

ABSTRACT

BLACK, DUSTIN JAMES. Efficacy and Crop Response of Herbicide Programs in Isoxaflutole Tolerant Soybeans [*Glycine max* (L.) Merr.]. (Under the direction of Drs. Keith Edmisten and Wesley Everman).

Studies were conducted in Clayton, NC and Pine Level, NC in 2019 to determine the length of residual efficacy of Isoxaflutole and other preemergent soybean herbicides in a bare ground scenario. Treatments consisted of 9 preemergence (PRE) soybean herbicides and one non-treated check. Two rates of IFT (70.15g ai/ha⁻¹, and 105.2 g ai/ha⁻¹), two rates of S-metolachlor (1069 g ai/ha⁻¹, and 2137 g ai/ha⁻¹), and single rates of mesotrione (105.2 g ai/ha⁻¹), 2,4-D (1064 g ai/ha⁻¹), dicamba (701.5 g ai/ha⁻¹), pyroxasulfone (89.3g ai/ha⁻¹), and fomesafen (280 g ai/ha⁻¹) were applied. IFT provided excellent control of Palmer amaranth, carpetweed, goosegrass, large crabgrass and crowfootgrass; and the difference between rates of IFT was marginal in early weeks. The results indicate that the maximum use rate of 105.2g ai/ha⁻¹ is required to maintain higher efficacy past 3 weeks after application. There were no observed statistical differences for most weeds between IFT, and S-metolachlor at their respective rates. Pyroxasulfone provides a long period of residual weed control similar to IFT and S-metolachlor. Fomesafen, 2,4-D and Dicamba lack any substantial residual activity past 1 to 3 weeks depending on the weed species.

Studies were conducted at two locations Clayton, NC in 2018 and Clayton, NC and Pikeville, NC in 2019 to compare the overall efficacy of isoxaflutole (IFT) herbicide programs with other common herbicide programs in IFT tolerant soybeans. Treatments included 16 different preemergence (PRE) and postemergence (POST) programs of IFT, S-metolachlor, glyphosate, glufosinate, flumioxazin, and fomesafen. Isoxaflutole PRE controlled Palmer amaranth 64 to 94% 1 week after POST application (WAP) and was more effective than S-

metolachlor PRE (51 to 93 %), but both underperformed compared to programs incorporating PRE and POST herbicides. While both herbicides experience a decline in efficacy, IFT appears to provide a longer period of control and suppression of Palmer amaranth. Annual grass control was similar for both IFT and *S*-metolachlor. Glyphosate control of Palmer amaranth and glufosinate control of annual grasses was poor. Glyphosate plus glufosinate provided 83 to 99% control of all weed species 2 WAP. Yield data showed that IFT herbicide programs performed statically similar to industry standard programs. In the absence of POST herbicides yields experienced a 28 to 49% reduction.

Studies were conducted at two locations in Clayton, NC in 2018 and in Clayton, NC and Pine level, NC in 2019 to determine crop response of isoxaflutole (IFT) tolerant soybeans to isoxaflutole herbicide programs and industry standard programs. Treatments included 16 different preemergence (PRE) and postemergence (POST) programs of IFT, *S*-metolachlor, glyphosate, glufosinate, flumioxazin, and fomesafen. Trials were kept weed-free for the duration of the season using a broadcast PRE application of acetochlor, hand weeding and a broadcast application POST of glyphosate and glufosinate after crop response ratings were completed. There were no observed visual injury or yield loss from IFT PRE. Only when flumioxazin was used as a PRE or fomesafen was used POST was visual injury greater than any other non-PPO treatment. Injury from IFT, *S*-metolachlor and IFT plus *S*-metolachlor ranged from 0 to 2% 1 week after emergence (WAE). Minor crop stunting was observed late in the season in plots treated with flumioxazin or fomesafen. Yields for all 16 treatments and non-treated control were not statically different from each other.

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Efficacy and Crop Response of Herbicide Programs in Isoxaflutole Tolerant Soybeans [*Glycine max* (L.) Merr.].

by
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DEDICATION

To all those who saw potential in me and encouraged me to follow my dreams.

BIOGRAPHY

Dustin James Black was born on November 16, 1994 in Franklinville NC to William and Julie Black. One of two children, he grew up with an early interest in agriculture helping in the family garden. Dustin was an active FFA member and worked on a row crop farm when he was in high school. He graduated from Providence Grove High School in 2013 and moved to North Dakota to work in canola breeding. In 2015 Dustin returned to North Carolina to attend North Carolina State University. In 2017, Dustin graduated from North Carolina State University with a Bachelor of Science Degree in Plant Biology. While at North Carolina State University Dustin further developed his passion for agriculture by working with Bayer CropScience in cotton and soybean trait development. Following his graduation from North Carolina State University Dustin stayed to pursue a Master of Science Degree Under the direction of Drs. Keith Edmisten, Wesley Everman, and Charlie Cahoon. Dustin conducted his research evaluating the efficacy of herbicide programs in isoxaflutole tolerant soybeans and crop response of herbicides programs on isoxaflutole tolerant soybeans.

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Central Crops Research Station, Clayton NC

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BASF Pikeville Research Station, Pikeville NC

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CHAPTER 1

Length of Residual Efficacy of Isoxaflutole and Other Preemergence Soybean [*Glycine max*

(L.) Merr.] Herbicides.

Length of Residual Efficacy of Isoxaflutole and Other Preemergence Soybean [*Glycine max* (L.) Merr.] Herbicides.

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Field studies were conducted at the Central Crops Research Station in Clayton, North Carolina and the BASF Pine Level Research Farm in Pinelevel, NC in 2019 to determine overall length of residual efficacy of isoxaflutole (IFT) herbicide and other common preemergence soybean herbicides in a bare ground scenario. Treatments consisted of 9 preemergence (PRE) soybean herbicides and one non-treated check. Two rates of IFT (70.15 g ai/ha⁻¹, and 105.2 g ai/ha⁻¹), two rates of *S*-metolachlor (1069 g ai/ha⁻¹, and 2137 g ai/ha⁻¹), and single rates of mesotrione (105.2 g ai/ha⁻¹), 2,4-D (1064 g ai/ha⁻¹), dicamba (701.5 g ai/ha⁻¹), pyroxasulfone (89.3 g ai/ha⁻¹), and fomesafen (280 g ai/ha⁻¹) were applied. IFT provided excellent control of Palmer amaranth, *Amaranthus palmeri* S. Wats, tropic croton, *Croton glandulosus* L., carpetweed, *Mullugo verticillata* L., goosegrass, *Eleusine indica* (L.) Gaertn., large crabgrass *Digitaria sanguinalis* (L.) Scop., crowfootgrass *Dactyloctenium aegyptium* (L.) Willd; and the difference between rates of IFT was marginal in early weeks. The results indicate that the maximum use rate of 105.2 g ai/ha⁻¹ is required to maintain higher efficacy past 3 weeks after application. There were no observed statistical differences for most weeds between IFT, and *S*-metolachlor at their respective rates. Pyroxasulfone provides a long period of residual weed control similar to IFT and *S*-metolachlor. Fomesafen, 2,4-D and Dicamba lack any substantial residual activity past 1 to 3 weeks depending on the weed species. Further research analyzing

tank mix partners and other weed species would provide more comprehensive results of soybean PRE herbicide programs.

Nomenclature: Dicamba; fomesafen; isoxaflutole; mesotrione; pyroxasulfone; *S*-metolachlor; 2,4-D; Palmer amaranth, *Amaranthus palmeri* S. Wats; tropic croton, *Croton glandulosus* L.; carpetweed, *Mullugo verticillata* L.; goosegrass, *Eleusine indica* (L.) Gaertn.; large crabgrass *Digitaria sanguinalis* (L.) Scop.; crowfootgrass *Dactyloctenium aegyptium* (L.) Willd.; soybean, *Glycine max* (L.) Merr.

Key words: Length of residual efficacy, efficacy, preemergent herbicides

Herbicide-resistant weeds continue to pose a credible threat to soybean yield and quality (Schwartz-Lazaro et al. 2018). Highly competitive species can reduce soybean yields by 78% (Bensch et al., 2003). In response to widespread glyphosate- and acetolactate synthase (ALS)-resistant Palmer amaranth (*Amaranthus palmeri* S. Wat.), extension specialists recommend the use of residual herbicides applied preplant, preemergence (PRE) and postemergence (POST) (Norsworthy, et al., 2012). Most current soybean PRE programs rely on combining a protoporphyrinogen oxidase (PPO)-inhibiting herbicide like fomesafen, with a long chain fatty acid (LCFA)-inhibiting herbicide like acetochlor or *S*-metolachlor (Sarangi, et al., 2017). These programs have provided growers with efficacious control of weeds (Norsworthy et al., 2008, Hay, Shoup, and Peterson, 2018), however, in recent years, problematic weeds such as Palmer amaranth and common ragweed (*Ambrosia artemisiifolia* L.) have developed resistance to PPO-inhibitors while the LCFA-inhibitors have been shown to underperform in controlling Palmer amaranth in many regions. (Salas et al., 2016, Heap 2020).

