized PRE or preplant incorporated (PPI), to control Palmer amaranth (Cahoon and York 2020; Culpepper 2019). Although numerous soil-residual herbicides and effective mechanisms of action (MOA) are available in cotton, it will be

critical to preserve current tools and adopt new strategies to manage troublesome weeds including Palmer amaranth and horseweed (*Erigeron canadensis*) (Van Wychen 2019)

Transgenic herbicide traits in cotton

In 2020, 96% of US cotton acreage included genetically engineered tolerance to one or more herbicides (USDA-NASS 2020b). The rapid adoption of transgenic herbicide-resistant cotton began with the commercialization of GR cotton in 1997. This ushered in an era of non-selective weed control and improved crop safety (Gianessi 2008). The efficacy and flexibility of glyphosate caused some cotton producers to abandon soil residual herbicides and switch to POST-only programs. Similar weed control and net profit could be achieved with glyphosate alone compared to the more complex programs that utilized soil applied residuals (Culpepper and York 1999; Faircloth et al. 2001). Simple and effective weed control with glyphosate also replaced time- and labor-intensive methods of weed control, such as between-row cultivation and other tillage practices and postemergence directed herbicide applications, while allowing adoption of reduced tillage systems (Duke and Powles 2008; Shurley 2006; Webster and Sosonskie 2010). These newfound efficiencies likely contributed to increased farm size in the years following adoption of GR cotton cultivars (USDA-ERS 2013). Despite the ease of effective weed management afforded by GR cotton, sole reliance on glyphosate abetted the evolution of GR weeds, ultimately creating need for additional weed management tools. (Duke and Heap 2017; Kniss 2018).

Transgenic cotton with tolerance to glufosinate was first introduced in 2004 branded as LibertyLink ®, and later was released in combination with tolerance to glyphosate as GlyTol® LibertyLink ® cotton. Tolerance to glufosinate provided a new mode of action for managing troublesome weeds, such as GR Palmer amaranth (Reed et al. 2014). Performance of glufosinate

is sometimes inconsistent (Steckel et al. 1997) and is dependent upon weed size, light, temperature, and relative humidity during application (Kumaratilake et al. 2002; Sellers et al. 2003; Takano et al. 2019; Takano and Dayan 2020; Tharp et al. 1999). Glufosinate effectively controls Palmer amaranth; however, it is less efficacious than glyphosate on glyphosate-susceptible biotypes and can antagonize control by glyphosate when applied in combination (Reed et al. 2014; Whitaker et al. 2011). Presently there are only five cases of herbicide resistance to glufosinate (Heap 2020). However, adoption of glufosinate-resistant crops, and therefore selection pressure, is increasing, creating concern over potential evolution of glufosinate-resistant weeds (Sosnoskie and Culpepper 2014; Takano and Dayan 2020).

Over-the-top applications of synthetic auxin herbicides were introduced to cotton with the commercialization of XtendFlex® and Enlist™ cultivars. XtendFlex® cotton was released in 2016, and confers tolerance to glyphosate, glufosinate, and dicamba (Anonymous 2020a). Dicamba provides control of troublesome weeds such as GR Palmer amaranth and horseweed when applied POST (Cahoon et al. 2015; Johnson et al. 2010). A challenge in dicamba-reistant cotton, dicamba can antagonize control of some grass species by graminicides or glyphosate when applied in combinations with these herbicides (Flint and Barrett 1989; Underwood et al. 2016). Dicamba-resistant transgenic crops have been scrutinized because of dicamba's potential off-target movement (Herrold 2020; Lipton 2017; Mueller and Steckel 2019). Low volatility formulations of dicamba have been registered (Anonymous 2018a; Anonymous 2018b; Anonymous 2018c) and additional training for applicators has been mandated by federal and state governments (EPA-OPP 2016; EPA-OPP 2019; Buhler 2020). However, widespread off-target movement to sensitive species has been reported in some states (Bradley 2017). Similarly to the adoption of GR crops two decades earlier, dicamba-resistant cotton and soybean have been

rapidly adopted and were planted on 24 million hectares in 2019 (Nandula 2019; Unglesbee 2019). With increased adoption of the technology, selection pressure for Palmer amaranth biotypes resistant to dicamba also increased and, in 2020, dicamba-resistant Palmer amaranth was confirmed by University of Tennessee researchers (Steckel 2020), further strengthening and validating the need for additional, diverse weed management tactics.

Enlist™ cotton tolerates glyphosate, glufosinate, and 2,4-D. (Anonymous 2020b). Commercialization of the Enlist trait was accompanied by the release of 2,4-D choline salt, a low-volatility formulation (Anonymous 2017a; Anonymous 2017b). Two,4-D controls small Palmer amaranth, while sequential POST applications may be required to control larger weeds (Manucheri et al. 2017; Merchant et al. 2014). An advantage of the Enlist weed management systems is the ability to tank mix glufosinate and 2,4-D to control large Palmer amaranth (Meyer and Norsworthy 2019), a potential tool for slowing the development of herbicide resistance. Herbicides with different MOA applied in combination have proven to decrease the likelihood of resistance when compared to applying the same herbicides sequentially (Diggle et al. 2003). In contrast, glufosinate has been shown to increase volatility of dicamba when applied in combination (Castner et al. 2020); therefore, this tank mixture is prohibited to dicamba-resistant cotton (Anonymous 2018a; Anonymous 2018b; Anonymous 2018c). Two,4-D has been used for over 70 years (Peterson et al. 2016) but Palmer amaranth resistant to 2,4-D was not discovered until a biotype from Kansas was discovered in 2015 (Kumar et al. 2019). Biotypes of common waterhemp resistant to 2,4-D have also been reported in Illinois and Nebraska (Heap 2020).

Research has commenced in anticipation of the future commercialization of HPPD-resistant cotton. In addition to tolerance to the HPPD-inhibiting herbicide isoxaflutole (IFT), this trait package is expected to include tolerance to glyphosate, glufosinate, and dicamba.

Commercial launch is projected for 2023 with IFT being evaluated for use both PRE and early POST (Gregory Baldwin, BASF Corporation, personal communication). Cotton tolerance to HPPD-inhibiting herbicides was achieved by insertion of an HPPD protein from *Pseudomonas fluorescens*. A single substitution of glycine for tryptophan at position 336 allows for reduced sensitivity to HPPD-inhibiting herbicides including IFT (Boudec et al. 2001; Matringe et al. 2005; USDA-APHIS 2017).

An overview of isoxaflutole

Isoxaflutole (5-cyclopropyl isoxazol-4-yl-2-mesyl-4-trifluoromethylphenyl ketone) inhibits the 4-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme and is a member of the isoxazole chemical family. Inhibition of the HPPD enzyme indirectly obstructs carotenoid biosynthesis, leading to bleaching of plant foliage followed by necrosis. Upon plant uptake, IFT is rapidly metabolized to the herbicidally active form diketonitrile (DKN) [2-cyclopropyl-3-(2-mesyl-4-trifluoromethylphenyl)-3-oxopropanenitrile] and is further metabolized to form a biologically inactive benzoic acid (2-mesyl-4-trifluoromethyl benzoic acid) (Rice et al. 2004; Viviani et al. 1998). Herbicide selectivity is achieved in tolerant, non-transgenic species by the metabolizing DKN into benzoic acid more rapidly than sensitive species (Pallett et al. 1998). Isoxaflutole and DKN are both considered highly mobile in soil and have been studied as potential ground water contaminants (Beltran et al. 2003; Rice et al. 2004). The most recent EPA registration of IFT restricts use in some US states and counties, citing concerns for endangered species and potential groundwater contamination (Unglesbee 2020b).

Isoxaflutole use

Isoxaflutole is presently labeled in corn for control of broadleaf and grass weeds. It is available as a standalone product or in a prepackaged mixture with the thiencarbazone methyl, an

ALS-inhibiting herbicide. Both the standalone and prepackaged mixture contain cyprosulfamide, a crop safener, and can be used preplant incorporated (PPI), preemergence (PRE) or early POST (Anonymous, 2019a; Anonymous, 2019b). Considerable corn injury has been documented following applications of IFT (Wicks et al. 2007; Wilson et al. 1999), which have in some cases been attributed to coarse soil texture, low organic matter content, and elevated soil pH (Vrabel 1998; Wicks et al. 2007). The propensity of IFT to injure corn in coarse, low organic matter soils raises concern for future use in cotton, as these soil characteristics are common to cotton production in the southeastern US (Garcia et al. 2011; NASA 2016; USDA-NASS 2019).

In 2017, HPPD-resistant soybean [Glycine max (L.) Merr.] was commercialized (Nandula 2019). Tolerance to IFT was conferred using a similar transgenic event as previously described in cotton, with the insertion of a HPPD protein from *Pseudomonas fluorescens* (Dufourmantel et al. 2007). Commercialization of HPPD-resistant soybean was accompanied by the release of Alite 27™, a new formulation of IFT, which does not contain the safener cyprosulfamide. Differing from anticipated use in cotton, IFT cannot be applied POST in soybean. (Anonymous 2019c).

Isoxaflutole efficacy

Management of Palmer amaranth is critical for maintaining cotton lint yield (Rowland et al. 1999; Morgan et al. 2001) and PRE applications of IFT have proven to be an effective tool for management of this troublesome weed. Stephenson and Bond (2012) evaluated IFT PRE in corn and reported control $\geq$ 93% 4 weeks after application (WAA). In the same study, Palmer amaranth control was 83% 20 WAA in the absence of any POST treatment. Despite results reported in corn, cotton is less competitive than corn (Bond 2010, Lassiter et al. 2009; Lassiter

and York 2009); therefore, research is warranted to explore integration of IFT into cotton weed management systems.

In preparation for the commercialization HPPD-resistant crops, Starkey et al. (2016) evaluated the efficacy of IFT, mesotrione, and tembotrione, in comparison to commonly used cotton and soybean PRE herbicides. Their research concluded that IFT and mesotrione provide greater control of Palmer amaranth, compared to tembotrione. Isoxaflutole controlled Palmer amaranth greater than pendimethalin and, in most experiments, performed similarly to *S*-metolachlor and fomesafen.

Isoxaflutole also controls other *Amaranthus* species. Knezevic et al. (1998) reported IFT at 100 g ha^{-1} controlled redroot pigweed [*Amaranthus retroflexus* (L.)], which was later confirmed by Zhao et al. (2017) in greenhouse and field trials. Zhao et al. (2017) and Vyn et al. (2006) were both encouraged by the ability of IFT to control PSII-resistant *Amaranthus* species.

Outside of *Amaranthus* species, IFT has demonstrated activity on other weeds, some of which are troublesome in cotton. Smith (2019) reported IFT controlled common lambsquarters [*Chenopodium album* (L.)], common ragweed [*Ambrosia artemisiifolia* (L.)] and velvetleaf [*Abutilon theophrasti* (Medik.)]. Sprague et al. (1999) also reported control of common lambsquarters; however, less consistent control has been observed in drier years (Chomas and Kells 2004; Smith 2019). Young et al. (1999) concluded that common ragweed could not be controlled 8 WAA by IFT alone. Velvetleaf was controlled $\geq$ 88% across multiple studies and use rates (Stephenson and Bond 2012; Young et al. 1999). Isoxaflutole has the potential to control large-seeded weeds more effectively than VLCFA-inhibiting herbicides (Bhowmik et al. 1999; Young et al. 1999; Zhao et al. 2017).

Some of the most common and troublesome weeds in cotton include grasses , such as barnyardgrass (*Echinochloa crus-galli* (L.), johnsongrass [*Sorghum halepense* (L.)], and goosegrass [*Eleusine indica* (L.) Gaertn.) (Van Wychen, 2019). Data suggests each of these troublesome weeds may be controlled by IFT applied PRE. Stephenson and Bond (2012) and Bhowmik et al. (1999) report ≥ 93% control of barnyardgrass. Stephenson and Bond reported similar success in controlling rhizomatous johnsongrass, with control ranging from 87 to 91% and 78%, 4 and 8 WAA, respectively. Goosegrass control of 99% has been observed 3 WAA; however by 5 WAA, residual control dissipated to 63% (Takano et al., 2018).

Though most often utilized as a residual herbicide, IFT has limited activity on emerged weeds. Despite its discovery over 30 years ago (Armel, 2002), literature is limited on POST efficacy of IFT. Starkey et al. (2016) evaluated IFT for POST control of Palmer amaranth and found that control was dependent upon weed size. Palmer amaranth less than 10 cm was controlled ≥ 94% whereas control of weeds greater than 30cm was ≤ 53%. In the same experiment, IFT controlled barnyardgrass ≥ 96% when applied POST. Young and Hart (1998) found an adjuvant improved adsorption and translocation of IFT in giant foxtail (*Setaria* faberi). Isoxaflutole alone controlled giant foxtail ≤ 36% control; however, efficacy improved with the addition of a non-ionic surfactant (NIS), crop oil concentrate (COC), or methylated seed-oil (MSO). Isoxaflutole plus MSO performed the greatest, controlling giant foxtail 71%. Proper adjuvant use could enhance POST efficacy of IFT while providing residual control of troublesome weeds. This could be especially relevant for controlling emerged weeds in reduced-tillage cotton. Similar assertions were made regarding potential use of IFT in no-till corn (Armel 2002; Vrabel et al. 1996).

Weed resistance to HPPD-inhibitors

Following commercialization of HPPD-resistant crops, careful stewardship of HPPD-inhibiting herbicides will be needed to sustain effectiveness of the MOA. Currently, resistance to HPPD-inhibiting herbicides has evolved in biotypes of Palmer amaranth and Tall waterhemp. Most HPPD-resistant *Amaranthus* spp. also have resistance to as many as 4 additional MOAs (Heap 2020). A recent North Carolina survey found nearly 40% of screened Palmer amaranth populations contained survivors following mesotrione POST at 105 g ai ha^{-1}, confirmation of HPPD-resistant Palmer amaranth in the southeastern U.S. (Mahoney 2020). Fecundity and competitiveness of *Amaranthus* spp. (Keely et al. 1987; Place et al. 2008; Ward et al. 2013) bring into question how long before HPPD-resistant biotypes are predominant.

The primary objective of this research was to determine how IFT would integrate into cotton weed management systems in North Carolina. This includes evaluation of weed control, proper application timing, use rate, and possible herbicide combinations. Additional research was completed to evaluate PRE and POST tolerance of HPPD-resistant cotton to IFT alone and combinations of IFT and commonly used cotton herbicides.

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CHAPTER II

Isoxaflutole Preemergence and Postemergence Control of Weeds in Cotton (*Gossypium hirsutum*)

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ABSTRACT

The commercialization of isoxaflutole (IFT) resistant cotton will allow for the use of a new MOA in cotton production. Isoxaflutole is a 4-hydroxyphenylpyrivate dioxygenase (HPPD) inhibiting herbicide that can provide a new tool for managing troublesome weeds and potentially delay the development of herbicide-resistant biotypes. Field research was conducted at research stations near Clayton, Lewiston, and Rocky Mount, NC to evaluate preemergence (PRE) and postemergence (POST) efficacy of IFT. Preemergence trials compared IFT at rates of 53, 70 and 105 g ha^{-1} to cotton PRE standards and evaluated IFT at 70 g ha^{-1} in combination with standard residual herbicides. Standard treatments included acetochlor (1261 g ha^{-1}), diuron (560 g ha^{-1}), fluometuron (1121 g ha^{-1}), fluridone (168 g ha^{-1}), fomesafen (210 g ha^{-1}), and pyrithiobac (59 g ha^{-1}). In postemergence trials, IFT at 53 and 105 g ha^{-1} was compared to glyphosate (1261 g ae ha^{-1}), glufosinate (656 g ha^{-1}), pyrithiobac (101 g ha^{-1}), glyphosate + acetochlor (1261 g ae ha^{-1} + 1261 g ha^{-1}), and glufosinate + acetochlor (656 + 1261 g ha). In addition, IFT at 53 and 105 g ha^{-1} was evaluated in combination with all the previously stated POST treatments. Both PRE and POST trials included a non-treated control for comparison. Visual estimates of percent weed control were collected 2, 3, 4, 5, 6, 8, and 10 weeks after application (WAA). Control by IFT varied considerably by year and location. Control of Palmer amaranth (*Amaranthus palmeri* S. Watson*)* 4 WAA by IFT alone or in combination with standards was greater in 2019, a year that included limited rainfall following application, compared to 2020 which received abundant rainfall. In most instances, control by IFT used in combination with standard treatments was equal to grower standard combinations acetochlor + fomesafen and fluridone + fomesafen. Preemergence control of common ragweed (*Ambrosia artemisiifolia*) was evaluated and IFT controlled the weed $\geq$ 88% 4 WAA, while control by standard residual herbicides was less

consistent (54 to 98%). In postemergence experiments, IFT never controlled Palmer amaranth > 54% 2 WAA, but in some cases did improve control when applied in combination with an effective postemergence herbicide such as glufosinate. These experiments demonstrate that combinations of IFT and cotton preemergence standards can improve control of Palmer amaranth and common ragweed. Additionally, research suggests IFT is ineffective when used postemergence but will serve as a viable postemergence residual option.