Herbicide-resistant in Palmer amaranth is a wide spread issue, with populations developing both single and multiple resistance throughout the southeast in states like Arkansas (Salas et al., 2016), South Carolina (Ward, Webster, and Steckel, 2013) and North Carolina (Poirier et al., 2014). Palmer amaranth is infamous for causing yield loss in all crops. The issues from Palmer amaranth arise from its highly competitive ability (Keeley et al., 1987) and its evolution of resistance to eight herbicide modes of action (MOAs) (Heap, 2020). Palmer amaranth is a species that can germinate throughout the growing season. As seeds mature and ripen throughout winter and into spring, seed experience reduced dormancy and an expansion of their thermal germination range (10 to 40 C) and fluctuating temperatures also improve germination (Jha et al., 2010). The ability to have season long emergence, overall competitive

nature, and its ability to quickly adapt to selection pressure makes this species extremely difficult to manage.

As the efficacy of herbicides is reduced, it becomes increasingly more difficult for a grower to profit from a crop riddled with weeds that cannot be adequately controlled. The industry has responded by creating crop varieties through genetic engineering with multiple herbicide resistance traits. These trait “stacks” allow for the use of multiple herbicides that would otherwise damage conventional varieties. Of these stacks, the most common include different combinations of the traits conferring tolerances to synthetic auxin (2,4-D and dicamba) traits glufosinate and glyphosate. However, a population of Palmer amaranth has recently developed resistance to a synthetic auxin (2,4-D within a sorghum field in Kansas (Kumar et al., 2019).

With the reduction in efficacy of the common soybean PRE herbicides and with concern for POST herbicide selection pressure, a shift to MOAs not previously implemented in soybeans has occurred. The latest of these herbicides is isoxaflutole (IFT). Isoxaflutole (5-cyclopropyl isoxazol-4-yl-2-mesyl-4-trifluoromethylphenyl ketone) is a 4-Hydroxyphenyl Pyruvate Dioxygenase (HPPD)-inhibiting herbicide that inhibits carotenoid synthesis. Isoxaflutole is actively converted into the metabolically active metabolite Diketonitrile (DKN) [2-cyclopropyl-3-(2-mesyl-4-trifluoromethylphenyl)-3-oxopropanenitrile] and targets the synthesis pathway of plastoquinone within the plant causing a reduction of plastoquinone and thus the indirect inhibition of carotenoid synthesis (Pallett et al. 2001). Further degradation of DKN into a biologically inactive benzoic acid derivative, 2-mesyl-4-trifluoromethyl benzoic acid occurs within the plants; however, this degradation step is slow in susceptible species, resulting in herbicidal selectivity (Alletto et al. 2012). Herbicidal injury is most noted in the emerging leaves of the plants, and as carotenoid synthesis is halted, solar radiation breaks down chlorophyll

molecules rendering the plant unable to photosynthesize and causing the leaves to turn white (Pallett et al. 1998). For this reason, IFT and other herbicides in the HPPD family are known as “bleachers”.

An additional characteristic of IFT that makes it a favorable herbicide is its “rechargeable” characteristic. The chemical properties of IFT and its metabolite DKN, indicate that it is mobile to highly mobile in soils (Rice, Koskinen, and Carrizosa, 2004). This mobility allows the compound to move with water molecules in the soil, and thus be reabsorbed into already emerged weeds, as well as be absorbed by newly emerging weeds after a rainfall allowing for continuous weed control and suppression. However, research has shown that when used as a PRE, IFT struggles to sufficiently control large established weeds over 5 cm (OMAFRA, 2016). Additionally, POST activity of IFT is limited (Spaunhorst and Johnson, 2016).

Isoxaflutole was first commercialized for use in corn production in 1998, as many current corn hybrids have adequate tolerances to the herbicide (Wicks, et al., 2007). The use of IFT in corn showed weed control of up to 94% for weeds like Palmer amaranth, barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) and velvetleaf (*Abutilon theophrasti* Medikus) 4 weeks after application (WAA) (Stephenson and Bond, 2012). The use of IFT has mostly been limited to areas of the country with high corn production, such as Iowa, Illinois, Indiana, Nebraska, the Dakotas; little of the herbicide has been used in the Southeast (Simmons and Kells, 2003). With most IFT use outside of North Carolina, the selection pressure for HPPD resistance in weed populations is dissimilar to high corn producing areas. There have been no HPPD-inhibiting herbicides previously used in soybean production because soybeans are highly susceptible to the herbicides.

BASF Corporation has recently commercialized their Credenz GT27 soybean varieties that are tolerant to IFT. The advent of IFT tolerant soybeans provides the prospect of controlling resistant weeds prior to emergence back to the table. Additional HPPD herbicide resistant varieties that were undergoing research by Syngenta Crop Protection LLC was the “MGI” soybean and corresponding mesotrione herbicide. Mesotrione is an herbicide very similar to IFT in its MOA and previous use in field corn. In 2013 Syngenta was conducting research on MGI soybeans, these varieties would confer resistance to mesotrione, glufosinate and isoxaflutole (Omstrom, 2013). However, in recent years the research has declined, and a potential launch of these varieties is not known.

As PRE herbicides become increasingly important, various forms of research are needed. The objective of this study was to determine the length of residual efficacy of IFT, mesotrione and other soybean PRE herbicides in a non-crop setting.

Materials and Methods

Studies were conducted in 2019 at the Central Crops Research Station in Clayton, NC, and the Pine level Research Farm in Pinelevel, NC. Soils at Central Crops consisted of a Norfolk sandy loam (Fine-loamy, kaolinitic, thermic Typic Kandiuldults) with 0.8% organic matter and pH of 6.3. Soil at the Pine level Research Farm consisted of poorly drained Rains loamy sand (Fine-loamy, siliceous, semiactive, thermic Typic Paleaquults) with 0.6% organic matter and a pH of 6.4.

Plots were 3.7m wide by 7.6 m long. Experiments were arranged in a randomized complete block design with treatments replicated 4 times. Herbicide treatments consisted of 9 PRE herbicides (Table 1.1). A nontreated check was included for comparison. Trials were initiated at Pine level and Clayton on 10 May and 4 June, respectively. Herbicide were applied

using a CO₂ – pressurized backpack sprayer equipped with flat-fan nozzles (TeeJet XR8002VS) delivering 140 L ha⁻¹ at 234 kPA. Pine level received 1.8cm of rainfall on 12 May and Clayton received 6.7cm of rainfall between 7 June and 9 June for adequate herbicide activation.

Visual estimates of Palmer amaranth, tropic croton (*Croton glandulosus* L.), carpetweed (*Mullugo verticillata* L.), goosegrass (*Eleusine indica* (L.) Gaertn.), large crabgrass (*Digitaria sanguinalis* (L.) Scop.), and crowfootgrass (*Dactyloctenium aegyptium* (L.) Willd.) control were collected weekly for 5 weeks using a 0 to 100 scale where 0 is no control and 100 is complete control.

Data were subjected to ANOVA using PROC GLIMMIX in SAS 9.4 (SAS Institute Inc., Cary, NC). Location by treatment interactions were identified and replicates were treated as random effects. Means were separated for location by treatment interaction using a Tukey's HSD at $P \leq 0.05$.

Results and Discussion

Isoxaflutole, mesotrione, S-metolachlor and pyroxasulfone all provided similar control of broadleaf weed species compared to other treatments 3 weeks after application (WAA) (Table 1.2-1.4). Control of all species varied significantly due to environmental factors. Isoxaflutole, S-metolachlor and pyroxasulfone showed no significant difference in weed control 1 to 3 WAA for all broadleaf species (Table 1.2- 1.4). Control of Palmer amaranth by the low rates of S-metolachlor and IFT began to decrease 3 WAA (Table 1.2). At 5 WAA, Palmer amaranth control by the low rate of S-metolachlor decreased to 15% at Pine level, while the low rate of IFT for the same location fell to just 30% control. While this is not statistically different, having half the control of a species as competitive as Palmer amaranth is a serious concern. Both rates of IFT controlled Palmer amaranth similarly at 5 WAA. Pyroxasulfone, a LCFA-inhibiting herbicide,

controlled Palmer similar to the high rates of IFT and S-metolachlor. At 5 WAA, control by pyroxasulfone ranged 42-88%. The synthetic auxins failed to provide longer control of Palmer amaranth dropping to 5 to 13% for 2,4-D, and 25 to 28% for dicamba. At the Pine level location, fomesafen performed similar to the synthetic auxins, controlling Palmer amaranth only 38% 5 WAA, but performed similar to pyroxasulfone and the high rates of IFT and S-metolachlor at the Central Crops Research Station (90%). Mesotrione controlled Palmer amaranth 5 WAA similar to the low rates of IFT and S-metolachlor at Pine Level but underperformed at Central Crops Research Station.

There was no statistical difference for any treatment across all rating for tropic croton control. However, it should be noted control quickly decreases for all herbicides between 2 and 3 WAA (Table 1.3). Similarly, carpetweed control decreases for some herbicides 4 WAA. S-metolachlor underperformed in carpet weed control from 4 to 5 WAA. The low rate provided similar control 5 WAA to that of the synthetic auxins (Table 1.4). At 5 WAA, carpetweed was best controlled by both rates of IFT.

Annual grass control was similar for all herbicides except the synthetic auxins just 1 WAA (1.5-1.7). Dicamba and 2,4-D provided just 48% and 50% control after the first week, respectively. Additionally, fomesafen provided lower control of large crabgrass and goosegrass than IFT, S-metolachlor, pyroxasulfone, and mesotrione (Table 1.5-1.6). Pertaining to large crabgrass, IFT outperformed S-metolachlor 4 WAA and continued to maintain the highest efficacy 5 WAA (Table 1.5). Pyroxasulfone control of large crabgrass is comparable to the low rate of IFT 5 WAA. Mesotrione control of large crabgrass decreases 3 WAA and continued to decrease to almost 0% 5 WAA. Large crabgrass control by 2,4-D decreases to 0 just 2 WAA, but dicamba did suppress large crabgrass out to 5 WAA.

A similar trend was documented for goosegrass control and crowfootgrass control for all herbicides (Table 1.6- 1.7). The high rate *S*-metolachlor controlled both species similar to IFT and pyroxasulfone 5 WAA. Mesotrione and fomesafen were less effective in controlling annual grasses than IFT, *S*-metolachlor, and pyroxasulfone. Control of annual grasses by synthetic auxins was poor 1 WAA (48-73%).

This research demonstrates the potential fit for IFT in soybean production. Like previous research, IFT controlled Palmer amaranth and annual grasses > 90% during the first 2 WAA. However, IFT did not effectively control tropic croton. Since large seeded broadleaves contain more stored seed energy, they can germinate below the herbicide layer, making these species more difficult to control with PRE herbicides compared to small seeded broadleaves. From this study, comparable control of annual grasses and broadleaf weed species by IFT and current soybean PRE herbicides was achieved. Compared to previous research by Stephenson and Bond (2012), IFT provided less control of Palmer amaranth past 4 WAA. However, there are many factors such as soil type, climate, and cropping system that affect herbicide efficacy. Research conducted by Stephenson and Bond (2012) was conducted in corn planted in a silt loam and very fine sandy loam, both uniquely different from the bare ground soils of this study and both with higher organic matter content. The binding affinity of IFT and its metabolite DKN increases as soil organic matter increases (Mitra, Bhowmik, and Xing, 1999). Preventing the herbicide from leaching through the soil profile allows more herbicide to be present and provides for longer control. Furthermore, IFT provides better control of annual grasses than mesotrione and fomesafen and provides similar control to current soybean PRE herbicides. Isoxaflutole provides a level of weed control of broadleaf and annual grass weeds that is comparable to standard LCFA herbicides on the market for soybeans. Additionally, IFT demonstrated superior length of

residual efficacy on these species compared to mesotrione. While mesotrione controlled broadleaf weeds well, control of annual grasses was poor.

This study can best be described as an early framework for more intensive research on soybean PRE herbicides. While the data often suggests little to no significant difference, it must be recognized that even a 5% difference in weed control can be crucial in resistance management. Growers and extension specialists have adopted zero-tolerance weed programs with a focus on early season weed control from PRE herbicides. While none of the herbicides in this study can be used as a standalone, they can be critical parts of an entire integrated weed management program.

Further research must be conducted on these treatments and their efficacy on other species such as tall waterhemp (*Amaranthus tuberculatus* (Moq.) Sauer), barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) and other common species in North Carolina; as well as other soil types that are found in North Carolina. Research evaluating the efficacy and compatibility of tank mixes of these and other PRE herbicides would allow for a greater understanding of potential herbicide programs that can be recommended to growers in the future for the control of problematic weeds. There are many factors that affect PRE herbicides and their efficacy, thus more research is required to understand how impactful IFT will be in herbicide programs in North Carolina soybean production.

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Figures and Tables

Table 1.1 Herbicide treatments of length of residual trials 2019.^a

Treatment	Tradename	Rate	Application time	Company	Location
		g ai ha ⁻¹			
Isoxaflutole (LR) ^b	Alite 27	70.15	PRE	BASF Corporation	Research Triangle Park, NC
Isoxaflutole (HR) ^c	Alite 27	105.2	PRE		
S-metolachlor (LR)	Dual II Magnum	1069	PRE	Syngenta Crop Protection, LLC	Greensboro, NC
S-metolachlor (HR)	Dual II Magnum	2137	PRE		
Mesotrione	Callisto 480SC	105.2	PRE	Syngenta Crop Protection, LLC	Greensboro, NC
Dicamba	Engenia	701.5	PRE	BASF Corporation	Research Triangle Park, NC
2,4-D	Enlist One	1064	PRE	Dow AgroSciences LLC	Indianapolis, IN
Pyroxasulfone	Zidua Herbicide WG	89.3	PRE	BASF Corporation	Research Triangle Park, NC
Fomesafen	Reflex	280	PRE	Syngenta Crop Protection, LLC	Greensboro, NC

^a Specimen labels for each product, mailing addresses and website addresses for each manufacturer can be found at www.cdms.net.

^b Low Rate (LR)

^c High rate (HR)

Table 1.2 Palmer amaranth control by treatment and location^a

Treatment	Palmer amaranth									
	1 WAA		2 WAA		3 WAA		4 WAA		5 WAA	
	Pine level	CCRS	Pine level	CCRS	Pine level	CCRS	Pine level	CCRS	Pine level	CCRS
Isoxaflutole (LR) ^b	96 a	100 a	91 abc	96 abc	79 abc	86 abc	44 fe	86 abc	30 def	86 a
Isoxaflutole (HR) ^c	100 a	100 a	96 abc	97 abc	90 ab	89 ab	59 b-e	87 abc	43 c-f	83 ab
S-metolachlor (LR)	98 a	100 a	83 c	97 abc	80 abc	84 abc	41 fe	79 a-d	15 def	76 abc
S-metolachlor (HR)	100 a	100 a	95 abc	99 a	93 ab	97 a	55 cde	90 b	45 b-e	87 a
Mesotrione	100 a	99 a	95 abc	95 abc	92 ab	74 bc	79 a-d	48 de	43 c-f	53 a-d
Dicamba	99 a	58 c	92 abc	31 e	88 abc	34 d	59 b-e	25 ef	28 def	25 def
2,4-D	97 a	79 b	83 bc	0 e	68 c	0 e	28 ef	13 f	5 f	13 ef
Pyroxasulfone	96 a	100 a	90 abc	98 ab	83 abc	95 ab	51 de	89 abc	42 c-f	88 a
Fomesafen	100 a	99 a	92 abc	98 abc	80 abc	98 a	40 ef	97 a	38 def	90 a

^a Means within a rating period within a column followed by the same letter are not statistically different according to Tukey's HSD test at P = 0.05.

^b Low Rate (LR)

^c High rate (HR)

Table 1.3 Tropic croton control by treatment^a

Treatment	Tropic croton				
	1 WAA	2 WAA	3 WAA	4 WAA	5 WAA
Isoxaflutole (LR) ^b	92 a	83 a	66 a	44 a	18 a
Isoxaflutole (HR) ^c	90 a	74 a	81 a	50 a	30 a
S-metolachlor (LR)	96 a	80 a	76 a	45 a	18 a
S-metolachlor (HR)	97 a	88 a	89 a	59 a	60 a
Mesotrione	93 a	91 a	85 a	71 a	35 a
Dicamba	97 a	92 a	88 a	69 a	43 a
2,4-D	97 a	83 a	73 a	45 a	0 a
Pyroxasulfone	96 a	89 a	85 a	68 a	43 a
Fomesafen	98 a	90 a	87 a	35 a	40 a

^a Means within a rating period within a column followed by the same letter are not statistically different according to Tukey's HSD test at P = 0.05.