Nomenclature: acetochlor; diuron; fluometuron; fluridone; fomesafen; glufosinate; glyphosate; isoxaflutole; pyrithiobac; Palmer amaranth, Amaranthus palmeri S. Watson; Common ragweed, Ambrosia artemisiifolia; Cotton, Gossypium hirsutum

Key words: HPPD-resistant; weed management systems

INTRODUCTION

Cotton weed management will become more complex as the prevalence of herbicide-resistant weeds increases, like Palmer amaranth (*Amaranthus palmeri* S. Watson), which continues to evolve resistance to additional mechanisms of action (MOA) (Heap 2020; Van Wychen 2019). Prior to glyphosate-resistant (GR) cotton, weed management relied heavily upon cultural and mechanical practices and extensive use of residual herbicides (Braswell et al. 2016; Wilcut et al. 1995). The safety and efficacy afforded by GR cotton led some growers to abandon residual herbicide for postemergence (POST) only glyphosate-based programs (Culpepper and York 1999; Faircloth et al. 2001). The discovery and subsequent spread of GR Palmer amaranth forced farmers to revisit previously utilized practices and created need for additional weed management tools. (Duke and Heap 2017; Kniss 2018). One strategy recommended by extension weed specialists is use of residual herbicides, such as protoporphyrinogen oxidase (PPO)- and

very long chain fatty acid (VLCFA)-inhibitors (Cahoon and York 2020; Culpepper 2020). However, resistance of Palmer amaranth to these widely used mechanisms of action (MOAs) has been documented in recent years, with resistance to PPO-inhibitors reported in TN, and resistance to PPO- and VLCFA-inhibitors documented in Arkansas (Brabham et al. 2019; Copeland et al. 2019; Copeland et al. 2018; Heap 2020; Mahoney et al. 2020; Varanasi et al. 2019; Ward et al. 2013).

In response to growing need for additional weed management strategies, the commercialization of transgenic cotton resistant to herbicides inhibiting the 4-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme is anticipated by 2023 (Gregory Baldwin, BASF Corporation, personal communication), providing a new tool for managing herbicide-resistant weeds (Starkey et al. 2016). This transgenic cotton will allow the HPPD-inhibiting herbicide isoxaflutole (IFT) to be applied PRE or early POST, while also including resistance to glyphosate, glufosinate, and dicamba. (Unglesbee 2020).

Presently, IFT is utilized in corn for residual control of monocot and dicot weed species and has proven to be an effective tool for controlling *Amaranthus* spp. (Knezevic et al. 1998; Starkey et al. 2016; Stephenson and Bond 2012; Vyn et al. 2006; Zhao et al. 2017). Additionally, it has the potential to provide control of larger-seeded weeds compared to VLCFA-inhibitors, such as common cocklebur [(*Xanthium strumarium* (L.)] and velvetleaf [*abutilon theophrasti* (Medik.)] (Bhowmik et al. 1999; Young et al. 1999; Zhao et al. 2017). Janak and Grichar (2016) found isoxaflutole effectively controls Texas millet (*Urochloa texana* (Buckley) R. Webster). This may be of particular importance where populations have shifted to Texas millet (*Urochloa texana* (Buckley) R. Webster) in response to repeated use of VLCFA-inhibitors; Texas millet

control by chloroacetamide herbicides is poor compared to other annual grasses (Cahoon, 2020b).

Despite use of IFT in corn for several decades (Armel, 2002), it has not been utilized extensively as a tool to control emerged weeds. Limited literature regarding its postemergence (POST) efficacy indicate control by IFT is highly dependent upon weed size (Starkey et al. 2016) and the inclusion of appropriate adjuvants (Young and Hart 1998).

The objectives of this research were to evaluate the efficacy of IFT when applied preemergence (PRE) or POST alone and in combination with herbicides presently used in cotton. Experiments sought to determine if the inclusion of IFT could enhance control of some the most common and troublesome weeds plaguing cotton production.

MATERIALS AND METHODS

Six PRE and five POST experiments were conducted in North Carolina during 2019 and 2020. Locations included the Upper Coastal Plain Research Station near Rocky Mount (35.89°N, -77.68°W), the Central Crops Research Station near Clayton (35.67°N, -78.49°W), and the Peanut Belt Research Station near Lewiston-Woodville (36.13°N, -77.17). Experimental sites at Rocky Mount and Clayton were in separate fields in 2019 and 2020, while the Lewiston-Woodville experiments were implemented in the same field.

All PRE and POST sites were prepared using conventional methods of seedbed preparation apart from Rocky Mount 2019, which was prepared using a strip-tillage system (Edmisten et al. 2020). Across all locations, beds were formed in 97-cm rows, except Rocky Mount, where 91-cm rows were formed. In 2019, plots were 3 rows by 9 m while in 2020 plots

were 4 rows by 9 m. The experimental design was a randomized complete block with three or four replications.

In PRE and POST experiments, visual estimates of weed control were rated on a 0-100 scale with 0% representing no control and 100% representing complete control. Ratings were collected at 2, 3, 4, 5, 6, 8, and 10 weeks after application (WAA). During 2020, individual weed density was collected 4 WAA for PRE experiments and 3 WAA for POST experiments from a 0.25 m^2 area within each plot. Data were analyzed using the PROC GLIMMIX procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) and means separated using Fisher's Protected LSD at $p = 0.05$.

Preemergence Experiment

Currently available cotton PRE herbicides were compared to IFT at three different rates. A nontreated control was included for comparison. Each site's soil is described in Table 1 and herbicide information is presented in Table 2. If emerged weeds were present at the time of PRE application, paraquat (Parazone® 3SL Herbicide, Makhteshim Agan of North America, Raleigh, NC) plus crop oil concentrate (Agri-Dex®, Helena Chemical Company, Collierville, TN) was applied at 840 g ai ha^{-1} plus 1% (v/v) to all plots, including the nontreated control. Application dates and rainfall data are available in Table 3.

All PRE sites were naturally infested with Palmer amaranth, with exception of Lewiston in 2019 and 2020. All sites with one exception were infested with Texas millet; common ragweed was present in Rocky Mount in 2020 and Lewiston in 2019 and 2020. Treatments were applied using a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} at 207 kPa pressure with flat-fan nozzles (TTI TeeJet® Turbo TeeJet Induction Flat Spray Tips, TeeJet Technologies, Wheaton, IL).

Postemergence Experiment

Glyphosate, glufosinate and pyrithiobac were compared to and evaluated in combination with IFT at two rates. Additionally, glyphosate and glufosinate were evaluated in combination with acetochlor and acetochlor + IFT. All treatments containing IFT included crop oil concentrate applied at 1.6% (v/v). A nontreated control was included for comparison. Herbicide and adjuvant information, soil data, and application date and rainfall are presented in Tables 2, 4, and 5, respectively.

All POST sites were naturally infested with Palmer amaranth, with exception of Lewiston. All but one site included Texas millet, and the Lewiston site was infested with common ragweed. Treatments were applied using a CO_2-pressurized backpack sprayer with a carrier volume of 140 L ha^{-1}. All locations with one exception, utilized TeeJet AIXR11002 flat-fan nozzles (TeeJet Air Induction XR Flat Spray Tips), with equipment calibrated to a pressure of 207 kPa. Applications at Clayton in 2019 were made using TeeJet TTI 11015 flat fan nozzles (TTI TeeJet Turbo TeeJet Induction Flat Spray Tips) and spray equipment was calibrated to a pressure of 138 kPa.

RESULTS AND DISCUSSION

Preemergence Experiment

The interaction of herbicide treatment by location was significant for PRE control of Palmer amaranth, common ragweed, and Texas millet; therefore, data are presented by location. All locations received timely activating rainfall within 7 d of PRE herbicide applications (Table 3).

With exception of Rocky Mount, Palmer amaranth control increased with increasing rate of IFT (Table 6). Isoxaflutole alone at 53, 70, and 105 g ha^{-1} controlled Palmer amaranth 77, 85,

and 92% at Clayton 2019 and 45, 53, and 63% at Clayton, 2020 4 WAA, respectively. Acetochlor (53%), diuron (43%), and fluometuron (58%) were less effective controlling Palmer amaranth than IFT at 70 or 105 g ha^{-1} at Clayton in 2019 whereas fomesafen (84%) and pyrithiobac (82%) were similar to IFT. At Clayton 2020, all residual herbicides applied alone, except fluometuron and pyrithiobac, controlled Palmer amaranth greater than or equal to IFT at 105 g ha^{-1}. Palmer amaranth density at Clayton 2020 (Table 7) followed similar trends as visual ratings. In general, plots treated with residual herbicides had fewer Palmer amaranth compared to stand-alone treatments. Rainfall patterns may explain why IFT was more effective at Clayton 2019 compared to Clayton 2020. Clayton 2019 only received 1 cm of rainfall during the first 14 d after application whereas Clayton 2020 received 5.9 and 1.8 cm of rain 0 to 7 and 8 to 14 d after PRE, respectively. Isoxaflutole, as well as its phytotoxic active metabolite diketonitrile (DKN), are water-soluble and known to be mobile in soil (Beltran et al. 2003; Rice et al. 2004). Thus, IFT and DKN have potential to receive sufficient activation despite limited rainfall conditions or move below the seed germination zone following periods of increased rainfall, the latter of which may have occurred at Clayton 2020 and reduced control of Palmer amaranth by IFT. At Rocky Mount 2020, IFT alone controlled Palmer amaranth 85 to 88% 4 WAA; all residual herbicides applied alone, with exception of fluometuron (58%), controlled Palmer amaranth similar to IFT. As expected, residual Palmer amaranth control decreased 8 WAA, but trends were similar to those observed 4 WAA. Isoxaflutole alone at 105 g ha^{-1} controlled Palmer amaranth 88% 8 WAA at Clayton 2019 and continued to provide superior control compared to all stand-alone residual herbicides with exception of fomesafen (87%) and pyrithiobac (78%). At Clayton 2020, IFT, diuron, fluometuron, and pyrithiobac controlled Palmer amaranth $\leq$ 61% 8 WAA and were less effective than acetochlor (90%) and fluridone (86%); fomesafen (75%) was

moderately effective at this time. All residual herbicides applied alone controlled Palmer amaranth 61 to 88% 8 WAA at Rocky Mount 2020.

In general, residual herbicide combinations controlled Palmer amaranth greater than stand-alone residual treatments. At Clayton 2019 and Rocky Mount 2020, all residual combinations including IFT controlled Palmer amaranth 81 to 96% and provided comparable control as standard residual combinations of acetochlor + fomesafen (87 to 98%) and fluridone + fomesafen (88 to 97%). At Clayton 2020 4 WAA, all IFT combinations, except IFT + fomesafen and IFT + pyrithiobac, controlled Palmer amaranth similar to acetochlor + fomesafen (96%) and fluridone + fomesafen (95%). Compared to Palmer amaranth control 4 WAA, similar trends were observed 8 WAA with residual herbicide combinations. Residual herbicide combinations applied PRE are encouraged by extension weed specialists for Palmer amaranth control (Cahoon and York 2020) and results of this experiment affirm this recommendation. In general, residual herbicide combinations controlled Palmer amaranth greater than residual herbicides applied alone. Additionally, IFT combinations controlled Palmer amaranth similar to acetochlor + fomesafen and fluridone + fomesafen. Therefore, it appears IFT combinations have a place in management of herbicide-resistant Palmer amaranth in cotton, though substantial rainfall following application may reduce control, as was observed at Clayton in 2020.

At Lewiston 2019, IFT alone at 53, 70, and 105 g ha $^{-1}$ controlled common ragweed 83 to 93% 4 WAA (Table 8). Other residual herbicides less effectively controlled common ragweed, resulting in $\leq$ 74% when applied alone. At Lewiston 2020, IFT at 53, 70, and 105 g ha $^{-1}$ controlled common ragweed 90, 98, and 100% control 4 WAA, respectively. Control by residual herbicides was greater at Lewiston in 2020, compared to 2019, with fluridone (96%) and fomesafen (98%) performing similarly to IFT at 70 and 105 g ha^{-1}. All herbicide treatments,

except acetochlor and pyrithiobac, controlled common ragweed ≥ 81% at Lewiston 2020. In 2019, Lewiston received 0.4 cm of rain 0-7 d after PRE, while Lewiston 2020 received 0.9 cm during the same interval. Total rainfall amounts 0-14 d after PRE differed by < 0.1 cm for each year, but greater precipitation 0-7 d after PRE in 2020 may explain improved control observed by fomesafen and fluridone compared to results observed in 2019. At Rocky Mount 2020, IFT alone controlled common ragweed 87 to 100% 4 WAA and was similar to all other residual herbicides, except acetochlor (57%). As anticipated, residual control of common ragweed decreased 8 WAA, but trends observed 4 WAA persisted. At Lewiston 2019, increased separation between IFT and other residual herbicides was observed 8 WAA. Isoxaflutole at 53, 70, and 105 g ha^{-1} controlled common ragweed 82, 90, and 96% 8 WAA, respectively; no other residual herbicide controlled the weed > 36%. At Lewiston 2020, IFT alone at 70 (90%) and 105 (92%) g ha^{-1} were the only herbicide treatments to control common ragweed > 85% 8 WAA. In general, common ragweed control was greater at Rocky Mount 2020, compared to Lewiston 2019 or 2020, which may be attributed to lower common ragweed density at the Rocky Mount 2020 location.

Common ragweed control improved when IFT was applied in combination with standard soil residual herbicides compared to the same herbicides without IFT. Across all locations 4 WAA, IFT in combination with other herbicides controlled common ragweed ≥ 88%. Common ragweed density reflected visual ratings, with combinations including IFT having common ragweed density ≤ 1 per m^2 (Table 7). At Lewiston 2019, combinations of acetochlor + fomesafen (62%) and fluridone + fomesafen (70%) did not adequately control common ragweed. However, these treatments were more effective in 2020, controlling common ragweed similar to IFT combinations. At Rocky Mount 2020, acetochlor + fomesafen (75%) controlled common

ragweed less than IFT combinations, while fluridone + fomesafen (90%) was comparable to combinations including IFT. IFT combinations remained effective controlling common ragweed 8 WAA, with control ranging 84 to 97% across locations. Residual herbicides are important for controlling common ragweed, and additional management tools are increasingly needed given the recent discovery of common ragweed in North Carolina with multiple resistance to ALS-inhibitors, PPO-inhibitors, and glyphosate (Heap 2020, Schrage 2018). Control of common ragweed by IFT alone or in combination with other residual herbicides observed in these experiments indicates IFT is useful in management of herbicide-resistant common ragweed.

Postemergence Experiment

The interactions of herbicide treatment by location were significant for POST control of Palmer amaranth and annual grasses. Data for annual grass control are presented by location. However, Palmer amaranth data for 2020 locations could be pooled; therefore data is presented for Clayton 2019 and pooled for 2020 (Rocky Mount and Clayton). Common ragweed POST control was only evaluated at Lewiston 2020.

At Clayton 2019, POST Palmer amaranth control by IFT at 53 and 105 g ha^{-1} was 21 and 46% 2 WAA, respectively (Table 10). Despite the presence of GR Palmer amaranth, glyphosate control (33%) was similar to that of IFT. Glufosinate (81%) controlled Palmer amaranth greater than any other herbicide applied alone. Surprisingly, pyrithiobac controlled Palmer amaranth 72% indicating a majority of the Palmer amaranth population at this site are sensitive to ALS-inhibiting herbicides. As expected, general trends observed 2 WAA remained 4 WAA, with glufosinate (66%) and pyrithiobac (64%) controlling Palmer amaranth > IFT and glyphosate. For pooled 2020 data from Rocky Mount and Clayton, IFT at 53 and 105 g ha^{-1} controlled Palmer amaranth 49 and 54% 2 WAA, respectively. This was greater than control by glyphosate (36%)

and pyrithiobac (19%) but less than glufosinate (91%). Trends in pooled data for Palmer amaranth control at Rocky Mount and Clayton in 2020 4 WAA were similar to those observed 2 WAA.