^b Low Rate (LR)

^c High rate (HR)

Table 1.4 Carpetweed control by treatment and location^a

Treatment	Carpetweed								
	1 WAA		2 WAA		3 WAA		4 WAA		5 WAA ^d
	Pine level	CCRS	Pine level	CCRS	Pine level	CCRS	Pine level	CCRS	Pooled
Isoxaflutole (LR) ^b	100 a	88 a	100 a	95 a	84 a	99 a	93 ab	99 a	81 a
Isoxaflutole (HR) ^c	100 a	90 a	100 a	94 a	98 a	93 a	95 ab	99 a	83 a
S-metolachlor (LR)	100 a	93 a	100 a	94 a	93 a	49 c	83 a-e	20 h	44 bc
S-metolachlor (HR)	100 a	93 a	100 a	93 a	99 a	70 b	91 abc	52 efg	71 ab
Mesotrione	100 a	95 a	100 a	94 a	99 a	95 a	98 ab	60 c-g	78 ab
Dicamba	100 a	75 a	100 a	35 b	90 a	30 d	91 abc	31 gh	44 bc
2,4-D	100 a	91 a	100 a	43 b	95 a	38 cd	89 a-d	40 fgh	36 c
Pyroxasulfone	100 a	93 a	100 a	94 a	100 a	96 a	95 ab	89 a-d	78 ab
Fomesafen	100 a	92 a	100 a	89 a	92 a	97 a	58 d-g	66 b-f	67 abc

^a Means within a rating period within a column followed by the same letter are not statistically different according to Tukey's HSD test at P = 0.05.

^b Low Rate (LR)

^c High rate (HR)

^d Location by treatment interaction was not significant for this rating, treatments were pooled across locations

Table 1.5 Large crabgrass control by treatment^a

Treatment	Large crabgrass				
	1 WAA	2 WAA	3 WAA	4 WAA	5 WAA
Isoxaflutole (LR) ^b	100 a	100 a	99 a	99 a	96 ab
Isoxaflutole (HR) ^c	100 a	100 a	100 a	98 a	98 a
S-metolachlor (LR)	98 a	99 a	94 a	74 b	65 bc
S-metolachlor (HR)	98 a	99 a	96 a	89 ab	81 ab
Mesotrione	94 ab	95 a	50 b	5 de	3 e
Dicamba	48 c	33 c	23 c	23 cd	25 de
2,4-D	50 c	0 d	0 c	0 e	0 e
Pyroxasulfone	99 a	100 a	95 a	90 ab	88 ab
Fomesafen	83 b	65 b	25 bc	35 c	48 cd

^a Means within a rating period within a column followed by the same letter are not statistically different according to Tukey's HSD test at P = 0.05.

^b Low Rate (LR)

^c High rate (HR)

Table 1.6 Goosegrass control by treatment^a

Treatment	Goosegrass				
	1 WAA	2 WAA	3 WAA	4 WAA	5 WAA
Isoxaflutole (LR) ^b	100 a	100 a	100 a	100 a	99 a
Isoxaflutole (HR) ^c	100 a	100 a	100 a	100 a	97 a
S-metolachlor (LR)	100 a	99 a	96 a	83 ab	75 a
S-metolachlor (HR)	100 a	100 a	98 a	100 a	99 a
Mesotrione	92 a	80 a	43 b	33 cd	0 b
Dicamba	60 c	38 b	31 b	33 cd	30 b
2,4-D	73 b	0 c	0 c	0 d	0 b
Pyroxasulfone	99 a	99 a	99 a	100 a	96 a
Fomesafen	73 b	45 b	30 b	30 cd	28 b

^a Means within a rating period within a column followed by the same letter are not statistically different according to Tukey's HSD test at P = 0.05.

^b Low Rate (LR)

^c High rate (HR)

Table 1.7 Crowfootgrass control by treatment^a

Treatment	Crowfootgrass				
	1 WAA	2 WAA	3 WAA	4 WAA	5 WAA
Isoxaflutole (LR) ^b	100 a	100 a	99 a	100 a	98 a
Isoxaflutole (HR) ^c	100 a	100 a	100 a	100 a	98 a
S-metolachlor (LR)	78 ab	100 a	96 a	83 a	75 a
S-metolachlor (HR)	95 a	100 a	98 a	100 a	97 a
Mesotrione	100 a	80 b	43 b	13 b	0 b
Dicamba	70 b	8 e	0 c	0 b	0 b
2,4-D	66 b	29 d	30 b	28 b	23 b
Pyroxasulfone	100 a	100 a	99 a	100 a	72 a
Fomesafen	99 a	48 c	0 c	25 b	25 b

^a Means within a rating period within a column followed by the same letter are not statistically different according to Tukey's HSD test at P = 0.05.

^b Low Rate (LR)

^c High rate (HR)

CHAPTER 2

Efficacy of Herbicide Programs in Isoxaflutole Tolerant FG-72xLL55 Soybeans [*Glycine max* (L.) Merr.].

Efficacy of Herbicide Programs in Isoxaflutole Tolerant FG-72xLL55 Soybeans [*Glycine max* (L.) Merr.].

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Field studies were conducted at the Central Crops Research Station near Clayton, North Carolina and the Bayer Environmental Science research station near Clayton, NC in 2018; and at the Central Crops Research Station near Clayton, NC and the BASF Pikeville Research Station near Pikeville, NC in 2019 to compare the overall efficacy of isoxaflutole (IFT) herbicide programs with other common herbicide programs in IFT tolerant soybeans, Credenz GT27(FG-72xLL55). Treatments included 16 different preemergence (PRE) and postemergence (POST) programs of IFT, *S*-metolachlor, glyphosate, glufosinate, flumioxazin, and fomesafen. Isoxaflutole PRE controlled Palmer amaranth, *Amaranthus palmeri* S. Wats., 64 to 94% 1 week after POST application (WAP) and was more effective than *S*-metolachlor PRE (51 to 93%), but both underperformed compared to programs incorporating PRE and POST herbicides. While both herbicides experience a decline in efficacy, IFT appears to provide a longer period of control and suppression of Palmer amaranth. Annual grass control was similar for both IFT and *S*-metolachlor. Glyphosate control of Palmer amaranth and glufosinate control of annual grasses was poor. Glyphosate plus glufosinate provided 83 to 99% control of all weed species 2 WAP. Yield data showed that IFT herbicide programs performed statically similar to industry standard programs. In the absence of POST herbicides yields experienced a 28 to 49 % reduction.

Nomenclature: Flumioxazin; fomesafen; glufosinate; glyphosate; isoxaflutole; *S*-metolachlor; Palmer amaranth; *Amaranthus palmeri* S. Wats.; soybean; *Glycine max* (L.) Merr.

Key words: Preemergent herbicides, post-emergent herbicides, isoxaflutole herbicide programs.

Soybean growers in the Southeastern United States battle problematic weeds and herbicide resistant weeds every year. Soybeans are highly susceptible to early season competition, having up to 92% yield loss from early season weeds (Knezevic et al. 2019). Research from Ontario has shown that weeds not in direct resource competition with soybeans will still cause the crop to initiate a shade avoidance response as ratios of far-red to red light shift; causing decreases in yields and biomass of soybeans (Green-Tracewicz et al., 2011). Green-Tracewicz et al. (2011), suggest that season long weed control is necessary to ensure maximum yield potential in soybeans.

Commercialization of glyphosate resistant (GR) soybeans in 1996 allowed growers to spray glyphosate postemergence (POST) over-the-top control most weeds present (Duke and Powles, 2008). The broad-spectrum activity of glyphosate on weeds led to the rapid adoption of GR crops, the reduction in the use of preemergent (PRE) herbicides, and repeated applications of glyphosate in a single season. As weed populations were subjected to repeated selection pressure, GR weeds evolved. Soybean producers in the Southeastern US have for years battled with glyphosate and ALS resistant weeds such as Palmer Amaranth, (*Amaranthus palmeri* S. Wats.) (Heap, 2020).

As GR weeds become more prevalent across soybean and other crop acres, extension weed specialists recommend a zero tolerance for weed escapes (Zimdahl, 1994). Proliferation of resistant biotypes have led to the understanding that a single weed left in a field, that can flower and produce seed, exacerbates the issue. A single female Palmer amaranth can produce upwards of 500,000 seeds (Webster and Grey, 2015) and in some cases produced over 1,000,000 seeds (A.C York, personal communication). Because of this, herbicide programs currently include a

focus on rotating modes of action (MOAs) and using multiple MOAs for both PRE and POST applications (Sarangi, et al., 2017).

The issues from Palmer amaranth arise from its highly competitive ability (Keeley et al., 1987) and its evolution of resistance to eight herbicide MOAs globally (Heap, 2020). Research has shown that Palmer amaranth at densities of 0.33, 3.33, and 10 plants m⁻¹ of row reduced soybean yield 17%, 64%, and 68%, respectively (Klingaman and Oliver 1994). Palmer amaranth produces more biomass and is more competitive than common waterhemp (*Amaranthus rudis* Sauer) or redroot pigweed (*Amaranthus retroflexus* L.) in soybean (Bensch et al., 2003). Globally this species has developed resistance to 8 MOAs, with a population in Arkansas having confirmed multiple resistance to 5 MOAs (Heap, 2020).

The overuse of protoporphyrinogen oxidase (PPO)-, acetolactate synthase (ALS)- and long chain fatty acid (LCFA)- inhibiting herbicides as PRE herbicides and glyphosate for over-the-top POST weed control has left growers with few options for Palmer amaranth control in soybean. Advancements in genetic engineering has led to the stacking of several POST herbicide traits within varieties, allowing growers to apply multiple chemistries, including glyphosate, glufosinate and the synthetic auxins (2,4-D and dicamba) (Meyer et al. 2015). With the launch of auxin-tolerant soybeans, growers can now apply 2,4-D and Dicamba over-the-top of 2,4-D- and dicamba-tolerant cultivars, respectively. However, in 2015, a population of Palmer amaranth was confirmed to have resistance to 2,4-D in a sorghum field in Kansas (Kumar et al., 2019). Current herbicide programs are placing tremendous amounts of selections pressure on PPO-inhibitors, synthetic auxins and glufosinate, where resistance has not developed. PPO-inhibitors have been used in row crops for over 50 years and the wide-spread use of PPO-inhibitors to control GR

Palmer amaranth can quickly result in the development of wide-spread resistant populations. (Copeland et al., 2018)

Developing new herbicides has become more arduous in recent decades and most “new” herbicides are premixes of old chemistries (Green, 2014). It has been theorized that the best and easiest to discover chemistries, have already been discovered (Duke, 2011). As the ability to produce new herbicides has been reduced, the focus has changed to producing genetically modified crops that express resistance traits to older chemistries that have not been widely used. Most recently, soybean varieties tolerant to the herbicide isoxaflutole (IFT) have been released.

Isoxaflutole (5-cyclopropyl isoxazol-4-yl-2-mesyl-4-trifluoromethylphenyl ketone) is a 4-Hydroxyphenylpyruvate Dioxygenase (HPPD) inhibiting herbicide. It was launched in 1998 for use as a PRE in small grains and corn, crops that have natural tolerance to the herbicide, and has been widely used in the Midwest, US (O’Brien et al., 2018). Isoxaflutole is quite injurious to dicotyledonous crops and weed species. Injury from IFT begins in newly formed leaves as carotenoid synthesis is stopped, and chlorophyll is degraded by solar radiation (Pallett et al., 1998). Isoxaflutole is metabolically processed in soil and plants into its herbicidally active metabolite diketonitrile [2-cyclopropyl-3-(2-mesyl-4-trifluoromethylphenyl)-3-oxopropanenitrile] (Pallett et al., 2001). Diketoneitrile (DKN) is further metabolized into a biologically inactive benzoic acid derivative, 2-mesyl-4-trifluoromethyl benzoic acid, but this degradation step is slow in susceptible species and thus, results in herbicidal selectivity (Alletto et al., 2012). Isoxaflutole targets the HPPD enzyme pathway that is responsible for the biosynthesis of the molecule plastoquinone (Pallett et al., 1998). Plastoquinone is an essential cofactor in the production of carotenoids and is also an electron transporter for photosynthetic

electron transport chains (Pallett et al., 1998). Without carotenoid synthesis, chlorophyll is promptly degraded by solar radiation, leaving affected leaves white or “bleached”.

Roots of young or newly germinating weeds absorb isoxaflutole. Research of the efficacy of IFT in corn showed weed control of up to 94% for weeds like Palmer amaranth, barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) and velvetleaf (*Abutilon theophrasti* Medikus) 4 weeks after application (WAA) (Stephenson and Bond, 2012). An additional trait of IFT that makes it an efficacious PRE herbicide is its mobility in soils. Isoxaflutole and its metabolite DKN are both mobile to highly mobile in soils (Rice, Koskinen, and Carrizosa, 2004). This mobility allows for the compounds to move with water molecules in the soil and be reabsorbed into already emerged small weeds, less than 5 cm, as well as absorbed by later germinating weeds. It has been documented that IFT cannot sufficiently control large established weeds, over 5 cm, when used as a PRE herbicide (OMAFRA, 2016). In contrast, POST activity of the herbicide is limited (Spaunhorst and Johnson, 2016).

Recently, the launch of Credezz GT27 (FG-72xLL55) soybean by BASF Corporation has allowed for the potential use of IFT in soybean production. The GT27 soybean varieties express a gene stack that confers resistance to glyphosate, glufosinate, and isoxaflutole (IFT). The specific mechanism of tolerance for these varieties to IFT comes from the hppdPF W336 gene of *Pseudomonas fluorescens* strain A32 (ISAAA, 2020). This gene confers a modified p-HPPD enzyme that allows for tolerance to IFT. Therefore, IFT is intended for PRE use in GT27 soybeans. The 2mepsps gene from corn and the pat gene from *Streptomyces viridochromogenes* confer tolerances to glyphosate and glufosinate, respectively. While the published research for efficacy of IFT herbicide programs provides a basis for its use in the Midwest and in corn

production, there is little published research on IFT use in tolerant soybean cultivars in North Carolina.

Materials and Methods

Experiments were conducted at the Central Crops Research station near Clayton, NC during 2018 and 2019, at the Bayer Environmental Science Research Station near Clayton, NC during 2018, and at the BASF Research Facility near Pikeville, NC during 2019. Soils at locations near Clayton was Norfolk sandy loam (Fine-loamy, kaolinitic, thermic Typic Kandiudults) 0.41 to 0.8% organic matter and pH 6.3 to 6.5. Soil at the Pikeville site consisted of a Wagram loamy sand (Loamy, kaolinitic, thermic Arenic Kandiudults) having 0.7% organic matter and pH 6.4. Irrigation was used to supplement rainfall at the Pikeville site.

Soybean cultivar ‘Credenz 4539GTLL’ were planted using a 2-row cone planter at a depth of 1.9 cm. Plots were 4 rows spaced 91 cm apart by 7.6 m long. Planting dates can be found in Table 2.1. Treatments were arranged in a randomized complete block design with 4 replications. Prior to planting, weed seed germination was encouraged by light field cultivation.

Herbicide treatments consisted of 16 herbicide programs and a nontreated check for comparison (Table 2.2). Preemergence applications occurred the day of planting and POST applications were applied at V2 to V4 growth stage or prior to weeds becoming too large for efficacious treatment. Herbicide were applied using a CO₂-pressurized backpack sprayer equipped with flat-fan nozzles (TeeJet XR8002VS, Spraying Systems Co. Wheaton, IL) delivering 140 L ha⁻¹ at 243 kPA.

Visual estimates of weed control were collected weekly beginning 1 week after emergence (1WAE) and ending 2 weeks after POST (WAP) in 2018 and 3 WAP in 2019 on

identified weed species using a 0 to 100% scale, where 0 is no control and 100 is complete control. Identified weed species were Palmer amaranth, pink purslane (*Portulaca Pilosa* L.), carpetweed (*Mullugo verticillata* L.), goosegrass (*Eleusine indica* (L.) Gaertn.), large crabgrass (*Digitaria sanguinalis* (L.) Scop.), and crowfootgrass (*Dactyloctenium aegyptium* (L.) Willd.) Final visual control estimates were collected 3 WAP and weeds were terminated with an application glyphosate at 1.26 kg ae ha⁻¹ plus glufosinate at 656 g ai ha⁻¹, and later hand weeding to remove escapes or newly emerged weeds. Insecticides were used as needed. At the end of the season, all plots received paraquat at 292 g ai ha⁻¹ to condition soybean for harvest. Plots were harvested using an Almaco small plot combine and weighed with a HarvestMaster grain system to determine soybean yield.