Across all locations, POST Palmer amaranth control by IFT alone was relatively low (≤ 56%). Furthermore, IFT, when combined with glufosinate, never improved Palmer amaranth control compared to glufosinate alone. In some cases IFT did improve Palmer amaranth control by glyphosate and pyrithiobac; however, control of the weed remained poor (≤ 68%). This may be explained by the presence of glyphosate- and ALS-resistant biotypes at each location and IFT's lack of POST efficacy. Reductions in Palmer amaranth density provide similar trends as visual estimates of Palmer amaranth control (Table 11). Isoxaflutole reduced density of Palmer amaranth compared to the nontreated control but reductions were not as great as observed with treatments containing glufosinate, which led to the greatest reductions in Palmer amaranth density. Despite poor control by IFT POST, the herbicide may be of value as a POST residual for Palmer amaranth control. At 4 and 8 WAA, IFT at 105 g ha^{-1} + glufosinate controlled Palmer amaranth similar to acetochlor + glufosinate. Acetochlor, *S*-metolachlor, or dimethenamid-*P* applied POST are widely recommended for residual Palmer amaranth control (Cahoon and York 2020; Culpepper 2020). The recent discovery of metabolic resistance to VLCFA-inhibitors in an Arkansas Palmer amaranth biotype (Brabham et al. 2019) and two Illinois tall waterhemp biotypes (Strom et al. 2020) indicates sole reliance upon VLCFA-inhibitors PRE and POST is not sustainable. Use of IFT as a POST residual could provide an effective alternative to the already heavily utilized VLCFA-inhibitors. Adoption of IFT or other HPPD-inhibiting herbicides also raises similar concerns for the development of herbicide resistance, which had previously been limited to the midwestern US, but was recently confirmed in North Carolina (Heap 2020,

Mahoney 2020). This discovery raises concern for the distribution of HPPD-inhibitor resistance Palmer amaranth biotypes in the cotton producing regions of the US.

Common ragweed was present only at Lewiston 2020. The experiment site received adequate rainfall for the activation of POST applied residual herbicide treatments (Table 5). At 2 WAA, all treatments, except pyrithiobac (64%), controlled common ragweed $\geq$ 97% (Table 12). Surprisingly, the predominant common ragweed biotype at Lewiston 2020 was sensitive to glyphosate, despite reports of resistance to these herbicides in North Carolina (Heap 2020; Schrage 2018). At 4 WAA, control by pyrithiobac remained relatively low (53%) whereas all other treatments controlled common ragweed > 94%. Similar to residual common ragweed control observed in PRE experiments, the residual value of IFT applied POST for common ragweed was evident 8 WAA. At this time, herbicide combinations containing IFT at 105 g ha^{-1} controlled common ragweed 90 to 95%. Comparatively, common ragweed control 8 WAA by combinations not including IFT was 80 to 85%. Postemergence applied VLCFA-inhibitors that are recommended for control of Palmer amaranth (Cahoon and York 2020; Culpepper 2020) do not effectively control common ragweed (Cahoon 2019). Use of IFT POST could provide greater residual control of common ragweed compared to commercial standards while also providing a unique, effective MOA for combatting herbicide resistant common ragweed biotypes.

Annual grasses were observed at Clayton 2019, Clayton 2020, and Lewiston 2020. Grass species included goosegrass (*Eleusine indica* (L.) Gaertn.), large crabgrass (*Digitaria sanguinalis* (L.) Scop.), and Texas millet; however, one composite rating for all annual grasses was recorded. At Clayton 2019, IFT at 53 and 105 g ha^{-1} controlled annual grasses 35 and 67% 2 WAA, respectively. Glyphosate (61%) nor glufosinate (68%) performed as anticipated and pyrithiobac did not effectively control annual grasses (36%). The addition of a soil-residual

herbicide improved control over POST herbicides alone, which may indicate grass emerged shortly after POST applications. Control was similar, when either acetochlor or IFT were added to glyphosate or glufosinate (80 to 83%). At Clayton 2020, IFT performed similarly to 2019, with 53 and 105 g ha^{-1} controlling annual grasses 57 and 55% 2 WAA, respectively. All treatments containing glyphosate completely controlled annual grasses 100%, while glufosinate (86%) controlled annual grasses more effectively than IFT and pyrithiobac (10%). IFT or acetochlor applied in combination with glufosinate (86 to 90%) did not improve control of annual grasses compared to glufosinate alone. Annual grass control at Lewiston 2020 was greater compared to other locations. IFT at 53 g ha^{-1} controlled annual grasses 89%, while increasing the rate of IFT to 105 g ha^{-1} improved control to 93% 2 WAA. Pyrithiobac (63%) was least effective but control was improved when IFT was combined with pyrithiobac (90 to 97%). Annual grass control by glyphosate (98%) and glufosinate (95%) increased when combined with IFT and/or acetochlor (99 to 100%). In general, IFT alone did not adequately control annual grasses but control by glyphosate or glufosinate was sometimes improved with the addition of acetochlor and/or IFT as a POST residual.

Conclusion

IFT can control some of the most troublesome weeds in cotton and performed similarly to many common PRE herbicides. In addition to widespread GR Palmer amaranth, isolated PPO- and VLCFA-resistant weeds (Brabham et al. 2019; Copeland et al. 2018; Copeland et al. 2019; Varanasi et al. 2019; Ward et al. 2013) demonstrate increasing need for additional effective MOAs. In general, IFT as a stand-alone treatment did not provide adequate control; however, when applied in combination with commonly used herbicides, control was $\geq$ current grower-standards. Residual control of common ragweed is especially difficult in some cotton producing

regions; however, IFT effectively controlled common ragweed, and was superior to current standards. Isoxaflutole is not suited for controlling emerged weeds compared to glyphosate or glufosinate but has potential to be applied as a POST residual prolonged control of both monocot and dicot weeds and can serve as a residual alternative to the heavily relied upon VLCFA-inhibiting herbicides but potential development of resistance to HPPD-inhibiting herbicides is of major concern. Discoveries of HPPD-inhibitor resistance in a screening of North Carolina Palmer amaranth biotypes (Mahoney 2020) demonstrates that herbicide resistance may challenge the adoption and effectiveness of HPPD-resistant cotton.

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Table 1. Description of soils at preemergence experiment sites.

Location	Year	Soil series[z]	Soil texture	Soil pH[y]	Soil humic matter[y]
					%
Rocky Mount	2019	Aycock	Sandy loam	6.1	0.3
Clayton	2019	Dothan	Loamy sand	6.1	0.2
Lewiston	2019	Goldsboro	Sandy loam	6.0	1.1
Rocky Mount	2020	Rains	Sandy loam	6.1	0.5
Clayton	2020	Dothan	Loamy sand	5.7	0.5
Lewiston	2020	Goldsboro	Sandy loam	6.2	1.1

[z] Aycock, fine-silty, siliceous, subactive, thermic Typic Paledults; Dothan, fine-loamy, kaolinitic, thermic Plinthic Kandiudults,; Goldsboro, fine –loamy, siliceous, subactive, thermic Aquic Paleudults; Rains, fine-loamy, siliceous, thermic, Typic Paleaquult.

[y] Soils charachterized by the Agronomic Services Division of the North Carolina Department of Agriculture and Consumer Services. Soil humic matter determined according to Mehlich (1984).

Table 2. Herbicides and adjuvants used in preemergence and postemergence experiments.[a]

Herbicides and adjuvants	Trade names	Formulation concentration	Manufacturer
Acetochlor	Warrant®	360 g ai L^{-1}	Bayer CropScience
ammonium sulfate	Amaze Gold®	34%	Loveland Products, Inc.
crop oil concentrate	Agri-Dex®	99%	Helena Chemical Co.
Diuron	Direx® 4L	480 g ai L^{-1}	Makhteshim Agan of North America
Fluometuron	Cotoran® 4L	480 g ai L^{-1}	Adama US
Fluridone	Brake®	144 g ai L^{-1}	SePRO Corporation
Glufosinate	Liberty® 280 SL	280 g ai L^{-1}	BASF Corporation
Glyphosate	Roundup PowerMAX® II	660 g ae L^{-1}	Bayer CropScience
fomesafen sodium salt	Reflex®	240 g ai L^{-1}	Syngenta Crop Protection
Isoxaflutole	Alite 27®	479 g ai L^{-1}	BASF Corporation
paraquat dichloride	Gramoxone® SL	240 g ae L^{-1}	Syngenta Crop Protection
pyrithiobac sodium	DuPont™ Staple® LX	384 g ai L^{-1}	Dupont Crop Protection Co.

[a] Specimen labels for each product and mailing addresses and web site addresses of each manufacturer can be found at www.cdms.net.

Table 3. Application dates and rainfall at preemergence experiment sites.

Location	Year	Application Date	Days After Application	
			0-7 DAA	8-14 DAA
			cm	
Rocky Mount	2019	May 3	0.4	1.6
Clayton	2019	May 9	1.0	0
Lewiston	2019	May 7	0.4	0.4
Rocky Mount	2020	May 18	9.6	3.0
Clayton	2020	May 15	5.9	1.8
Lewiston	2020	May 4	0.9	0

Table 4. Description of soils at postemergence experiment sites.

Location	Year	Soil series[z]	Soil texture	Soil pH[y]	Soil humic matter[y]
					%
Rocky Mount	2019	Aycock	Sandy loam	6.1	0.3
Clayton	2019	Dothan	Loamy sand	6.1	0.2
Rocky Mount	2020	Goldsboro	Sandy loam	5.9	0.5
Clayton	2020	Dothan	Loamy sand	5.7	0.5
Lewiston	2020	Goldsboro	Sandy loam	6.1	1.1

[z] Aycock, fine-silty, siliceous, subactive, thermic Typic Paledults; Dothan, fine-loamy, kaolinitic, thermic Plinthic Kandiudults,; Goldsboro, fine –loamy, siliceous, subactive, thermic Aquic Paleudults; Rains, fine-loamy, siliceous, thermic, Typic Paleaquult.

[y] Soils charachterized by the Agronomic Services Division of the North Carolina Department of Agriculture and Consumer Services. Soil humic matter determined according to Mehlich (1984)

Table 5. Application dates and rainfall at postemergence experiment sites.

Location	Year	Application Date	Days After Application	
			0-7 DAA	8-14 DAA
			cm	
Rocky Mount	2019	May 30	6.1	4.1
Clayton	2019	May 29	3.2	8.0
Rocky Mount	2020	June 1	0.1	2.8
Clayton	2020	June 9	9.0	5.9
Lewiston	2020	June 8	0.8	9.6

Table 6. Palmer amaranth control by residual herbicides applied preemergence.[a]

		Clayton 2019		Clayton 2020		Rocky Mount 2020	
Treatment[b]	Application Rate	4 WAA	8 WAA	4 WAA	8 WAA	4 WAA	8 WAA
	g ha^{-1}	%					
IFT	53	77 ef	79 bcd	45 fg	46 fgh	85 abc	71 a-d
IFT	70	85 b-e	86 abc	53 ef	42 gh	86 abc	77 a-d
IFT	105	92 a-d	88 abc	63 cde	61 d-g	88 abc	77 a-d
aceto.	1261	58 g	61 ef	95 a	90 ab	80 abc	88 a
diuron	560	43 h	48 g	62 de	57 efg	69 cd	77 a-d
fluom.	1121	58 g	54 fg	37 g	35 h	58 d	61 d
fluri.	168	73 f	69 de	94 a	86 abc	92 a	81 a-d
fome.	210	84 cde	87 abc	75 bcd	75 a-e	87 abc	81 a-d
pyrith.	59	82 def	78 cd	35 g	27 h	71 bcd	65 bcd
IFT + aceto.	70 + 1261	94 abc	92 a	94 a	85 abc	92 a	85 ab
IFT + diuron	70 + 560	92 a-d	90 a	86 ab	78 a-d	81 abc	71 a-d
IFT + fluom.	70 + 1121	93 abc	93 a	82 ab	66 c-f	83 abc	63 cd
IFT + fluri.	70 + 168	95 ab	92 a	82 ab	72 b-e	98 a	87 a
IFT + fome.	70 + 210	92 a-d	89 ab	77 bc	75 a-e	87 abc	73 a-d
IFT + pyrith.	70 + 59	96 a	95 a	63 cde	56 efg	91 ab	74 a-d
aceto. + fome.	1261 + 210	98 a	96 a	96 a	93 a	87 abc	88 a
fluri. + fome.	168 + 210	88 a-d	88 abc	95 a	88 ab	97 a	85 ab

Table 6. cont.

[a] Means within a column followed by the same letter are not different according to

Fisher's Protected LSD test at $p < 0.05$.

[b]Abbreviations: aceto., acetochlor; fome., fomesafen; fluom., fluometuron; fluri., fluridone; IFT, isoxaflutole; pyrith., pyrithiobac; WAA, weeks after application; Paraquat applied with PRE herbicides at 840 g ha^{-1}.

Table 7. Weed species and density at preemergence experiment sites.[a,b]

		Weed density, 2020				
		Palmer Amaranth		Common ragweed		Texas Millet
Treatment[c]	Application Rate	Clayton	Rocky Mount	Lewiston	Rocky Mount	Clayton
	g ha^{-1}	no. m^{2}				
Nontreated	None	79 ab	7 NS	13 ab	16 NS	164 ab
IFT	53	96 a	8	3 c	0	187 a
IFT	70	68 abc	13	0 c	9	139 ab
IFT	105	33 d	9	5 bc	4	100 ab
aceto.	1261	0 d	5	17 a	81	127 ab
diuron	560	25 bcd	8	13 ab	0	211 a
fluom.	1121	70 abc	32	8 bc	5	201 a
fluri.	168	0 d	8	1 c	0	71 ab
fome.	210	17 cd	13	1 c	1	159 ab
pyrith.	59	61 abc	24	11 abc	9	132 ab
IFT + aceto.	70 + 1261	4 d	4	0 c	1	132 ab
IFT + diuron	70 + 560	4 d	13	0 c	0	99 ab
IFT + fluom.	70 + 1121	17 cd	24	0 c	0	39 b
IFT + fluri.	70 + 168	7 d	0	0 c	0	79 ab
IFT + fome.	70 + 210	7 d	13	1 c	0	184 ab

Table 7 cont.

IFT + pyrith.	70 + 59	32 bcd	1	1 c	0	112 ab
aceto. + fome.	1261 + 210	0 d	11	3 c	17	123 ab
fluri. + fome.	168 + 210	0 d	3	3 c	3	115 ab

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $p < 0.05$.

[b]Weed density taken 37 to 46 days after application.

[c]Abbreviations: aceto., acetochlor; fome., fomesafen; fluom., fluometuron; fluri., fluridone; IFT, isoxaflutole; pyrith., pyrithiobac; Paraquat applied with PRE herbicides at 840 g ha^{-1}.

Table 8. Common ragweed control by residual herbicides applied preemergence.[a]

Treatment[b]	Application Rate	Lewiston, 2019		Lewiston, 2020		Rocky Mount, 2020	
		4 WAA	8 WAA	4 WAA	8 WAA	4 WAA	8 WAA
	g ha^{-1}	%					
IFT	53	83 cd	82 a	90 bc	74 d	100 a	100 a
IFT	70	93 abc	90 a	98 a	90abc	97 ab	92 a
IFT	105	93 abc	96 a	100 a	92 abc	87 ab	80 ab
aceto.	1261	61 f	21 cd	75 d	54 e	57 c	48 c
diuron	560	66 ef	36 c	81 d	48 e	95 ab	99 a
fluom.	1121	70 ef	35 c	89 c	77 cd	92 ab	91 a
fluri.	168	74 de	36 c	96 abc	76 cd	93 ab	79 ab
fome.	210	65 ef	30 c	98 a	85 bcd	90 ab	87 ab
pyrith.	59	54 g	10 d	74 e	62 de	90 ab	83 ab
IFT + aceto	70 + 1261	88 bc	88 a	99 a	96 ab	98 a	89 ab
IFT + diuron	70 + 560	91 abc	84 a	99 a	91 ab	98 a	95 a
IFT + fluom.	70 + 1121	96 ab	94 a	94 abc	97 a	100 a	96 a
IFT + fluri.	70 + 168	99 a	97 a	100 a	94 ab	100 a	97 a
IFT + fome.	70 + 210	91 abc	86 a	98 a	96 ab	99 a	89 ab
IFT + pyrith.	70 + 59	92 abc	89 a	98 a	89 ab	100 a	95 a
aceto. + fome.	1261 + 210	62 f	58 b	99 a	87 abc	75 bc	62 bc
fluri. + fome.	168 + 210	70 ef	54 b	99 a	91 ab	90 ab	90 ab

Table 8. cont.

[a] Means within a column followed by the same letter are not different according to

Fisher's Protected LSD test at $p < 0.05$.

[b] Abbreviations: aceto., acetochlor; fome., fomesafen; fluom., fluometuron; fluri., fluridone; IFT, isoxaflutole; pyrith., pyrithiobac;

Paraquat applied with PRE herbicides at 840 g ha^{-1}.