Data were subjected to ANOVA using PROC GLM procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). Location and year were combined in the analysis due to significant interactions and classified as an environment. Means for each species and rating periods were separated for their appropriate main effects, environment, treatment, and environment by treatment interactions. Only ratings that were statistically significant are presented and discussed. Means were separated using a Tukey's HSD test, $P \leq 0.05$.

Results and Discussion

Prior to application of POST herbicides, environmental conditions influenced weed control (Table 2.3-2.4). Herbicide efficacy ranged from 78% to 99% solely due to environment. Weed control by PRE herbicide treatments were not statistically different for most species and ratings except for Palmer amaranth control 3 weeks after emergence (WAE) for, and carpetweed control 1 and 3 WAE. At 3 WAE, IFT (90-99%) controlled Palmer amaranth more effectively than *S*-metolachlor (70-93%) across pooled environments (Table 2.5). Combinations of IFT and

S-metolachlor controlled Palmer amaranth 84 to 99% (Table 2.5). Control from the tank mix of the two herbicides is comparable to that of flumioxazin.

In the absence of POST herbicides, IFT PRE controlled Palmer amaranth 64 to 94% 1 week after POST application (WAP) and was more effective than *S*-metolachlor PRE which only controlled the weed 51 to 93% (Table 2.9). This trend continued 2 WAP (Table 2.6). At the same time, IFT PRE controlled carpetweed 43% better than *S*-metolachlor (Table 2.6) while annual grass control by the two herbicides was similar (Table 2.7). Both herbicides experienced a decline in efficacy 2WAP, but from these results, IFT appears to maintain a longer period of Palmer amaranth control than *S*-metolachlor. Both herbicides maintain good long-term residual control of annual grass species.

Treatments of POST herbicides showed the limitations of both glyphosate and glufosinate in controlling escapes of certain species. As expected, Palmer amaranth control by glyphosate was poor as locations were infested with GR Palmer amaranth (Table 1.6). Glyphosate resistant Palmer amaranth can be seen in Figure 1.1. Additionally, as expected, glufosinate control of annual grasses was poor (Table 2.7). Glufosinate control of annual grasses is variable and variability increases with weed size (Ritter and Menbere 2001; Steckel et al. 1997; Thomas et al. 2007). Glufosinate effectively control broadleaf weed species when application is made in a timely manner. Glyphosate plus glufosinate controlled all weed species 83 to 99% 2 WAP (Table 2.6-2.7). Post-emergent applications that included fomesafen provided 99 to 100% control of all weed species 2 WAP, demonstrating the strength of PRE programs plus POST applications that include PPO-inhibiting herbicides.

In the absence of POST herbicides soybean yield was reduced 28 to 49% (Table 2.10). This illustrates the importance of eliminating weeds that escape PRE herbicides. In the absence

of PRE herbicides, yields from POST only treatments showed a similar yield reduction trend. When only POST herbicides are used to remove weeds after 3 WAE, yield were reduced 15 to 32 %. The large variation in yields is once again a function of environmental conditions. Additionally, as can be expected, the lowest yielding treatment was the nontreated check (143-1693 kg ha⁻¹) further emphasizing the importance of weed control.

This study suggests that PRE only and POST only treatments fail to maximize yield potential. While IFT provides better control of Palmer amaranth than *S*-metolachlor and is comparable to it and flumioxazin for other weed species, IFT cannot be relied on as a standalone. IFT and *S*-metolachlor provide excellent weed control when used in combination and a robust POST program provides for continued weed control later into the season. Isoxaflutole PRE fb fomesafen plus *S*-metolachlor plus glufosinate POST provided excellent weed control and utilize several MOAs. However, it can be hypothesized that efficacy could be reduced if PPO tolerant weeds are present. Within these trial fields, no known PPO resistant weeds were found.

The potential use of IFT in soybean gives growers access to a MOA not currently used in production. Having the additional MOA could alleviate selection pressure on current herbicides. The ability to add a third, new MOA to soybean PRE herbicide programs can alleviate selection pressure on PPO-inhibiting herbicides where wide-spread PPO resistance is not present. Furthermore, IFT can protect the efficacy of LCFA-inhibiting, synthetic auxins and glufosinate from resistance development. Eliminating more weeds prior to their emergence results in fewer weeds to control with POST herbicides and overall reduction in the selection pressure on these populations.

Best management practices of including multiple MOAs and rotating MOAs should still be implemented with the launch of GT27 soybeans and herbicide programs utilizing IFT. Using

IFT in combination with *S*-metolachlor provides excellent PRE control of problematic weeds, and the use of glyphosate plus glufosinate as well as having the option to use PPO-inhibitors, allow for control of escapes and later emerging weeds. Having the ability to utilize five different MOAs gives growers the ability to tailor an herbicide plan to their fields and limit selection pressure on a single MOA.

To understand fully the efficacy of IFT programs in GT27 soybeans, further research needs to be conducted to determine efficacy on other weed species. Many factors affect the efficacy and duration of PRE herbicides; additional experiments across soil types, tank mixes and climates must be conducted. Similar research with these species as well as other problematic species found in North Carolina such as ivy-leaf morning glory (*Ipomoea hederacea* Jacq.), horseweed (*Conyza canadensis* (L.) Cronquist,) tall waterhemp (*Amaranthus tuberculatus* (Moq.) Sauer), and barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) should be conducted. Further research would confirm the efficacy and utility of IFT in soybean production across geographic regions and on multiple problematic species.

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Figures and Tables

Table 2.1 Locations, planting, treatment, and harvest dates.

Location	Planting Date	PRE	POST	Harvest
Central Crops 2018 ^a	24-May	24-May	14-Jun	18-Oct
Bayer ES 2018 ^b	7-May	7-May	28-May	4-Oct
Central Crops 2019	5-Jun	5-Jun	26-Jun	19-Oct
Pikeville 2019 ^c	3-Jun	3-Jun	24-Jun	17-Oct

^a Central Crops Research Station, near Clayton, NC

^b Bayer Environment Science Research Station, near Clayton, NC

^c BASF Pikeville Research Station, near Pikeville, NC

Table 2.2 Herbicides, rates and manufactures^a

Herbicide	Tradename	Rate g ai ha ⁻¹	Company	Location
Isoxaflutole	Alite 27	105.2	BASF Corporation	Research Triangle Park, NC
S-metolachlor	Dual II Magnum	1069	Syngenta Crop Protection, LLC	Greensboro, NC
Glyphosate	Roundup Power max	1263	Bayer CropScience LP	St. Louis, MO
Glufosinate	Liberty 280 SL	655	BASF Corporation	Research Triangle Park, NC
Flumioxazin	Valor SX	107.2	Valent U.S.A Corp	Walnut Creek, CA
Fomesafen+ S-metolachlor	Prefix	1483	Syngenta Crop Protection, LLC	Greensboro, NC

^a Specimen labels for each product, mailing addresses and web site addresses of each manufacture can be found at www.cdms.net

Table 2.3 Broadleaf weed control by environment^a

Environment	Palmer amaranth			Carpetweed	
	1 WAE ^e	2 WAE	3 WAE	1 WAE	3 WAE
CCRS ^b 2018	72 b	87 b	83 b	N/A	N/A
Bayer ^c 2018	N/A	N/A	N/A	N/A	N/A
CCRS 2019	97 a	94 a	92 a	96 b	97 b
Pikeville ^d 2019	99 a	97 a	97 a	99 a	99 a

^a Means within a column within a rating period and species followed by the same letter are not different according to Tukey's HSD test at P=0.05

^b Central Crops Research Station, Near Clayton, NC

^c Bayer Environmental Science Location, Near Clayton, NC

^d BASF Pikeville Research Station, near Pikeville, NC

^e Abbreviation: WAE, weeks after emergence

Table 2.4 Grass weed control by environment^a

Environment	Goosegrass				Large Crabgrass			Crowfootgrass
	1 WAE ^c	3 WAE	1 WAP ^f	2WAP	1 WAE	3 WAE	2 WAP	1 WAE
CCRS ^b 2018	78 b	94 a	93 b	91 b	78 b	94 a	91a	78 b
Bayer ^c 2018	N/A	N/A	N/A	N/A	98 a	80 b	78b	N/A
CCRS 2019	100 a	93 a	96 ab	97 ab	98 a	99 a	97a	100 a
Pikeville ^d 2019	100 a	99 a	98 a	98 a	N/A	N/A	N/A	N/A

^a Means within a column within a rating period and species followed by the same letter are not different according to Tukey's HSD test at P=0.05