Table 9. Texas millet control by residual herbicides applied preemergence.[a]

		Rocky Mount, 2019	Clayton, 2020
Treatment[b]	Application Rate	4 WAA	4 WAA
	g ha^{-1}	%	
IFT	53	68 a-d	13 efg
IFT	70	67 b-e	20 c-f
IFT	105	73 ab	31 bc
aceto.	1261	62 def	13 efg
diuron	560	66 b-e	4 g
fluom.	1121	72 abc	10 efg
fluri.	168	74 ab	22 b-e
fome.	210	57 f	6 g
pyrith.	59	67 b-e	7 fg
IFT + aceto	70 + 1261	66 b-e	23 b-e
IFT + diuron	70 + 560	70 a-d	35 ab
IFT + fluom.	70 + 1121	72 abc	45 a
IFT + fluri.	70 + 168	76 a	33 abc
IFT + fome.	70 + 210	74 ab	8 fg
IFT + pyrith.	70 + 59	73 ab	31 bc
aceto. + fome.	1261 + 210	59 ef	16 d-g
fluri. + fome.	168 + 210	64 c-f	27 bcd

Table 9. cont.

[a] Means within a column followed by the same letter are not different according to

Fisher's Protected LSD test at $p < 0.05$.

[b] Abbreviations: aceto., acetochlor; fome., fomesafen; fluom., fluometuron; fluri., fluridone; IFT, isoxaflutole; pyrith., pyrithiobac;

Paraquat applied with PRE herbicides at 840 g ha^{-1}.

Table 10. Palmer amaranth control by herbicides applied postemergence.[a]

		Clayton 2019		Rocky Mount 2020 and Clayton 2020		Clayton 2020
Treatments[b,c]	Application Rate	2 WAA	4 WAA	2 WAA	4 WAA	8 WAA[d]
	g ha^{-1}	%				
IFT	53	21 k	19 g	49 de	48 ef	15 de
IFT	105	46 hij	30 fg	54 cd	56 cf	17 cde
glyph.	1261	33 jk	19 g	36 f	54 f	11 e
gluf.	656	81 a-d	66 ab	91 a	80 abc	48 a
pyrith.	101	72 cde	64 ab	19 g	38 f	17 cde
glyph. + aceto.	1261 + 1261	38 j	29 fg	39 ef	41 ef	6 e
gluf. + aceto.	656 + 1261	87 ab	71 a	91 a	83 ab	60 a
IFT + glyph.	53 + 1261	53 ghi	41 def	52 cd	48 ef	5 e
IFT + gluf.	53 + 656	70 de	50 cde	93 a	78 a-d	39 a-d
IFT + pyrith.	53 + 101	64 efg	56 bc	48 def	35 f	12 e
IFT + glyph.	105 + 1261	40 ij	29 fg	67 b	65 a-e	19 b-e
IFT + gluf.	105 + 656	85 abc	66 ab	98 a	84 a	42 abc
IFT + pyrith.	105 + 101	68 def	57 bc	56 bcd	55 c-f	14 de
IFT + glyph. + aceto.	53 + 1261 + 1261	73 b-e	40 ef	57 bcd	51 ef	12 e
IFT + gluf. + aceto.	53 + 656 + 1261	92 a	62 abc	94 a	85 a	45 ab
IFT + glyph. + aceto.	105 + 1261 + 1261	62 bc	58 bcd	62 bc	58 b-f	19 b-e
IFT + gluf + aceto	105 + 656 + 1261	94 a	72 a	94 a	83 ab	57 a

Table 10. cont.

[a] Means within a column followed by the same letter are not different according to

Fisher's Protected LSD test at $p < 0.05$.

[b] Abbreviations: aceto., acetochlor; glyph., glyphosate; gluf., glufosinate; IFT, isoxaflutole; pyrith., pyrithiobac

[c] Treatments containing IFT were applied with ammonium sulfate at 2.517 kg ha^{-1}; Treatments containing IFT were applied with Crop Oil Concentrate at 1.6% (v/v)

[d] Data were not significant at Rocky Mount, 2020 8WAA

Table 11. Weed species and density at postemergence experiment sites.[a,b]

		Weed density, 2020					
		Palmer Amaranth		common ragweed		annual grasses	
Treatment[c]	Application Rate	Clayton	Rocky Mount	Lewiston	Rocky Mount	Clayton	Lewiston
	g ha^{-1}	no. m^{2}					
Nontreated	none	291 a	94 NS	12 b	13 NS	213 a	43 NS
IFT	53	84 de	9	0 c	0	207 a	8
IFT	105	125 b-e	4	0 c	0	180 ab	9
glyph.	1261	271 ab	31	0 c	1	4 e	245
gluf.	656	19 e	5	0 c	0	64 cde	149
pyrith.	101	53 e	13	16 a	9	164 a-d	33
glyph. + aceto.	1261 + 1261	219 a-d	32	0 c	0	0 c	1
gluf. + aceto.	656 + 1261	11 e	13	0 c	0	57 de	4
IFT + glyph.	53 + 1261	253 abc	12	0 c	0	32 e	34
IFT + gluf.	53 + 656	25 e	0	0 c	0	64 cde	8
IFT + pyrith.	53 + 101	136 a-e	1	0 c	0	92 b-e	17
IFT + glyph.	105 + 1261	115 de	5	0 c	0	8 e	20
IFT + gluf.	105 + 656	16 e	1	0 c	0	37 e	1
IFT + pyrith.	105 + 101	137 a-e	15	0 c	1	179 abc	9
IFT + glyph. +	53 + 1261 + 1261	87 de	11	0 c	0	11 e	4

Table 11 cont.

IFT + gluf. +	53 + 656 + 1261	15 e	3	0 c	0	108 a-e	3
IFT + glyph. +	105 + 1261 + 1261	49 e	8	0 c	0	11 e	1
IFT + gluf +	105 + 656 + 1261	3 e	1	0 c	0	92 b-e	0

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $p < 0.05$.

[b]Weed density taken 21 to 24 days after application.

[c]Abbreviations: aceto., acetochlor; fome., fomesafen; fluom., fluometuron; fluri., fluridone; IFT, isoxaflutole; pyrith., pyrithiobac; Paraquat applied with PRE herbicides at 840 g ha^{-1}.

Table 12. Common ragweed control by herbicides applied postemergence.[a]

POST herbicide[b,c]	Application Rate	Lewiston, 2020		
		2 WAA	4 WAA	8 WAA
	g ha^{-1}	%		
IFT	53	97 b	94 a	82 bc
IFT	105	100 a	100 a	92 ab
glyph.	1261	99 ab	98 a	86 abc
gluf.	656	100 a	98 a	87 abc
pyrith.	101	62 c	53 b	35 d
glyph. + aceto.	1261 + 1261	100 a	99 a	80 c
gluf. + aceto.	656 + 1261	100 a	99 a	85 abc
IFT + glyph.	53 + 1261	100 a	99 a	83 bc
IFT + gluf.	53 + 656	100 a	100 a	88 abc
IFT + pyrith.	53 + 101	99 ab	98 a	90 abc
IFT + glyph.	105 + 1261	98 ab	99 a	91 abc
IFT + gluf.	105 + 656	100 a	100 a	95 a
IFT + pyrith.	105 + 101	99 ab	98 a	90 abc
IFT + glyph. + aceto.	53 + 1261 + 1261	100 a	99 a	85 abc
IFT + gluf. + aceto.	53 + 656 + 1261	100 a	99 a	88 abc
IFT + glyph. + aceto.	105 + 1261 + 1261	100 a	100 a	95 a
IFT + gluf + aceto glufosinate	105 + 656 + 1261	100 a	100 a	95 a

Table 12. cont.

[a] Means within a column followed by the same letter are not different according to

Fisher's Protected LSD test at $p < 0.05$.

[b] Abbreviations: aceto., acetochlor; glyph., glyphosate; gluf., glufosinate; IFT, isoxaflutole; pyrith., pyrithiobac

[c] Treatments containing IFT were applied with ammonium sulfate at 2.517 kg ha^{-1}; Treatments containing IFT were applied with Crop Oil Concentrate at 1.6% (v/v)

Table 13. Annual grass control by herbicides applied postemergence.[a]

		Clayton, 2019		Clayton, 2020		Lewiston, 2020	
POST herbicide[b,c]	Application Rate	2 WAA	4 WAA	2 WAA	4 WAA	2 WAA	4 WAA
	g ha^{-1}	%					
IFT	53	35 i	16 g	57 cd	17 cd	89 c	79 cd
IFT	105	67 fg	53 de	55 d	20 cd	93 abc	88 abc
glyph.	1261	61 gh	63 cd	100 a	98 a	98 a	81 bcd
gluf.	656	68 efg	53 de	86 b	68 b	95 abc	76 d
pyrith.	101	36 i	26 f	10 e	9 d	63 d	40 e
glyph. + aceto.	1261 + 1261	82 b-f	83 abc	100 a	98 a	100 a	96 a
gluf. + aceto.	656 + 1261	80 c-f	78 abc	89 b	71 b	99 a	95 a
IFT + glyph.	53 + 1261	83 a-e	79 abc	100 a	97 a	100 a	91 a
IFT + gluf.	53 + 656	78 def	74 bc	87 b	65 b	99 a	90 ab
IFT + pyrith.	53 + 101	58 gh	40 ef	49 d	18 cd	90 bc	75 d
IFT + glyph.	105 + 1261	80 c-f	78 abc	100 a	93 a	100 a	97 a
IFT + gluf.	105 + 656	95 abc	91 ab	90 b	73 b	100 a	97 a
IFT + pyrith.	105 + 101	49 hi	43 def	65 c	27 c	97 ab	90 ab
IFT + glyph. + aceto.	53 + 1261 + 1261	90 a-d	89 ab	100 a	97 a	100 a	96 a
IFT + gluf. + aceto.	53 + 656 + 1261	83 a-e	77 abc	86 b	66 b	99 a	96 a
IFT + glyph. + aceto.	105 + 1261 + 1261	98 a	95 a	100 a	95 a	100 a	98 a
IFT + gluf + aceto	105 + 656 + 1261	96 ab	92 ab	89 b	66 b	100 a	96 a

Table 13. cont.

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $p < 0.05$.

[b] Treatments containing IFT were applied with Ammonium sulfate at 2.517 kg ha^{-1}.

[c] Treatments containing IFT were applied with Crop Oil Concentrate at 1.6% (v/v

CHAPTER III

HPPD-tolerant cotton (*Gossypium hirstrum*) Response to Isoxaflutole Applied Preemergence and Postemergence

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ABSTRACT

Studies were conducted in 2019 and 2020 at Lewiston, NC to determine the crop response of 4-hydroxyphenylpyrivate dioxygenase (HPPD)-tolerant cotton to IFT and other cotton herbicides as part of a cotton weed management program, which included preemergence (PRE), early postemergence (EPOST), and mid-postemergence (MPOST) herbicides. Isoxaflutole was applied preemergence (PRE) (105 g ha^{-1}) alone and in various combinations with acetochlor, diuron, fluometuron, fluometuron, fluridone, fomesafen, pendimethalin and pyrithiobac. Early postemergence (EPOST) treatments included IFT (53 or 105 g ha^{-1}) alone or in combination with glyphosate or glufosinate, or dimethenamid-*P* + glufosinate. Glyphosate + glufosinate was applied MPOST to all treatments, except the nontreated control. Cotton injury from IFT PRE was minimal (0 to 3%). Across both years, injury EPOST was greater for dimethenamid-*P* + glufosinate and ranged 3 to 5% and 6 to 9% in 2019 and 2020, respectively. In both years, injury from IFT EPOST alone or in combination with glyphosate or glufosinate never exceeded injury from dimethenamid-*P* + glufosinate. Isoxaflutole fb IFT EPOST at 105 g ha^{-1} resulted in miniscule cotton injury (0 to 2%), indicating IFT can be applied PRE or EPOST with minimal risk to cotton. Late-season cotton height and cotton lint yield were not affected by any herbicide treatment. The experimental HPPD-tolerant cotton cultivar was minimally injured by IFT PRE and EPOST, adequately tolerated standard cotton herbicides, and yield loss was not observed. Given these results, HPPD-tolerant cotton and IFT may be integrated into cotton weed management systems with minimal risk for cotton injury and provide an additional effective mechanism of action (MOA) for managing troublesome weeds in cotton.

Nomenclature: acetochlor; dimethenamid-*P*; diuron; fluometuron; fluridone; fomesafen; glufosinate; glyphosate; isoxaflutole; pendimethalin, pyrithiobac; Cotton, Gossypium hirstrum

Key words: cotton tolerance, cotton injury

INTRODUCTION

In 2020, 96% of US cotton acreage included genetically engineered tolerance to one or more herbicides (USDA-NASS 2020). This trend began with rapid adoption of glyphosate-tolerant (GT) cotton, which allowed cotton growers to control troublesome weeds postemergence (POST) with minimal risk for crop injury. (Gianessi 2008). Extensive use of glyphosate hastened the evolution of glyphosate resistant (GR) weeds, thus creating need for a return to integrated weed control systems and additional management tools. (Duke and Heap 2017; Kniss 2018).

In the era prior to GT cotton, it was commonplace to utilize layered residual herbicides with multiple effective mechanisms of action (MOA). A typical recommendation of the time would have included a preplant incorporated (PPI) herbicide, such as pendimethalin or trifluralin, followed by a photosystem II (PSII) inhibiting herbicide PRE, such as diuron or fluometuron. In some instances, a POST-directed herbicide would follow to provide additional late season control (Wilcut et al. 1995). Like the aforementioned strategy, which utilizes multiple effective MOA and layered soil-residual herbicides, similar programs are once again encouraged by extension weed scientists to control GR Palmer amaranth (*Amaranthus palmeri* S. Watson) (Cahoon and York 2020; Culpepper 2019). Soil-residual herbicides are a critical component of an integrated approach to weed management (Culpepper et al. 2010; Norsworthy et al. 2014; Whitaker et al. 2011 Wiggins et al. 2016) and can delay further evolution of herbicide-resistant weed biotypes (Busi et al. 2019; Neve et al. 2011). In recent years, fluridone, an inhibitor of phytoene desaturase and new MOA to cotton (Bartels and Watson 1978; Cahoon et al. 2015a; Chamovitz et al. 1993; Kowalczyk-Schroder and Sandmann 1992), was commercialized specifically for controlling herbicide-resistant Palmer amaranth (Anonymous 2019a; York 2014;

York 2016). Protoporphyrinogen oxidase (PPO)-inhibitors applied preplant and/or PRE and very long chain fatty acid (VLCFA)-inhibitors applied PRE and/or early POST also control GR Palmer amaranth (Bond et al. 2006; Cahoon et al. 2015b; Cahoon and York 2020; Culpepper 2019; Whitaker et al. 2010). However, Palmer amaranth biotypes resistant to PPO- and VLCFA-inhibitors have been discovered in Tennessee and Arkansas, respectively, bringing into question the longevity of these important MOAs as tools for managing Palmer amaranth (Brabham et al. 2019; Copeland et al. 2018; Copeland et al. 2019; Varanasi et al. 2019; Ward et al. 2013). With the looming threat of additional herbicide resistance and the pace of herbicide discovery at a near standstill (Beckie et al. 2017; Dayan 2019; Duke 2012), there is great need to incorporate new effective weed control strategies into cotton production.

Research is underway in anticipation of the commercialization of cotton tolerant to 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides, for which commercial launch is projected by 2023 (Gregory Baldwin, BASF Corporation, personal communication). In addition to tolerance to the HPPD-inhibiting herbicide Isoxaflutole (5-cyclopropyl isoxazol-4-yl-2-mesyl-4-trifluoromethylphenyl ketone (IFT), these cultivars are anticipated to have tolerance to glyphosate, glufosinate, and dicamba. Isoxaflutole is being evaluated for use both PRE and early POST (Unglesbee 2020). Cotton tolerance to HPPD-inhibiting herbicides was achieved by insertion of an HPPD protein from *Pseudomonas fluorescens*. A single substitution of glycine for tryptophan at position 336 allows for reduced sensitivity to IFT (Boudec et al. 2001; Matringe et al. 2005; USDA-APHIS 2017).

Isoxaflutole is presently labeled in corn (*Zea mays* (L.) for control of broadleaf and grass weeds and can be used PPI, PRE or early POST (Anonymous 2019b; Anonymous 2019c) and in transgenic HPPD-tolerant soybean [*Glycine max* (L.) Merr.] PPI or PRE (Anonymous 2019d).

Considerable corn injury has been documented following applications of IFT (Wicks et al. 2007; Wilson et al. 1999), which have in some cases been attributed to coarse soil texture, low organic matter content, and elevated soil pH (Vrabel 1996; Wicks et al. 2007). The propensity of IFT to injure corn in coarse, low organic matter soils raises concern for future use in cotton, as these soil characteristics are common to cotton production in the southeastern US (Garcia et al. 2011; NASA 2016; USDA-NASS 2019).

Isoxaflutole could be a valuable tool for management of *Amaranthus* species in cotton as previous research reports effective residual control (Knezevic et al. 1998; Starkey et al. 2016; Stephenson and Bond 2012; Zhao et al. 2017). Outside of *Amaranthus* species, IFT has also demonstrated activity on other weeds, some of which are troublesome in cotton. Smith (2019) reported IFT controlled common lambsquarters [*Chenopodium album* (L.)], common ragweed [*Ambrosia artemisiifolia* (L.)] and velvetleaf [*Abutilon theophrasti* (Medik.)]. Zhao et al. (2017) noted IFT has the potential to control large-seeded weeds more effectively than VLCFA-inhibiting herbicides. Some of the most common and troublesome weeds in cotton include grasses, such as barnyardgrass (*Echinochloa crus-galli* (L.), johnsongrass [*Sorghum halepense* (L.)], and goosegrass [*Eleusine indica* (L.) Gaertn.) (Van Wychen, 2019). Data suggests each of these troublesome weeds may be controlled by IFT applied PRE (Bhowmik et al. 1999; Stephenson and Bond 2012; Takano et al. 2018).

Research observing control by IFT on emerged weeds is limited. Starkey et al. (2016) evaluated IFT for POST control of Palmer amaranth and found that control was highly dependent upon weed size. Palmer amaranth $\leq$ 10 cm was controlled $\geq$ 94%. Young and Hart (1998) observed adjuvants applied in combination with IFT improved adsorption and translocation of the herbicide in giant foxtail (*Setaria* faberi); methylated seed-oil (MSO) was reported to

improve POST activity of IFT the greatest. Weed control by IFT POST could be a tool for managing emerged weeds in reduced-tillage cotton while still providing residual control. Similar assertions were made regarding potential use of IFT in no-till corn (Armel 2002; Vrabel et al. 1996).

Following commercialization of HPPD-tolerant crops, careful stewardship of HPPD-inhibiting herbicides will be needed to avoid evolution of HPPD-resistant weed biotypes. Currently, resistance to HPPD-inhibiting herbicides has evolved in biotypes of Palmer amaranth and tall waterhemp (Heap 2020). Most notably, a recent North Carolina survey found nearly 40% of screened Palmer amaranth populations contained survivors following mesotrione applied POST at 105 g ai ha^{-1} (Mahoney 2020). Confirmation of HPPD-resistant Palmer amaranth in the southeastern U.S. brings into question the longevity of this MOA.

The main objective of this research was to determine how IFT would integrate into cotton weed management systems in North Carolina. The goal was to evaluate PRE and POST tolerance of HPPD-tolerant cotton to IFT alone and in combination with commonly used cotton herbicides.

MATERIALS AND METHODS

A weed-free experiment was conducted at the Peanut Belt Research Station near Lewiston-Woodville, NC (36.14° N, -77.16° W) in 2019 and 2020. Soil in 2019 was a Goldsboro sandy loam (fine-loamy, siliceous, subactive, thermic Aquic Paleudults) with 1.1% humic matter and pH 6.0, while the soil in 2020 was a Lynchburg sandy loam (fine-loamy, siliceous, thermic Aeric Paleaquults) with 0.7% humic matter and a pH of 6.0 (Mehlich 1984).

Sites were prepared using conventional tillage and then bedded on 91 cm rows, with plots 4 rows by 7.6 m in 2019 and 4 rows by 9 m in 2020. Experimental design was a

randomized complete block with four replications. An experimental GLIXTP cotton cultivar (BASF Corportation, Research Triangle Park, NC) with tolerance to dicamba, IFT, glufosinate and glyphosate was planted on 14 May in 2019 and 13 May in 2020. Cotton was seeded at a rate of 107,500 and 123,500 seeds ha^{-1} at a depth of 2 and 2.5 cm in 2019 and 2020, respectively. Treatments consisted of IFT, alone or in combination, applied PRE and early postemergence (EPOST) compared to commercial standards. A nontreated control was included for comparison. All treatments, except the nontreated control, received glyphosate + glufosinate + ammonium sulfate (AMS) mid-postemergence (MPOST). Application dates and rainfall data are presented in Table 1 whereas herbicides, adjuvants, and rates can be found in Table 2; herbicide treatments can be found in Table 3.

Preemergence herbicides were applied immediately following planting with a CO_2-pressurized backpack sprayer equipped with flat-fan nozzles (TTI TeeJet® Turbo TeeJet Induction Flat Spray Tips, TeeJet Technologies, Wheaton, IL) delivering 140 L ha^{-1} at 207 kPa. In 2019, EPOST treatments were applied 31 d after planting (DAP) when cotton was 3- to 4-leaf whereas MPOST herbicides were applied to 10-leaf cotton 18 d after (DA) EPOST. In 2020, EPOST treatments were applied to 1- to 2-leaf cotton (26 DAP); MPOST herbicides were applied to 6-leaf cotton (15 DA EPOST). Postemergence herbicides were applied using a CO_2-pressurized backpack sprayer delivering 140 L ha^{-1} equipped with DG 11002 (TeeJet® Drift Guard flat-spray nozzles, TeeJet Technologies, Wheaton, IL) and AIXR 11002 nozzles in 2019 and 2020, respectively. To avoid weed interference influencing cotton development and yield, hand-weeding was employed as necessary.

Cotton stand was evaluated 21 DAP by counting all emerged cotton in the two center rows of each plot. Visual estimates of cotton injury (Frans et al. 1986) were collected 9 to 14

DAP, 21 DAP, 7 DA EPOST, 15 to 18 DA EPOST, 13 to 14 DA MPOST, and 27 to 28 DA MPOST. In addition, cotton height was collected 15 DA EPOST and late season, prior to harvest, by measuring the height of 10 plants from the two center rows of each plot. The center two rows of each plot were mechanically harvested and weighed to determine seedcotton yield. Seedcotton grab samples from each plot were collected and ginned using a table-top gin to calculate lint percentage and thus determine lint yield. To evaluate effects of herbicide treatments on fiber length, fiber length uniformity, fiber strength, and micronaire, lint sub-samples with a minimum of 10 g were subjected to high volume instrumentation (HVI) analysis (Sasser 1981). Data were subjected to analysis of variance utilizing the PROC GLIMMIX procedure of SAS (Version 9.4; SAS Institute Inc. Cary, NC) and means were separated using Fisher's Protected LSD at $p = 0.05$ where appropriate.

RESULTS AND DISCUSSION

Main effects of herbicide treatment and year-by-herbicide treatment interactions were only observed for visual estimates of cotton injury. Therefore, all data, except cotton injury, are presented pooled over years. Cotton stand in the nontreated control was 10 plants m row^{-1} 21 DAP (Table 3). Cotton stand in plots treated with a residual herbicide PRE ranged 9 to 10 plants m row^{-1}. Residual herbicides applied PRE, including the grower standard of acetochlor + diuron + fomesafen, IFT alone, and IFT + acetochlor, diuron, diuron + pendimethalin, fluometuron, fluridone, fomesafen, and pyrithiobac, had no effect on cotton stand.

Year-by-herbicide treatment interactions for cotton injury 9 to 14 DAP, 7 DA EPOST, and 15 to 18 DA EPOST were significant; therefore, data are presented by year (Table 4). The grower standard PRE treatment, acetochlor + diuron + fomesafen (4%), injured cotton the greatest 9 to 14 DA PRE in 2019. At this same time, only IFT + diuron and IFT + diuron +

pendimethalin injured cotton similar to acetochlor + diuron + fomesafen. All other treatments, including IFT alone (0%), were less injurious than the grower standard PRE. Cotton injury observed PRE in 2020 was less than in 2019 and ranged just 0 to 2%. It is important to note, like 2019, IFT alone caused 0% cotton injury 9 to 14 DA PRE.

In 2019, cotton injury 7 DA EPOST was minimal across all treatments (0 to 5%) but cotton injury was greater from dimethenamid-*P* + glufosinate (3 to 5%) compared to IFT low rate (LR; 53 g ha^{-1}) or IFT high rate (HR; 105 g ha^{-1}), which injured cotton 1 and 2%, respectively. Isoxaflutole HR + glyphosate (0%) and IFT HR + glufosinate (2%) were no more injurious than IFT alone 7 DA EPOST. Additionally, IFT PRE followed by (fb) IFT HR EPOST only caused 2% injury. Isoxaflutole alone applied EPOST included crop oil concentrate (COC) and ammonium sulfate (AMS); when IFT was applied in combination with glyphosate or glufosinate, only AMS was added. Regardless of adjuvant or herbicide partner, cotton response to IFT applied EPOST was minimal. In 2020, similar trends in cotton injury were observed; however, injury from dimethenamid-*P* + glufosinate was greater than in 2019 and ranged 6 to 9%. Injury from dimethenamid-*P* + glufosinate again exceeded injury from IFT EPOST. Regardless of IFT rate or herbicide combination, cotton was injured 0 to 1%, with the exception of IFT HR + glufosinate, which injured cotton 4%. No injury was observed from IFT PRE fb IFT HR EPOST in 2020, again confirming the safety of IFT when applied both PRE and EPOST.

In 2019, injury 15 to 18 DA EPOST ranged 2 to 3% across all treatments and was not significant. Contrarily, herbicide treatments did affect cotton injury at this timing in 2020, though injury was less than that observed 7 DA EPOST. Dimethenamid-*P* + glufosinate injured cotton 2 to 4% whereas IFT applied EPOST injured cotton 0 to 2% Isoxaflutole LR and IFT HR applied EPOST injured cotton 0 and 1% 15 to 18 DA EPOST, respectively. Isoxaflutole HR +

glyphosate (0%) and IFT + glufosinate (2%) applied at the same timing also caused little injury. Additionally, IFT PRE fb IFT HR EPOST again caused minimal injury (1%). When cotton was 6- to 10-leaf (MPOST), glyphosate + glufosinate was applied to all treatments, except the nontreated control (data not shown), injuring cotton $\leq$ 1%.

Cotton in the nontreated control plots averaged 19 cm in height 15 DA EPOST; all herbicide treatments reduce cotton height compared to the nontreated control at this time. Cotton in plots treated with IFT PRE fb dimethenamid-*P* + glufosinate, acetochlor fb IFT LR, and IFT fb IFT HR averaged 18 cm in height. Cotton was shortest in plots treated with pendimethalin PRE (14 cm). In general, residual combinations applied PRE fb dimethenamid-*P* resulted in shorter cotton compared to treatments not receiving dimethenamid-*P*. Despite early season differences in height, cotton recovered, and by the date that harvest aids were applied, no difference in cotton height was observed. Final cotton height across all treatments ranged 75 to 81 cm.

Cotton lint yield ranged 1300 to 1470 kg ha^{-1} (Table 5) and no treatment differences were observed. Like cotton yield, herbicide treatments did not affect micronaire, fiber length, fiber length uniformity, or fiber strength (data not shown).

The HPPD-tolerant cotton cultivar used in this experiment demonstrated excellent tolerance to IFT PRE and EPOST. Isoxaflutole, applied alone or in combination with other herbicides, caused minimal cotton injury, thus demonstrating potential for safe use at either timing. Additionally, the experimental cotton cultivar demonstrated tolerance to commonly utilized cotton herbicides PRE and POST. These findings are further reinforced by research from Arkansas and Texas, which also report minimal cotton injury and no significant reductions in lint yield from IFT PRE or EPOST when used alone or combined with other herbicides (Foster et al.

2021; Fleming et al. 2020). Despite considerable evidence that HPPD-tolerant cotton responds minimally to IFT applied PRE and EPOST at rates up to 105 g ha^{-1}, further research is warranted to explore response of HPPD-tolerant cultivars to higher rates of IFT. Presently, 105 g ha^{-1} is expected to be the 1X rate of IFT for use in cotton (John Sanderson, BASF Corporation, personal communication); however, evaluation of cotton response exceeding this rate is needed to ensure a considerable margin of crop safety is present.

Although further research is required, tolerance of the experimental HPPD-tolerant cultivar observed in this experiment indicates IFT can likely be integrated safely into cotton weed management systems and used alongside common PRE and POST cotton herbicides with minimal risk to producers, while providing another effective MOA for controlling troublesome weeds in cotton.

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Table 1. Planting and herbicide application dates and rainfall following preemergence herbicides applied at cotton planting.[a]

					Rainfall	
	Planting	Herbicide application date			0-7 DAP	8-14 DAP
Year	Date	PRE	EPOST	MPOST	cm	
2019	May 14	May 14	June 14	July 18	0.4	0
2020	May 13	May 13	June 8	July 23	1.1	1.6

[a] Abbreviations: PRE, preemergence; EPOST, early postemergence; MPOST, mid postemergence; DAP, days after planting.

Table 2. Herbicides and adjuvants used preemergence and postemergence.[a]

Herbicides and adjuvants	Trade names	Formulation concentration	Manufacturer
Acetochlor	Warrant®	360 g ai L^{-1}	Bayer CropScience
ammonium sulfate	Amaze Gold®	34%	Loveland Products, Inc.
crop oil concentrate	Agri-Dex®	99%	Helena Chemical Co.
dimethenamid-P	Outlook®	719 g ai L^{-1}	BASF Corporation
Diuron	Direx® 4L	480 g ai L^{-1}	Makhteshim Agan of North America
Fluometuron	Cotoran® 4L	480 g ai L^{-1}	Adama US
Fluridone	Brake®	144 g ai L^{-1}	SePRO Corporation
fomesafen sodium salt	Reflex®	240 g ai L^{-1}	Syngenta Crop Protection
Glufosinate	Liberty® 280 SL	280 g ai L^{-1}	BASF Corporation
Glyphosate	Roundup PowerMAX® II	660 g ae L^{-1}	Bayer Crop Science
Isoxaflutole	Alite 27®	479 g ai L^{-1}	BASF Corporation
pendimethalin	Prowl® H2O	395 g ai L^{-1}	BASF Corporation
pyrithiobac sodium	Staple® LX	384 g ai L^{-1}	Corteva Agriscience

[a] Specimen labels for each product and mailing addresses and web site addresses of each manufacturer can be found at www.cdms.net.

Table 3. Cotton stand as affected by isoxaflutole alone and isoxaflutole combinations applied preemergence and early postemergence.[a]

Herbicides and application times[bc]		Cotton Stand
		21 DAP
PRE	EPOST[d]	plants m row^{-1}
None	none	10 NS
acet + diuron + fome	dimet + gluf	9
IFT + diuron	dimet + gluf	10
IFT + pendi	dimet + gluf	10
IFT + diuron + pendi	dimet + gluf	10
IFT	dimet + gluf	10
IFT + acet	dimet + gluf	10
IFT + fome	dimet + gluf	10
IFT + fluometuron	dimet + gluf	10
IFT + fluridone	dimet + gluf	10
IFT + pyrithiobac	dimet + gluf	10
none[e]	IFT low rate (LR)[f]	10
none[e]	IFT high rate (HR)[f]	10
none[e]	IFT HR + glyph	10
none	IFT HR + gluf	10
IFT	IFT HR[f]	10

[a] Data are averaged over two years. Means within a column followed by the same letter are not different according to Fisher's Protected LSD Test at p = 0.05

[b] Abbreviations: acet, acetochlor; fome, fomesafen; IFT, isoxaflutole; pendi, pendimethalin; dimet, dimethenamid-P; gluf, glufosinate; glyph, glyphosate; PRE, preemergence; EPOST, early postemergence; DAP, days after planting.

Table 3 Cont.

[c] Acetochlor, diuron, fomesafen, fluometuron, fluridone, isoxaflutole, pendimethalin, and pyrithiobac applied at 1261, 560, 210, 1121, 168, 105, 925, and 59 g ha^{-1} PRE. Dimethenamid-P, glufosinate, isoxaflutole low rate, and isoxaflutole high rate applied at 841, 881, 53, and 105 g ha^{-1} EPOST. Glyphosate applied at 1734 g ae ha^{-1} EPOST.

[d] All treatments, except nontreated control, received glufosinate at 881 g ha^{-1} plus glyphosate at 1734 g ae ha^{-1} mid-POST.

[e] Treatments received no PRE in 2019 and acetochlor at 1261 g ha^{-1} PRE in 2020.

[f] Isoxaflutole applied alone EPOST included crop oil concentrate at 1% v/v; no adjuvant was included when IFT was applied in combination with glufosinate or glyphosate. Glufosinate and/or IFT applied EPOST included AMS at 2.5 kg ha^{-1}.