^b Central Crops Research Station, Near Clayton, NC

^c Bayer Environmental Science Location, Near Clayton, NC

^d BASF Pikeville Research Station, near Pikeville, NC

^e Abbreviation: WAE, weeks after emergence

^f Abbreviation: WAP, weeks after POST

Table 2.5 Weed control by pre-emergence treatment^{ab}

Herbicide Treatment		Palmer amaranth	Carpetweed	
PRE ^c	POST ^d	3 WAE ^e	1 WAE	3 WAE
Isoxaflutole	None	90 ab	98 abc	94 a
Isoxaflutole	Glyphosate	91 ab	98 abc	99 a
Isoxaflutole	Glufosinate	96 a	99 ab	99 a
Isoxaflutole	Glyphosate + Glufosinate	97 a	98 abc	99 a
<i>S</i> -metolachlor	None	70 b	98 abc	96 a
<i>S</i> -metolachlor	Glyphosate	87 ab	94 c	97 a
<i>S</i> -metolachlor	Glufosinate	93 ab	95cb	98 a
<i>S</i> -metolachlor	Glyphosate + Glufosinate	82 ab	96 abc	98 a
Isoxaflutole + <i>S</i> -metolachlor	Glyphosate	99 a	98 abc	99 a
Isoxaflutole + <i>S</i> -metolachlor	Glufosinate	92 ab	99 ab	100 a
Isoxaflutole + <i>S</i> -metolachlor	Glyphosate + Glufosinate	84 ab	99 ab	99 a
Flumioxazin	Fomesafen + <i>S</i> -metolachlor + Glyphosate	99 a	100 a	100 a
Isoxaflutole	Fomesafen + <i>S</i> -metolachlor + Glufosinate	99 a	99 ab	100 a

^a PRE herbicides applied immediately following planting, POST herbicides labeled for treatment differentiation

^b Means within a rating period within a species followed by the same letter are not different according to Tukey's HSD test at P=0.05

^c Abbreviation: PRE, preemergence herbicide treatment

^d Abbreviation: POST, postemergence herbicide treatment

^e Abbreviation: WAE, weeks after emergence

Table 2.6 Broadleaf weed control by pre-emergence and post-emergence treatment^{ab}

Herbicide Treatment		Palmer amaranth		Carpetweed	
PRE	POST	1 WAP	2 WAP	1WAP	2WAP
Isoxaflutole	None	89 ab	88 abc	100 a	100 a
Isoxaflutole	Glyphosate	92 a	92 abc	100 a	100 a
Isoxaflutole	Glufosinate	95 a	95 abc	100 a	100 a
Isoxaflutole	Glyphosate + Glufosinate	97 a	97 ab	100 a	100 a
<i>S</i> -metolachlor	None	78 ab	70 bc	73 b	70 b
<i>S</i> -metolachlor	Glyphosate	89 ab	89 abc	99 a	98 a
<i>S</i> -metolachlor	Glufosinate	97 a	97 ab	99 a	98 a
<i>S</i> -metolachlor	Glyphosate + Glufosinate	83 ab	82 abc	100 a	100 a
Isoxaflutole + <i>S</i> -metolachlor	Glyphosate	98 a	98 a	100 a	100 a
Isoxaflutole + <i>S</i> -metolachlor	Glufosinate	92 a	92 abc	100 a	100 a
Isoxaflutole + <i>S</i> -metolachlor	Glyphosate + Glufosinate	87 ab	87 abc	99 a	99 a
Flumioxazin	Fomesafen + <i>S</i> -metolachlor + Glyphosate	99 a	99 a	100 a	100 a
Isoxaflutole	Fomesafen + <i>S</i> -metolachlor + Glufosinate	99 a	99 a	100 a	100 a
None	Glyphosate	69 b	67 c	97 a	98 a
None	Glufosinate	91 ab	82 abc	98 a	95 a
None	Glyphosate + Glufosinate	93 a	81 abc	98 a	99 a

^a PRE herbicides applied immediately following planting

^b Means within a rating period within a species followed by the same letter are not different according to Tukey's HSD test at P=0.05

^c Abbreviation: PRE, preemergence herbicide treatment

^d Abbreviation: POST, postemergence herbicide treatment

^e Abbreviation: WAE, weeks after emergence

^h Abbreviation: WAP, weeks after POST

Table 2.7 Grass weed control by pre-emergence and post-emergence treatment^{ab}

Herbicide Treatment		Large crabgrass	Goosegrass		Crowfootgrass
PRE ^c	POST ^c	2 WAP ^e	1 WAP	2WAP	2 WAP
Isoxaflutole	None	92 a	97 a	96 ab	96 ab
Isoxaflutole	Glyphosate	94 a	99 a	99 a	99 a
Isoxaflutole	Glufosinate	86 ab	97 ab	97 ab	97 a
Isoxaflutole	Glyphosate + Glufosinate	86 ab	99 a	99 a	98 a
S-metolachlor	None	89 a	96 ab	97 ab	95 ab
S-metolachlor	Glyphosate	94 a	98 a	98 a	99 a
S-metolachlor	Glufosinate	88 ab	97 ab	97 ab	97 a
S-metolachlor	Glyphosate + Glufosinate	86 ab	91 ab	91 ab	86 ab
Isoxaflutole + S-metolachlor	Glyphosate	93 a	100 a	99 a	99 a
Isoxaflutole + S-metolachlor	Glufosinate	93 a	100 a	99 a	99 a
Isoxaflutole + S-metolachlor	Glyphosate + Glufosinate	85 ab	91 ab	91 ab	87 ab
Flumioxazin	Fomesafen + S-metolachlor + Glyphosate	99 a	99 a	99 a	99 a
Isoxaflutole	Fomesafen + S-metolachlor + Glufosinate	98 a	99 a	99 a	98 a
None	Glyphosate	90 a	96 ab	96 ab	95 ab
None	Glufosinate	63 b	81 b	75 b	63 b
None	Glyphosate + Glufosinate	83 ab	93 ab	88 ab	84 ab

^a PRE herbicides applied immediately following planting

^b Means within a rating period within a species followed by the same letter are not different according to Tukey's HSD test at P=0.05

^c Abbreviation: PRE, preemergence herbicide treatment

^d Abbreviation: POST, postemergence herbicide treatment

^e Abbreviation: WAP, weeks after POST

Table 2.8 Weed control by environment by pre-emergence treatment interaction^{ab}

Herbicide Treatment		Environment	
		CCRS ^c 2019	Pikeville ^d 2019
		Carpetweed	
PRE ^e	POST ^f	2 WAE ^g	
Isoxaflutole	None	98 abc	99 ab
Isoxaflutole	Glyphosate	98 abc	99 ab
Isoxaflutole	Glufosinate	97 a-d	99 ab
Isoxaflutole	Glyphosate + Glufosinate	96 a-d	100 a
<i>S</i> -metolachlor	None	95 a-d	92 cd
<i>S</i> -metolachlor	Glyphosate	93 a-d	90 d
<i>S</i> -metolachlor	Glufosinate	95 a-d	93 a-d
<i>S</i> -metolachlor	Glyphosate + Glufosinate	97 a-d	92 bcd
Isoxaflutole + <i>S</i> -metolachlor	Glyphosate	98 abc	100 a
Isoxaflutole + <i>S</i> -metolachlor	Glufosinate	96 a-d	100 a
Isoxaflutole + <i>S</i> -metolachlor	Glyphosate + Glufosinate	97 a-d	100 a
Flumioxazin	Fomesafen + <i>S</i> -metolachlor + Glyphosate	100 a	100 a
Isoxaflutole	Fomesafen + <i>S</i> -metolachlor + Glufosinate	97 a-d	98 abc

^a PRE herbicides applied immediately following planting, POST herbicides are labeled for treatment differentiation

^b Means within an environment column within a species rating period followed by the same letter are not different according to Tukey's HSD test at P=0.05