Table 4. Cotton injury from isoxaflutole alone and isoxaflutole combinations applied preemergence and early postemergence.[a]

Herbicides and application times[bc]		Cotton Injury					
		2019			2020		
		9 to 14 DA PRE	7 DA EPOST	15 to 18 DA EPOST	9 to 14 DA PRE	7 DA EPOST	15 to 18 DA EPOST
PRE	EPOST[d]	%					
acet + diuron + fome	dimet + gluf	4 a	5 a	2 NS	2 a	8 ab	4 a
IFT + diuron	dimet + gluf	3 ab	4 b	2	1 b	6 bc	3 ab
IFT + pendi	dimet + gluf	2 b	5 a	2	0 c	9 a	4 a
IFT + diuron + pendi	dimet + gluf	3 ab	3 c	2	0 c	7 ab	2 bc
IFT	dimet + gluf	1 cd	4 b	3	0 c	7 ab	3 ab
IFT + acet	dimet + gluf	1 cd	3 c	2	1 b	7 ab	3ab
IFT + fome	dimet + gluf	1 cd	4 b	2	2 a	7 ab	3 ab
IFT + fluometuron	dimet + gluf	1 cd	4 b	2	0 c	7 ab	4 a
IFT + fluridone	dimet + gluf	2 bc	4 b	2	1 b	6 bc	4 a
IFT + pyrithiobac	dimet + gluf	1 cd	4 b	2	0 c	7 ab	4 a
none[e]	IFT low rate (LR)[f]	---	2 d	3	---	0 d	0 d
none[e]	IFT high rate (HR)[f]	---	1 e	3	---	1 d	1 c
none[e]	IFT HR + glyph	---	0 f	2	---	1 d	0 d
none	IFT HR + gluf	---	2 d	2	---	4 c	2 bc
IFT	IFT HR[f]	0 d	2 d	2	0 c	0 d	1 c

Table 4 Cont.

[a] Data are averaged over two years. Means within a column followed by the same letter are not different according to Fisher's Protected LSD Test at p = 0.05

[b] Abbreviations: acet, acetochlor; fome, fomesafen; IFT, isoxaflutole; pendi, pendimethalin; dimet, dimethenamid-P; gluf, glufosinate; glyph, glyphosate; PRE, preemergence; EPOST, early postemergence.

[c] Acetochlor, diuron, fomesafen, fluometuron, fluridone, isoxaflutole, pendimethalin, and pyrithiobac applied at 1261, 560, 210, 1121, 168, 105, 925, and 59 g ha^{-1} PRE. Dimethenamid-P, glufosinate, isoxaflutole low rate, and isoxaflutole high rate applied at 841, 881, 53, and 105 g ha^{-1} EPOST. Glyphosate applied at 1734 g ae ha^{-1} EPOST.

[d] All treatments, except nontreated control, received glufosinate at 881 g ha^{-1} plus glyphosate at 1734 g ae ha^{-1} mid-POST.

[e] Treatments received no PRE in 2019 and acetochlor at 1261 g ha^{-1} PRE in 2020.

[f] Isoxaflutole applied alone EPOST included crop oil concentrate at 1% v/v; no adjuvant was included when IFT was applied in combination with glufosinate or glyphosate. Glufosinate and/or IFT applied EPOST included AMS at 2.5 kg ha^{-1}.

Table 5. Cotton height and yield as affected by isoxaflutole alone and isoxaflutole combinations applied preemergence and early postemergence.[a]

Herbicides and application times		Cotton Height		Lint Yield
		15 DA EPOST	Final Plant Height	
PRE	EPOST	cm		kg ha^{-1}
none	none	19 a	80 NS	1470 NS
acet + diuron + fome	dimet + gluf	15 e	78	1340
IFT + diuron	dimet + gluf	16 d	76	1370
IFT + pendi	dimet + gluf	14 f	75	1370
IFT + diuron + pendi	dimet + gluf	14 f	76	1300
IFT	dimet + gluf	18 b	81	1440
IFT + acet	dimet + gluf	16 d	76	1340
IFT + fome	dimet + gluf	16 d	76	1380
IFT + fluometuron	dimet + gluf	17 c	75	1430
IFT + fluridone	dimet + gluf	17 c	77	1400
IFT + pyrithiobac	dimet + gluf	16 d	76	1340
none[e]	IFT low rate (LR)[f]	18 b	77	1440
none[e]	IFT high rate (HR)[f]	17 c	79	1470
none[e]	IFT HR + glyph	17 c	76	1470
none	IFT HR + gluf	17 c	76	1380
IFT	IFT HR[f]	18 b	81	1430

Table 5 Cont.

[a] Data are averaged over two years. Means within a column followed by the same letter are not different according to Fisher's Protected LSD Test at p = 0.05

[b] Abbreviations: acet, acetochlor; fome, fomesafen; IFT, isoxaflutole; pendi, pendimethalin; dimet, dimethenamid-P; gluf, glufosinate; glyph, glyphosate; PRE, preemergence; EPOST, early postemergence.

[c] Acetochlor, diuron, fomesafen, fluometuron, fluridone, isoxaflutole, pendimethalin, and pyrithiobac applied at 1261, 560, 210, 1121, 168, 105, 925, and 59 g ha^{-1} PRE. Dimethenamid-P, glufosinate, isoxaflutole low rate, and isoxaflutole high rate applied at 841, 881, 53, and 105 g ha^{-1} EPOST. Glyphosate applied at 1734 g ae ha^{-1} EPOST.

[d] All treatments, except nontreated control, received glufosinate at 881 g ha^{-1} plus glyphosate at 1734 g ae ha^{-1} mid-POST.

[e] Treatments received no PRE in 2019 and acetochlor at 1261 g ha^{-1} PRE in 2020.

[f] Isoxaflutole applied alone EPOST included crop oil concentrate at 1% v/v; no adjuvant was included when IFT was applied in combination with glufosinate or glyphosate. Glufosinate and/or IFT applied EPOST included AMS at 2.5 kg ha^{-1}.

CHAPTER IV

Injury Potential of Herbicide Combinations and Acephate to Cotyledon Stage XtendFlex™ and Enlist™ Cotton (*Gossypium hirstrum*)

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ABSTRACT

Early season management of glyphosate-resistant (GR) Palmer amaranth (*Amaranthus palmeri* S. Watson) and thrips (Thysanoptera: Thripidae) resistant to neonicotinoid seed treatments is critical for successful cotton production in the southeastern US. In response to these challenges, cotton growers sometimes need to apply POST herbicides, soil-residual herbicides, and foliar insecticides in combination to cotyledon stage cotton. There is concern over the potential for these combinations to injure cotton. Experiments were conducted at Clayton and Rocky Mount, North Carolina to evaluate cotton injury from glyphosate, glufosinate, dicamba, *S*-metolachlor, and acephate in dicamba-resistant cotton and combinations of glyphosate, glufosinate, 2,4-D, *S*-metolachlor, and acephate in 2,4-D-resistant cotton. A planting date component was included to determine if injury to delayed-planted cotton increased potential for yield loss. Injury occurred almost exclusively as necrosis and therefore are used interchangeably henceforth. In dicamba-resistant cotton, injury from glyphosate + glufosinate (1 to 6%) and glyphosate + dicamba (0 to 2%) caused minimal injury. In general, injury was greatest from treatments containing *S*-metolachlor, and sometimes increased with the addition of acephate (0 to 8%). Cotton injury was greater in 2020 than 2019, with treatments containing *S*-metolachlor causing greatest cotton injury. The timely planting date at Rocky Mount demonstrated the potential for considerable cotton injury, where treatments that included *S*-metolachlor injured cotton 21 to 32% and reduced cotton height and seedcotton yield. In the 2,4-D-resistant cotton experiment, injury varied across timings and locations; treatments containing both 2,4-D and *S*-metolachlor caused the greatest injury, while the inclusion of acephate did not typically increase injury. Reductions in cotton height were sometimes observed, but no reductions in seedcotton yield occurred. Injury observed in dicamba and 2,4-D resistant cotton experiments was often

greatest when treatments contained *S*-metolachlor, but injury did not always increase when acephate was added. Seedcotton yields were reduced in some instances, but in general these experiments indicate POST herbicides, soil-applied residuals and acephate can be applied safely to cotyledon cotton.

Nomenclature: acephate; dicamba; glufosinate; glyphosate; *S*-metolachlor; 2,4-D; Palmer amaranth, Amaranthus palmeri S. Watson; Thrips, Thysanoptera: Thripidae; Cotton, Gossypium hirsutum

Key words: cotton injury; insecticides; residual herbicides; postemergence

INTRODUCTION

Palmer amaranth (*Amaranthus palmeri* S. Watson) is the most troublesome weed in U.S. cotton (*Gossypium Hirsutum* L.) (Van wychen 2019) and early season weed management strategies are critical for maintaining cotton lint yield and reducing Palmer amaranth seed production (MacRae et al. 2013). Glyphosate-resistant (GR) cotton, coupled with use of glyphosate postemergence (POST), provided a simple method for controlling emerged weeds with minimal risk of cotton injury (Gianessi 2008, Light et al. 2003). However, glyphosate resistant (GR) Palmer amaranth is now present in every cotton producing state in the U.S. (Heap 2020; USDA-NASS 2020), forcing cotton growers to adopt alternative methods of weed control. Soil-applied residual herbicides are a major component of successful management of GR Palmer amaranth in cotton and are strongly recommended by extension weed scientists (Cahoon and York 2020; Culpepper 2019). A common strategy is “layering” soil-residual herbicides to prevent weed emergence throughout the season (Robinson 2017), and POST applied very long chain fatty acid- (VLCFA)-inhibiting herbicides are often utilized in this capacity. Acetochlor ,

dimethenamid-*P*, and *S*-metolachlor can be used topically for preemergence (PRE) control of Palmer amaranth and have proven effective; however, these herbicides do not control emerged weeds. (Cahoon et al. 2015a; Grier et al. 2006; Hay 2017; Steele et al. 2005). Postemergence applications of VLCFA-inhibitors (WSSA Group 15) can sometimes cause injury to cotton, which appears as necrotic speckling and often diminishes by 21 days after application (DAA). Cahoon and York (2020) note increased cotton injury from *S*-metolachlor when applied in the presence of dew or warm temperatures and high humidity. Despite moderate, and sometimes severe, early season injury from Group 15 herbicides, cotton yield is unaffected (Cahoon et al. 2014; Collie et al. 2014; Eure et al. 2014; Everman et al. 2007; Everman et al. 2009; Inman et al. 2014; Samples, 2020).

In addition to utilizing residual herbicides, cotton growers have been forced to adopt POST herbicides aside from glyphosate to manage GR Palmer amaranth; in particular, use of glufosinate has increased (Raper et al. 2019; Sosnoskie and Culpepper 2014). Glufosinate effectively controls Palmer amaranth but has been found to be less effective than glyphosate on glyphosate-susceptible biotypes (Reed et al. 2014; Whitaker et al. 2011). Glufosinate resistance in LibertyLink®/GlyTol LibertyLink® and XtendFlex® cultivars is achieved with the bar gene, which encodes for phosphinothiricin acetyltransferase (PAT) and confers complete tolerance (Blair-Kerth et al. 2001; Carbonari et al. 2016; Perez-Jones et al. 2018; Raper et al. 2019). Tolerance to glufosinate in Widestrike® cotton was conferred by the pat gene and was less complete than compared to cultivars containing the bar gene (Barnett et al. 2013; OECD 2002; Stewart et al. 2013a; Tan et al. 2006). Injury from glufosinate early POST (EPOST) was sometimes observed; however, injury was typically transient and did not adversely affect lint yield (Culpepper et al. 2009; Dodds et al. 2015; Steckel et al. 2012; Whitaker et al. 2011).

Recently commercialized Enlist™ cotton contains the bar gene, thus making its tolerance to glufosinate equal to that of LibertyLink/GlyTol LibertyLink and XtendFlex cultivars (ISAAA 2020; Catchot et al. 2017)

In more recent years, over-the-top applications of synthetic auxin herbicides were introduced to cotton with the commercialization of XtendFlex and Enlist™ cultivars. XtendFlex cotton was released in 2016, and confers tolerance to glyphosate, glufosinate, and dicamba (Anonymous 2020a). Dicamba effectively controls small (≤ 10 cm tall) Palmer amaranth (Cahoon et al. 2015b; Inman et al. 2016; Inman et al. 2020; Johnson et al. 2010) while causing minimal injury to dicamba-resistant cotton (Cahoon et al. 2015b; Dotray et al. 2014). Enlist cotton resists glyphosate, glufosinate and 2,4-D (Anonymous 2020b); 2,4-D alone or combined with glyphosate or glufosinate are reported to control small Palmer amaranth well (Manucheri et al. 2017; Merchant et al. 2014; Meyer and Norsworthy 2019). In both dicamba- and 2,4-D-resistant cotton, low-volatility formulations of each herbicide were commercialized in effort to mitigate risk of herbicide volatility and injury to sensitive non-target species (Anonymous 2017a; Anonymous 2017b, Anonymous 2018a; Anonymous 2018b; Anonymous 2018c). Due to increased risk of volatility, dicamba is not labeled for use in combination with glufosinate; however, 2,4-D choline salt, the low-volatility formulation of 2,4-D, plus glufosinate is a legal tank mixture and effectively controls Palmer amaranth (Meyer and Norsworthy 2019).

In addition to early season management of weeds, proper cotton development is dependent upon successful management of thrips (Thysanoptera: Thripidae) to avoid delays in maturity and potential yield loss (Cook et al. 2011; Reisig 2020). In the southeastern US, tobacco thrips (*Frankliniella fusca* (Hinds.)) are a particularly troublesome pest of cotton. Tobacco thrips have previously been well controlled with in-furrow insecticides such as aldicarb

(Graham et al. 1995; Layton and Reed 2002; Reed and Jackson 2002; Cook et al. 2011; Samples 2020; Stewart et al. 2013b). In 2010, the registration of aldicarb was voluntarily canceled because of concerns regarding its toxicity to humans (USEPA-OPP 2010). Aldicarb has since been re-registered (USEPA-OPP 2016); however, in its absence, neonicotinoid seed treatments became the relied upon method of thrips control (Akin et al. 2012; Herbert et al. 2012; Huseth et al. 2016; Nino and Kerns 2010; Roberts et al. 2012). Widespread neonicotinoid resistance has since rendered these seed treatments marginally effective forcing cotton growers to adopt additional strategies. Presently, control of thrips requires combinations of neonicotinoid seed treatments, along with in-furrow and topically applied insecticides (Chappel et al. 2020; Huseth et al. 2016; Huseth et al. 2018; Reisig 2020). Postemergence applications targeting thrips commonly include acephate, cyantraniliprote, dicrotophos, dimethoate, or spinetoram (Reisig and Huseth 2021), and are most effective when sprayed to cotton just before the 1-leaf stage (Reisig, 2020).

Given the importance of managing early season weed and insect pests, as well as the necessity for efficiency of large-acreage producers, cotton growers routinely apply herbicides and insecticides simultaneously. Despite the need to apply these combinations, cotton growers are concerned with potential injury when mixing EPOST herbicides and foliar insecticides, with specific concern when adding a Group 15 herbicide (Cahoon 2018; Catchot et al. 2017; Dodds 2015; Hollifeld 2014; Steckel 2020). In general, injury from EPOST herbicides alone is minimal, but increases when used in combination with soil-residuals, such as *S*-metolachlor or acetochlor (Cahoon 2018; Cahoon et al. 2014, 2018; Dodds 2011, 2015; Everman et al. 2007; Steckel et al. 2012; Steckel 2020). North Carolina researchers evaluated combinations of glyphosate, glufosinate, acetochlor, *S*-metolachlor, and acephate and found *S*-metolachlor caused greater

injury than acetochlor but the addition of acephate did not increase cotton injury compared to combinations lacking the insecticide (Inman et al. 2014). Despite early season injury, Inman et al. (2014) reported yield was unaffected. In a similar study, Arkansas researchers reported minimal (< 8%) cotton injury, no delays in maturity, nor yield loss when evaluating cotton response to glyphosate, glufosinate and *S*-metolachlor combined with the insecticides acephate, dicrotophos, and spinetoram (Clarkson 2014). Steckel et al. (2012), in a study investigating herbicide combinations with dimethoate, observed a 3 to 5% increase in Widestrike cotton injury when *S*-metolachlor was applied with glyphosate or glufosinate compared to glyphosate or glufosinate alone. Glyphosate or glufosinate plus dimethoate increased injury $\leq$ 2 % over the two herbicides alone, while the most injurious treatment was glyphosate + glufosinate + *S*-metolachlor + dimethoate, resulting in 38% injury. Regardless of early season injury, cotton yield was not affected; however, maturity of the Widestrike cotton was delayed by treatments containing glufosinate.

Despite substantial research regarding cotton tolerance to herbicides and insecticides independent of one another, few studies have evaluated cotton injury from POST herbicides, soil residuals, and foliar insecticides applied in combination. Prior literature suggests such combinations rarely affect cotton lint yield (Clarkson 2014; Inman et al. 2014; Steckel et al. 2012); however, delays in maturity have been observed (Steckel et al. 2012). Data from North Carolina indicates optimum cotton yield is attainable when planting between 29 April and 10 June. Later plantings require timely management (Edmisten and Collins 2020) and delays in growth or maturity can lead to reduced lint yield (Collins et al. 2017, 2018; Copeland et al. 2017). Furthermore, yield penalties could be exacerbated by the already short growing season in the northernmost regions of the cotton belt. Therefore, the objective of these experiments was to

evaluate cotton injury from EPOST applications of herbicide combinations with and without acephate. In addition, a planting date component was included to determine if cotton injury from these combinations may translate into yield loss when applied to late planted cotton in North Carolina.

MATERIALS AND METHODS

Experiments were conducted in North Carolina during 2019 and 2020, to determine cotton response to herbicide combinations and acephate in dicamba resistant and 2,4-D resistant cotton. Locations included the Central Crops Research Station near Clayton (35.67°N, -78.49°W) and the Upper Coastal Plain Research Station near Rocky Mount (35.89°N, -77.68°W). Both Rocky Mount and Clayton sites were prepared using conventional methods of seedbed preparation with 91- or 97-cm rows. Cotton was seeded at rates ranging from 123,500 to 143,000 seeds ha^{-1}. Cotton cultivar, planting date, and soil data are summarized in Table 1 and Table 2, for the dicamba- and 2,4-D-resistant cotton experiments, respectively. Fluridone (Brake® Herbicide SePRO Corporation, Carmel IN) was applied to all plots PRE at 200 g ha^{-1} and hand weeding was employed throughout the season as necessary to keep plots weed-free. Cotton was maintained throughout the season utilizing agronomic practices recommended by cotton extension specialists in North Carolina (Edmisten et al. 2020).

Plots were 4 rows by 9 m, replicated four times and designed as a 2x8 factorial arrangement. Factors included timely or delayed planting by eight POST herbicide by insecticide combinations including acephate, dicamba, glufosinate, glyphosate or *S*-metolachlor in the dicamba-resistant cotton experiment, or acephate, glufosinate, glyphosate, S-metolachlor or 2,4-D in the 2,4-D-resistant cotton experiment. In both experiments, a non-treated control was also

included for comparison. Treatments were applied to cotyledon cotton, just prior to first true-leaf emergence. Pesticide application rates are presented in Table 3, while application date and environmental conditions at time of application are available in Table 4 and Table 5 for the dicamba- and 2,4-D-resistant cotton experiments, respectively. Three to 5 days before application of treatments, spinetoram (Radiant® SC, Corteva Agrisciences, Wilmington, DE) was applied to all plots at 70 g ha^{-1} to control possible infestations of thrips and limit potential positive response of cotton to thrips control from treatments including acephate. Treatments were applied using a CO2-pressurized backpack sprayer calibrated to deliver 140 L ha-1 of spray volume at 207 and 138 kPa pressure for dicamba- and 2,4-D-resistant cotton experiment, respectively. Backpack sprayers were outfitted with TTI11015 flat-fan nozzles (TTI TeeJet® Turbo TeeJet Induction Flat Spray Tips, TeeJet Technologies, Wheaton, IL) for dicamba-resistant cotton experiment whereas AIXR11002 flat-fan nozzles (TeeJet Air Induction XR Flat Spray Tips, TeeJet Technologies, Wheaton, IL) were used for the 2,4-D-resistant cotton experiments.

Visual estimates of cotton injury were conducted according to Frans et al. (1986), where 0 = no visible injury and 100% = complete plant death. Visual estimates of cotton injury were collected 3, 7, 14, and 21 days after application (DAA) and late season (mid-late September prior to cotton defoliation). Cotton height of 10 plants (5 from each center rows) was collected 28 DAA. The center two rows of each plots were mechanically harvested and weighed during late October to mid-November to determine seedcotton yield. Data were subjected to ANOVA using the PROC GLIMMIX procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) and means were separated using Fisher's Protected LSD at $p \leq 0.05$. Because treatments were applied on different

dates for each planting date, data for timely- and delayed-planted cotton were analyzed separately.

RESULTS AND DISCUSSION

Dicamba-resistant Cotton Response to Pesticide Combinations

The interaction of treatment by location was significant; therefore, data are presented by location. In general, cotton injury observed was almost exclusively necrosis; therefore, percent injury henceforth should be assumed to be necrosis unless otherwise noted. At Clayton 2019, injury was ≤ 9% 3 DAA for cotton planted at both planting dates (Table 6). Glyphosate + glufosinate (0 to 5%) and glyphosate + dicamba (0 to 3%) caused little cotton injury. Glyphosate + *S*-metolachlor caused 2 to 3% injury, but injury increased when other herbicides and acephate were added to this combination. Glyphosate + glufosinate + *S*-metolachlor + acephate caused the greatest injury (9%) whereas glyphosate + dicamba + *S*-metolachlor + acephate caused 5 to 8% injury. Acephate, when added to some herbicide combinations, caused minor increases in cotton injury (0 to 3%). However, increases in injury due to acephate were transient and not observed 14 (data not shown) nor 21 DAA. Across cotton planting dates, cotton injury was ≤ 3% for all treatments 21 DAA. Cotton injury observed at Rocky Mount 2019 was similar to Clayton 2019. Glyphosate + glufosinate and glyphosate + dicamba caused ≤ 3% injury 3 DAA. Glyphosate + glufosinate + *S*-metolachlor + acephate (2 to 8%) and glyphosate + dicamba + *S*-metolachlor + acephate (2 to 8%) were among the most injurious treatments but did not differ from combinations lacking acephate (2 to 7%) or from glyphosate + *S*-metolachlor (1 to 5%). At this location, cotton planted at the timely and delayed planting dates differed in response to treatments 21 DAA; injury ranged 0 to 1% for timely-planted cotton and 2 to 5% for delayed-planted cotton.

Greater cotton injury was observed in 2020 compared to 2019 (Table 7). Furthermore, treatments applied to timely-planted cotton caused more injury in 2020 than when cotton planting was delayed. At Clayton 2020, glyphosate + glufosinate (4 to 6%) and glyphosate + dicamba (1 to 3%) were the least injurious treatments 3 DAA whereas treatments containing *S*-metolachlor (8 to 18%) caused the greatest injury. In most cases, cotton injury resulting from glyphosate + *S*-metolachlor did not differ from glyphosate + *S*-metolachlor + glufosinate or dicamba. In the absence of acephate, glyphosate + *S*-metolachlor + glufosinate or dicamba injured cotton similarly to combinations including acephate. Cotton recovered quickly from injury observed 3 DAA. At 21 DAA, all treatments at Clayton 2020 caused $\leq$ 7% injury.

Of all experiments, injury was greatest at Rocky Mount 2020 when treatments were applied to cotton planted at the timely planting date. Visual estimates of injury to delayed-planted cotton at this location was confounded by necrosis resulting from nitrogen splash during a layby fertilizer application (data not shown). Glyphosate + glufosinate (4%) and glyphosate + dicamba (2%) were the least injurious treatments. The most injurious treatment was glyphosate + dicamba + *S*-metolachlor + acephate (32%) but injury resulting from this treatment was similar to all treatments containing *S*-metolachlor, except glyphosate + glufosinate + *S*-metolachlor + acephate (21%). Furthermore, acephate, added to glyphosate + *S*-metolachlor + glufosinate or dicamba, did not increase cotton injury compared to the same treatments without acephate.

Cotton height 28 DAA ranged 25 to 62 cm and was unaffected by treatments across planting dates and locations, except for the timely planting at Rocky Mount 2020 (Table 8). At this location, cotton measured 26 cm tall 28 DAA in nontreated control plots, which was greater than all other treatments. Cotton height in plots treated with glyphosate + dicamba (22 cm) was comparable to glyphosate + glufosinate (21 cm) and glyphosate + *S*-metolachlor (20 cm). Cotton

height in plots treated with all other treatments ranged 18 to 19 cm 28 DAA and was less than glyphosate + dicamba. Though height reductions are numerically small, they mirror the substantial necrosis observed at this timing and location resulting from treatments containing *S*-metolachlor (21 to 32% 3 DAA).

Similar to trends observed for cotton height 28 DAA, seedcotton yield reduction due to treatments was only noted for cotton planted at the timely planting date at Rocky Mount in 2019 and 2020 (Table 9). At Rocky Mount 2020, seedcotton yield was greatest in nontreated control plots (3538 kg ha^{-1}), which yielded similar to glyphosate + dicamba (3447 kg ha^{-1}) and glyphosate + dicamba + *S*-metolachlor (3254 kg ha^{-1}). In general, seedcotton yield was reduced in plots receiving *S*-metolachlor compared to plots not receiving the herbicide, averaging 3379 and 2995 kg ha^{-1}, respectively. Despite minimal early season injury like other locations, treatments were found to significantly affect yield of cotton planted at the timely planting date at Rocky Mount 2019. Yield loss at this location was unexpected given injury 3 DAA was $\leq$ 8% across all treatments. In addition, plots receiving glyphosate + dicamba yielded 2853 kg ha^{-1} and was less than yield from all other treatments despite causing injury $\leq$ 1%. Clayton 2020 yields were poor ($\leq$ 1342 kg ha^{-1}) and may be explained by above normal precipitation (150% greater than historical average for May through November; data not shown) which complicated management of cotton, leading to insect injury and excessive cotton growth (Anonymous 2020c). Furthermore, unseasonably cool, wet weather in September and early October inhibited maturation of bolls set late in the season.

In general, cotton injury observed in 2019 was less than in 2020, but injury remained transient, decreasing greatly by 21 DAA. Furthermore, treatment differences were not visually distinguishable late season. Differential response of cotton to treatments in 2019 and 2020 may

be explained by differing environmental conditions. Air and soil temperature and relative humidity across locations and planting dates varied (Tables 4 and 5) but did not seem correlated with visual cotton injury. However, environmental conditions prior to pesticide applications may explain greater cotton injury in 2020 compared to 2019. Rainfall was limited in 2019 with totals of 0 and 1.7 cm at Clayton and 1.6 cm and 0.8 cm at Rocky Mount in the 7 days preceding applications to timely- and delayed-planted cotton, respectively. Contrarily, in 2020, rainfall totaled 2.3 and 13.1 cm at Clayton in the 7 days before pesticide applications to timely- and delayed-planted cotton, respectively, while cotton planted at the timely planting date at Rocky Mount received 2.9 cm of rainfall. Norsworthy et al. (2016) and Oosterhuis et al. (1991) found that cotton leaf cuticular thickness increases in water limited environments and can restrict herbicide absorption, thus providing a possible explanation for decreased injury in 2019. In 2020, all locations also encountered greater relative humidity compared to the year prior. In 2019, average relative humidity 7 days prior to applications was 59 and 67% at Clayton and 69 and 80% at Rocky Mount for timely and delayed plantings, respectively. Contrast this with 2020, where average relative humidity was $\geq$ 83% for all locations and timings. Increased cotton response from these environmental conditions is consistent with the findings of Cathey (1986), who reported increased time of chemical solvency on cotton leaf surfaces when relative humidity is high. These findings are also supported by observations from Cahoon and York (2020), who report that warm temperatures and high humidity increase the likelihood of cotton injury from topically applied chloroacetamide herbicides. Likewise, Steckel (2014 and 2015) reported increased cotton response to topically applied *S*-metolachlor and acetochlor when soils are wet, contributing to increased cotton canopy humidity.

2,4-D-resistant Cotton Response to Pesticides Combinations

The interaction of treatment by location was significant; therefore, data are presented by location. Injury occurred as foliar necrosis; therefore, descriptions of cotton injury should be assumed necrosis, unless otherwise noted. In 2019, cotton response to herbicide treatments was minimal, with injury $\leq$ 12 and 14% for timely and delayed plantings 3DAA, respectively (Table 10). For the timely planting date, glyphosate + glufosinate (1%) and glyphosate + 2,4-D (4%) were least injurious, while treatments containing *S*-metolachlor caused more injury (5 to 12%). Combinations of glufosinate and *S*-metolachlor (5 to 8%) caused less injury than treatments containing 2,4-D and *S*-metolachlor (10 to 12%). Acephate, added to glyphosate + *S*-metolachlor + glufosinate or dicamba, did not increase injury compared to these combinations without acephate. For the delayed planting date, treatments containing 2,4-D (7 to 14%), caused greater injury than all other treatments (1 to 2%). In this instance, glyphosate + 2,4-D + *S*-metolachlor + acephate (14%) caused greater injury than glyphosate + 2,4-D + *S*-metolachlor (10%). Across both planting dates, injury diminished considerably over time, ranging 0 to 7% and 0 to 4% 14 and 21 DAA, respectively.

Like dicamba-cotton response to herbicide treatments, greater cotton injury was observed in 2020 compared to 2019. Furthermore, herbicide treatments applied to cotton planted at the timely planting date caused greater injury compared to delayed-planted cotton. For the timely planting date, glyphosate + glufosinate and glyphosate + 2,4-D caused 1 and 4% injury and were the least injurious treatments. Contrarily, treatments containing *S*-metolachlor caused the greatest injury (12 to 26%). Similar to observations for the timely planting date in 2019, 2,4-D and *S*-metolachlor containing treatments (26%) caused greater injury than treatments containing *S*-metolachlor in combinations with glyphosate, glufosinate, or acephate (12 to 18%). Acephate did

not increase injury when added to glyphosate + *S*-metolachlor + glufosinate or 2,4-D applied to cotton planted at the timely date compared to the same treatments in the absence of acephate. Early season cotton injury was transient and by 21 DAA injury was ≤ 7% for all treatments applied to cotton planted at the timely planting date in 2020. Additionally, increased injury from 2,4-D + *S*-metolachlor combinations 3 DAA was transient, with injury from all treatments containing *S*-metolachlor causing similar injury 21 DAA (5 to 7%).

Injury from treatments applied to the delayed planting date in 2020 was less than that following treatments applied to timely-planted cotton. However, combinations of glyphosate + 2,4-D + *S*-metolachlor caused greater injury (6 to 8%) than glyphosate + glufosinate + *S*-metolachlor (3 to 5%). Again, acephate did not increase cotton injury when added to glyphosate + *S*-metolachlor + glufosinate or 2,4-D compared to these combinations without acephate, and cotton injury quickly dissipated to ≤ 3% 14 and 21 DAA.

Differences in cotton height were observed 28 DAA (Table 11) for cotton planted at the timely planting date in both years, while treatment was found to have no significant effect on height when cotton planting was delayed. For timely-planted cotton in 2019, cotton in the nontreated control was shortest (24 cm), while cotton height resulting from all other treatments ranged 26 to 32 cm. Reduced height in the nontreated control may be explained early season weed competition in response to delayed hand removal of weeds. Cotton height in plots treated with glyphosate + glufosinate + *S*-metolachlor (26 cm) and glyphosate + glufosinate + *S*-metolachlor + acephate (27 cm) were comparable to the nontreated control; all other treatments were taller than the nontreated control. In general, trends in cotton height did not reflect visual injury ratings. The reverse was observed for cotton planted at the timely planting date in 2020. At this timing, cotton in the nontreated control (26 cm) was taller than cotton in any treated plots.

Cotton in treated plots ranged in size from 20 to 23 cm. Height differences again did not mirror trends observed in visual ratings. Regardless of treatment effects on cotton height, yield was not affected herbicide treatments (Table 12). Seedcotton yields were greater in 2019 (2802 to 3902 kg ha^{-1}) compared to 2020, where yields were particularly low (1257 to 2060 kg ha^{-1}). As previously mentioned, low seedcotton yield in 2020 may be attributed to excessive rainfall, which hindered proper management of insect pests, timeliness of plant growth regulator (PGR) applications, and unseasonably cool, wet weather during September and October which limited boll maturation.

Conclusion

Across both dicamba and 2,4-D resistant cotton experiments, injury varied greatly by year and timing. Generally, injury was greater with surplus soil moisture and increased relative humidity. In general, cotton injury increased when *S*-metolachlor was included POST compared to combinations lacking the herbicide. Additionally, of POST herbicide treatments evaluated, treatments containing 2,4-D generally caused more injury, than those containing dicamba or glufosinate. Future testing is warranted to determine cotton response to 2,4-D, glufosinate and *S*-metolachlor, as this mixture was not evaluated in this experiment, but is permitted (Anonymous, 2017a). Acephate, added to POST herbicide combinations, did not typically increase cotton injury compared to the same combinations lacking acephate. When averaged across locations and planting dates, acephate increased cotton injury approximately 1% compared to corresponding treatments not containing the insecticide, thus indicating acephate and herbicides may be coapplied EPOST with little risk to cotton. This is further supported by previous research, which determined herbicides and insecticides may be applied together without yield loss (Costello et al. 2005, Inman et al. 2014; Miciniski 1985; Miller et al. 2008; Stewart et al.

2013a). Treatment effects on seedcotton yield observed during this research were unanticipated and was unexplained by treatments.

Delayed-planted cotton always yielded less than cotton planted at the timely planting date; however, was not found to be more susceptible to yield loss from EPOST herbicide and insecticide combinations. Despite these findings, further work should be conducted, given the minor cotton injury observed in 2019 and limited yield at Clayton in 2020. Injury observed in the dicamba-resistant cotton experiment at Rocky Mount in 2020 from treatments containing *S*-metolachlor (18 to 32%) indicates substantial injury is possible in certain environmental conditions and may influence cotton yield. Yield loss at this location contradicts most literature evaluating combinations of POST herbicides and *S*-metolachlor, which find yield is unaffected, despite observable visual injury (Cahoon et al. 2014; Collie et al. 2014; Eure et al. 2014; Everman et al. 2007; Everman et al. 2009; Inman et al. 2014; Raper et al. 2019). Stewart et al. (2013a) found that glufosinate or glufosinate + an insecticide caused a delay in maturity and decreased yield in Widestrike cotton. However, the Widestrike 3 and XtendFlex cotton used in these experiments have improved tolerance to glufosinate compared to Widestrike cotton. Regardless, Stewart et al. (2013a) indicates early season injury can delay cotton maturity and ultimately reduce yield. In general, these experiments found early season cotton injury to be transient and planting date to be unrelated to treatment effect on seedcotton yield. However, further work is warranted as injury in 2019 was much less than 2020. Though the consensus among cotton researchers is that POST herbicides, soil residuals and acephate can be coapplied safely, further research is needed to better understand environmental conditions that contribute to increased cotton injury from herbicide and insecticide combinations.

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Table 1. Dicamba-resistant cultivars, planting dates, and soil information.

	2019		2020	
Site Characteristics	Clayton	Rocky Mount	Clayton	Rocky Mount
Cultivar[a]	DP 1646 B2XF	DP 1646 B2XF	DP 1646 B2XF	DP 1840 B3XF
Timely planting date	May 10	May 3	May 15	May 14
Delayed planting date	June 3	May 31	June 5	June 8
Soil series[b]	Dothan	Rains	Dothan	Norfolk
Soil texture	Loamy sand	Sandy loam	Loamy sand	Loamy sand
Soil humic matter (%)[c]	0.2	0.6	0.3	0.3
Soil pH	6.1	6.5	5.6	5.9

[a] DP 1646 B2XF, Deltapine® (Bayer Cropscience, St. Louis, MO); DP 1840 B3XF (Bayer Cropscience, St. Louis, MO)

[b] Dothan, fine-loamy, kaolinitic, thermic Plinthic Kandiudults,; Rains, fine-loamy, siliceous, thermic, Typic Paleaquult; Norfolk, fine-loamy, kaolinitic, thermic, Typic Knadiudults.

[c] Soils characterized by the Agronomic Services Division of the North Carolina Department of Agriculture and Consumer Services. Soil humic matter determined according to Mehlich (1984).

Table 2. 2,4-D-resistant cultivars, planting dates, and soil

	Clayton	
Site characteristics	2019	2020
Cultivar[a]	PHY 340 W3FE	PHY 350 W3FE
Timely planting date	May 10	May 15
Delayed planting date	June 3	June 5
Soil series	Dothan	Dothan
Soil texture	Loamy sand	Loamy sand
Soil humic matter (%)[c]	0.6	0.6
Soil pH	6.1	5.8

[a] PHY 340 W3FE, Phytogen® Corteva Agriscience (Wilmington, DE); PHY 350 W3FE, (Corteva Agriscience, Wilmington, DE)

[b] Dothan, fine-loamy, kaolinitic, thermic Plinthic Kandiudults.[c] Soils characterized by the Agronomic Services Division of the North Carolina Department of Agriculture and Consumer Services. Soil humic matter determined according to Mehlich (1984).

Table 3. Common names, trade names, application rates, and manufacturer information for pesticides used in experiments.

Common names	Trade names	Application rates	Manufacturer
acephate	Orthene 97®	420 g ai ha^{-1}	AMVAC Chemical Corporation, Newport Beach, CA https://www.adama.com
dicamba	Engenia®	560 g ai ha^{-1}	BASF Corporation, Research Triangle Park, NC https://www.basf.com
glufosinate	Liberty® 280SL	656 g ai ha^{-1}	BASF Ag Products
glyphosate	Roundup Powermax® II	1261 g ai ha^{-1}	Bayer CropScience, St. Louis, MO, https://www.cropscience.bayer.com
S-metolachlor	Dual Magnum®	1070 g ai ha^{-1}	Syngenta Crop Protection, Greensboro, NC https://www.syngenta.com
2,4-D	Enlist One®	1064 g ai ha^{-1}	Corteva, Inc. Wilmington, DE https://www.corteva.us

Table 4. Application date and environmental conditions at application for dicamba-resistant cotton experiments.

	2019			
	Clayton		Rocky Mount	
Site Characteristics	Timely PD[a]	Delayed PD	Timely PD	Delayed PD
EPOST application date[b]	May 23	June 14	May 16	June 26
Relative humidity (%)	65	75	56	46
Air temperature (°C)	30	29	19	27
Soil temperature (°C)	33	27	18	29
Cloud cover (%)	0	0	0	0

	2020			
	Clayton		Rocky Mount	
Site Characteristics	Timely PD	Delayed PD	Timely PD	Delayed PD
EPOST application date	June 1	June 22	June 1	June 26
Relative humidity (%)	74	90	39	72
Air temperature (°C)	18	30	24	28
Soil temperature (°C)	19	27	26	27
Cloud cover (%)	0	0	10	0

Table 4 cont.

[a] Abbreviations: EPOST, early postemergence; PD, planting date.

[b] Treatments applied to cotyledon cotton.

Table 5. Application date and environmental conditions at application for 2,4-D-resistant cotton experiments.

	Clayton			
	2019		2020	
Site Characteristics	Timely PD[a]	Delayed PD	Timely PD	Delayed PD
EPOST Application[b]	May 23	June 14	June 1	June 22
Relative humidity (%)	65	75	50	90
Air temperature (°C)	30	29	23	30
Soil temperature (°C)	33	27	20	32
Cloud cover (%)	0	0	0	10

[a] Abbreviations: EPOST, early postemergence; PD, planting date.

[b] Treatments applied to cotyledon cotton.

Table 6. Dicamba-resistant cotton response to combinations of acephate, postemergence herbicides, and *S*-metolachlor early postemergence at timely and delayed planting dates.[a]

	Cotton Necrosis, 2019							
	Clayton				Rocky Mount			
	Timely PD[b]		Delayed PD		Timely PD		Delayed PD	
Treatments[cd]	3 DAA	21 DAA	3 DAA	21 DAA	3 DAA	21 DAA	3 DAA	21 DAA
	%							
glyph + *S*-met.	3 cd	1 c	2 d	2 NS	1 d	1 a	5 c	5 a
glyph. + gluf.	1 d	0 d	5 bc	3	3 bc	0 b	1 e	2 b
glyph + gluf + *S*-met.	7 ab	3 a	6 b	3	4 b	1 a	2 d	3 b
glyph + gluf + *S*-met. + aceph.	9 a	3 a	9 a	2	8 a	1 a	2 d	3 b
glyph. + dic.	0 d	1 c	3 cd	1	1 d	0 b	1 e	3 b
glyph + dic. + *S*-met.	5 bc	2 b	6 b	3	2 cd	0 b	7 b	5 a
glyph + dic. + *S*-met. + aceph.	5 bc	2 b	8 a	3	3 bc	0 b	8 a	5 a

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD Test at p = 0.05.

[b] Abbreviations: aceph, acephate; DAA, days after application; dic, dicamba; gluf, glufosinate; glyph, glyphosate; PD, planting Date; *S*-met, *S*-metolachlor

Table 6 cont.

[c] Acephate, dicamba, glufosinate, *S*-metolachlor applied at 420, 560, 656, 1070 g ha^{-1}. Glyphosate applied at 1261 g ae ha^{-1}.

[d] Treatments applied to cotyledon cotton.

Table 7. Dicamba-resistant cotton response to combinations of acephate, postemergence herbicides and *S*-metolachlor early postemergence at timely and delayed planting dates.[a]

	Cotton Necrosis, 2020					
	Clayton				Rocky Mount	
	Timely PD[b]		Delayed PD		Timely PD	
Treatments[cd]	3 DAA	21 DAA	3 DAA	21 DAA	3 DAA	21 DAA
	%					
glyph + *S*-met.	15 abc	4 bc	8 bc	3 NS	24 ab	4 NS
glyph. + gluf.	6 d	6 ab	4 c	2	4 c	5
glyph + gluf + *S*-met.	14 bc	5 ab	11 ab	4	25 ab	7
glyph + gluf + *S*-met. + aceph.	12 c	4 bc	13 ab	4	21 b	6
glyph. + dic.	1 d	2 c	3 c	1	2 c	7
glyph + dic. + *S*-met.	17 ab	7 a	15 a	2	24 ab	8
glyph + dic. + *S*-met. + aceph.	18 a	6 ab	11 ab	2	32 a	8

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD Test at p = 0.05.

[b] Abbreviations: aceph, acephate; DAA, days after application; dic, dicamba; gluf, glufosinate; glyph, glyphosate; PD, planting date; *S*-met, *S*-metolachlor.

Table 7 cont.

[c] Acephate, dicamba, glufosinate, *S*-metolachlor applied at 420, 560, 656, 1070 g ha^{-1}. Glyphosate applied at 1261 g ae ha^{-1}.

[d] Treatments applied to cotyledon cotton.

Table 8: Dicamba-resistant cotton height 28 days after applications of acephate, postemergence herbicides, and *S*-metolachlor early postemergence at timely and delayed planting dates[a]

	Cotton height						
	2019				2020		
	Clayton		Rocky Mount		Clayton		Rocky Mount
Treatments[bc]	Timely PD[d]	Delayed PD	Timely PD	Delayed PD	Timely PD	Delayed PD	Timely PD
	cm						
Nontreated	25 NS	55 NS	29 NS	30 NS	30 NS	45 NS	26 a
glyph + *S*-met.	25	58	26	26	25	45	20 bcd
glyph. + gluf.	28	58	30	31	27	45	21 bc
glyph + gluf + *S*-met.	26	59	27	29	25	48	18 d
glyph + gluf + *S*-met. + aceph.	29	59	28	25	27	47	19 cd
glyph. + dic.	26	62	27	26	34	49	22 b
glyph + dic. + *S*-met.	26	61	28	28	25	44	18 d
glyph + dic. + *S*-met. + aceph.	32	57	27	27	30	42	19 cd

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD Test at p = 0.05.

[b] Treatments applied to cotyledon cotton.

Table 8 cont.

[c] Acephate, dicamba, glufosinate, *S*-metolachlor applied at 420, 560, 656, 1070 g ha^{-1}. Glyphosate applied at 1261 g ae ha^{-1}.

[d] Abbreviations: aceph, acephate; dic, dicamba; gluf, glufosinate; glyph, glyphosate; PD, planting date; *S*-met, *S*-metolachlor.

Table 9: Dicamba-resistant seedcotton yield response to combinations of acephate, postemergence herbicides, and *S*-metolachlor early postemergence at timely and delayed planting dates.[a]

	Seedcotton yield						
	2019				2020		
	Clayton		Rocky Mount		Clayton		Rocky Mount
Treatments[bc]	Timely PD[d]	Delayed PD	Timely PD	Delayed PD	Timely PD	Delayed PD	Timely PD
	kg ha^{-1}						
Nontreated	3450 NS	3160 NS	3470 ab	3170 NS	1130 NS	990 NS	3540 a
glyph + *S*-met.	3520	2690	3270 ab	2930	966	780	3015 c
glyph. + gluf.	3540	2940	3790 a	2930	1190	730	3150 bc
glyph + gluf + *S*-met.	3390	2816	3400 ab	2790	1260	660	3103 c
glyph + gluf + *S*-met. + aceph.	4080	2980	3240 bc	2630	1170	660	2946 c
glyph. + dic.	3970	3460	2850 c	2870	1520	430	3450 ab
glyph + dic. + *S*-met.	3870	3130	3780 a	2800	1270	430	3250 abc
glyph + dic. + *S*-met. + aceph.	4070	3000	3470 ab	2880	1340	380	2660 c

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD Test at p = 0.05.

Table 9 cont.

[b] Treatments applied to cotyledon cotton.

[c] Acephate, dicamba, glufosinate, *S*-metolachlor applied at 420, 560, 656, 1070 g ha^{-1}. Glyphosate applied at 1261 g ae ha^{-1}.

[d] Abbreviations: aceph, acephate; dic, dicamba; gluf, glufosinate; glyph, glyphosate; PD, planting date; *S*-met, *S*-metolachlor.

Table 10. 2,4-D-resistant cotton response to combinations of acephate, postemergence herbicides, and *S*-metolachlor early postemergence at timely and delayed planting dates.[a]

	Cotton Necrosis							
	2019				2020			
	Timely PD[b]		Delayed PD		Timely PD		Delayed PD	
Treatments[cd]	3 DAA	21 DAA	3 DAA	21 DAA	3 DAA	21 DAA	3 DAA	21 DAA
	%							
glyph + *S*-met.	5 cd	1 bc	1 c	1 c	12 b	7 a	2 d	1 c
glyph. + gluf.	1 e	0 c	1 c	1 c	1 c	0 b	2 d	0 d
glyph + gluf + *S*-met.	5 cd	1 bc	2 c	1 c	18 b	7 a	3 cd	1 c
glyph + gluf + *S*-met. + aceph.	8 bc	3 a	2 c	1 c	15 b	5 ab	5 bc	1 c
glyph. + 2,4-D	4 de	1 bc	7 b	1 c	4 c	3 ab	4 bcd	1 c
glyph + 2,4-D + *S*-met.	12 a	3 a	10 b	3 b	26 a	7 a	6 ab	2 b
glyph + 2,4-D + *S*-met. + aceph.	10 ab	3 a	14 a	4 a	26 a	7 a	8 a	3 a

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD Test at p = 0.05.

[b] Abbreviations: aceph, acephate; DAA, Days after application; dic, dicamba; gluf, glufosinate; glyph, glyphosate; PD, planting date; *S*-met, *S*-metolachlor.

[c] Acephate, glufosinate, *S*-metolachlor, 2,4-D applied at 420, 656, 1070, 1064 g ha^{-1}. Glyphosate applied at 1261 g ae ha^{-1}.

Table 10 cont.

[d] Treatments applied to cotyledon cotton.

Table 11: 2,4-D-resistant cotton height 28 days after applications of acephate, postemergence herbicides, and *S*-metolachlor early postemergence at timely and delayed planting dates,[a]

	Cotton height			
	2019		2020	
Treatments[bc]	Timely PD[d]	Delayed PD	Timely PD	Delayed PD
	cm			
Nontreated	24 d	55 NS	26 a	44 NS
glyph + *S*-met.	28 bc	57	22 bc	45
glyph. + gluf.	29 abc	59	23 b	45
glyph + gluf + *S*-met.	26 cd	60	20 c	46
glyph + gluf + *S*-met. + aceph.	27 cd	57	22 bc	45
glyph. + 2,4-D	31 ab	58	21 bc	46
glyph + 2,4-D + *S*-met.	28 bc	59	22 bc	46
glyph + 2,4-D + *S*-met. + aceph.	32 a	59	22 bc	46

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD Test at p = 0.05.

[b] Treatments applied to cotyledon cotton. [c] Acephate, glufosinate, *S*-metolachlor, 2,4-D applied at 420, 656, 1070 1064 g ha^{-1}.

Glyphosate applied at 1261 g ae ha^{-1}.

[d] Abbreviations: aceph, acephate; dic, dicamba; gluf, glufosinate; glyph, glyphosate; PD, planting date; *S*-met, *S*-metolachlor.

Table 12. 2,4-D-resistant cotton seedcotton yield response to combinations of acephate, postemergence herbicides, and *S*-metolachlor early postemergence at timely and delayed planting dates.[a]

	Seedcotton yield			
	2019		2020	
Treatments[bc]	Timely PD[d]	Delayed PD	Timely PD	Delayed PD
	kg ha^{-1}			
Nontreated	3328 NS	2961 NS	1705 NS	1412 NS
glyph + *S*-met.	3712	2802	1816	1342
glyph. + gluf.	3730	3230	1813	1346
glyph + gluf + *S*-met.	3836	3107	1955	1448
glyph + gluf + *S*-met. + aceph.	3902	3103	1864	1257
glyph. + 2,4-D	3893	3196	2060	1263
glyph + 2,4-D + *S*-met.	3439	2972	1894	1349
glyph + 2,4-D + *S*-met. + aceph.	3778	2802	1941	1427

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD Test at p = 0.05.

[b] Treatments applied to cotyledon cotton.

[c] Acephate, glufosinate, *S*-metolachlor, 2,4-D applied at 420, 656, 1070 1064 g ha^{-1}. Glyphosate applied at 1261 g ae ha^{-1}.

[d] Abbreviations: aceph, acephate; dic, dicamba; gluf, glufosinate; glyph, glyphosate; PD, planting date