^c Central Crops Research Station, Near Clayton, NC

^d BASF Pikeville Research Station, Near Pikeville, NC

^e Abbreviation: PRE, preemergence herbicide treatment

^f Abbreviation: POST, postemergence herbicide treatment

^g Abbreviation: WAE, weeks after emergence

Table 2.9 Weed control by environment by treatment interaction^{ab}

Herbicide Treatment		Environment							
		CCRS ^c 2018	CCRS 2019	Pikeville ^d 2019	CCRS 2018	CCRS 2019	CCRS 2018	Bayer ^e 2018	CCRS 2019
		Palmer amaranth			Crowfootgrass		Large crabgrass		
PRE ^f	POST ^g	1 WAP ^h			1 WAP		1 WAP		
Isoxaflutole	None	64 abc	78 abc	94 ab	94 a	98 a	97 ab	99 ab	99 ab
Isoxaflutole	Glyphosate	96 ab	93 ab	95 ab	98 a	100 a	98 ab	84 abc	100 a
Isoxaflutole	Glufosinate	89 ab	98 a	100 a	94 a	100 a	95 abc	71 abc	100 a
Isoxaflutole	Glyphosate + Glufosinate	92 ab	98 a	100 a	96 a	100 a	97 ab	84 abc	100 a
S-metolachlor	None	51 bc	62 abc	93 ab	94 a	96 a	98 ab	59 c	98 ab
S-metolachlor	Glyphosate	79 abc	78 abc	97 a	99 a	100 a	98 ab	84 abc	100 a
S-metolachlor	Glufosinate	92 ab	99 a	99 a	72 a	100 a	72 abc	71 abc	100 a
S-metolachlor	Glyphosate + Glufosinate	92 ab	97 a	100 a	93 a	100 a	93 abc	88 abc	100 a
Isoxaflutole + S-metolachlor	Glyphosate	76 abc	97 a	98 a	99 a	100 a	99 ab	81 abc	100 a
Isoxaflutole + S-metolachlor	Glufosinate	99 a	100 a	100 a	74 a	100 a	96 ab	81 abc	100 a
Isoxaflutole + S-metolachlor	Glyphosate + Glufosinate	88 ab	97 a	100 a	99 a	100 a	99 ab	80 abc	100 a
Flumioxazin	Fomesafen + S- metolachlor + Glyphosate	98 a	100 a	100 a	98 a	100 a	98 ab	98 ab	100 a
Isoxaflutole	Fomesafen+S- metolachlor+Glufosinate	98 a	99 a	100 a	97 a	100 a	98 ab	97 a	100 a

Table 2.9 Continued.

None	Glyphosate	84 abc	40 c	73 abc	98 a	89 a	98 ab	99 ab	90 abc
None	Glufosinate	95 ab	90 ab	99 a	95 a	62 a	74 abc	63 bc	67 abc
None	Glyphosate + Glufosinate	89 ab	93 ab	98 a	94 a	88 a	98 ab	99 ab	87 abc

^a PRE herbicides applied immediately following planting

^b Means within an environment column within a species rating period followed by the same letter are not different according to Tukey's HSD test at P=0.05

^c Central Crops Research Station, Near Clayton, NC

^d BASF Pikeville Research Station, Near Pikeville, NC

^e Bayer Environmental Science Location, Near Clayton, NC

^f Abbreviation: PRE, preemergence herbicide treatment

^g Abbreviation: POST, postemergence herbicide treatment

^h Abbreviation: WAP, weeks after POST

Table 2.10 Yield by environment by treatment interaction^{ab}

Herbicide Treatment		Environment			
		Yield kg ha ⁻¹			
PRE ^c	POST ^d	CCRS ^e 2018	Bayer ^f 2018	CCRS 2019	Pikeville ^g 2019
None	None	143 h	1605 d-h	1069 fgh	1693 c-h
Isoxaflutole	None	876 gh	2809 a-f	2295 a-g	2604 a-g
Isoxaflutole	Glyphosate	3766 a	3176 a-d	2867 a-e	2571 a-g
Isoxaflutole	Glufosinate	3077 a-d	2679 a-f	1916 b-h	3200 a-d
Isoxaflutole	Glyphosate + Glufosinate	3340 a-d	3029 a-d	2545 a-g	2650 a-g
<i>S</i> -metolachlor	None	1171 e-h	2605 a-g	2541 a-g	2278 a-g
<i>S</i> -metolachlor	Glyphosate	3628 ab	3061 a-d	2941 a-e	2935 a-d
<i>S</i> -metolachlor	Glufosinate	3889 a	3397 a-d	2381 a-g	3158 a-d
<i>S</i> -metolachlor	Glyphosate + Glufosinate	3475 abc	3327 a-d	2585 a-g	3215 a-d
Isoxaflutole + <i>S</i> -metolachlor	Glyphosate	3624 ab	3379 a-d	2619 a-g	2953 a-e
Isoxaflutole + <i>S</i> -metolachlor	Glufosinate	3454 abc	3472 abc	2545 a-g	2819 a-f
Isoxaflutole + <i>S</i> -metolachlor	Glyphosate + Glufosinate	3351 a-d	2825 a-f	2572 a-g	3085 a-d
Flumioxazin	Fomesafen + <i>S</i> -metolachlor + Glyphosate	3280 a-d	3049 a-d	1744 c-h	2888 a-e
Isoxaflutole	Fomesafen + <i>S</i> -metolachlor + Glufosinate	2982 a-d	3163 a-d	2694 a-f	2981 a-d
None	Glyphosate	3322 a-d	2670 a-g	1942 b-g	3022 a-d
None	Glufosinate	2449 a-g	2422 a-g	1172 e-h	3143 a-d
None	Glyphosate + Glufosinate	3472 abc	3094 a-d	2203 a-g	2822 a-f

^a PRE herbicides applied immediately following planting

^b Means within an environment column within a species rating period followed by the same letter are not different according to Tukey's HSD test at P=0.05

^c Abbreviation: PRE, preemergence herbicide treatment

^d Abbreviation: POST, postemergence herbicide treatment

^e Central Crops Research Station, Near Clayton, NC

^f Bayer Environmental Science Location, Near Clayton, NC

^g BASF Pikeville Research Station, Near Pikeville, NC

Figure 2.1 Glyphosate resistant Palmer amaranth in glyphosate treated plot^a



^a Glyphosate treated plot 2 weeks after postemergence application. Note: dead and chlorotic Palmer amaranth mixed in with healthy plants.

CHAPTER 3**FG-72xLL55 Soybean [Glycine max (L.) Merr.] Tolerance to Isoxaflutole**

FG-72xLL55 Soybean [*Glycine max* (L.) Merr.] Tolerance to Isoxaflutole

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Field studies were conducted at the Central Crops Research Station near Clayton, North Carolina and the Bayer Environmental Science Research Station near Clayton, NC in 2018; and at the Central Crops Research Station near Clayton, NC and the Pine level Research Farm near Pine level, NC in 2019 to determine crop response of isoxaflutole (IFT) tolerant soybeans, Credezz GT27, to isoxaflutole programs and industry standard programs. Treatments included 16 different preemergence (PRE) and postemergence (POST) programs of IFT, *S*-metolachlor, glyphosate, glufosinate, flumioxazin, and fomesafen (Table 1.1). Trials were kept weed-free for the duration of the season using a broadcast PRE application of acetochlor, hand weeding and a broadcast application POST of glyphosate and glufosinate after crop response ratings were completed. Trials were harvested using a small plot combine once the crop had reached maturity. There were no observed visual injury or yield loss from IFT PRE. Only when flumioxazin was used as a PRE or fomesafen was used POST was visual injury greater than any other non-PPO treatment. Injury from IFT, *S*-metolachlor and IFT plus *S*-metolachlor ranged from 0 to 2% 1 week after emergence (WAE). Minor crop stunting was observed late in the season in plots treated with flumioxazin or fomesafen. Yields for all 16 treatments and non-treated control were not statically different from each other.

Nomenclature: Flumioxazin; fomesafen; glufosinate; glyphosate; isoxaflutole; *S*-metolachlor; soybean, *Glycine max* (L.) Merr.

Key words: crop tolerance, crop injury, stunting, herbicide tolerance in soybean.

The commercialization of glyphosate resistant (GR) soybeans in 1996 allowed growers to spray glyphosate over the top of their crop and control most weeds (Duke and Powles, 2008). The success of glyphosate as a postemergence (POST) herbicide led to repeated applications within a season. As weed populations were subjected to this immense selection pressure, GR weeds evolved. Soybean producers in North Carolina for years have battled with glyphosate- and ALS-resistant weeds such as Palmer Amaranth (Heap, 2020).

The focus of weed control has shifted to a zero tolerance for weeds in a cropping system (Zimdahl, 1994). Understanding that leaving a single weed in a field that can flower and produce seed exacerbates the struggle against weeds. A single female Palmer amaranth plant can produce upwards of 500,000 seeds (Webster and Grey, 2015) and in some cases produced over 1,000,000 seeds (A.C York, personal communication). This mentality has led to the development of large herbicide programs utilizing multiple modes of action both preemergence (PRE) and POST. Common PRE herbicide combinations used are the long chain fatty acid (LCFA) inhibiting-herbicides, protoporphyrinogen oxidase (PPO) inhibitors and, to a lesser extent, dinitroaniline (DNA) herbicides. The problem that many growers face now in North Carolina is that weeds like Palmer Amaranth, tall waterhemp and, others have developed resistance to PPO inhibitors (Keeley et al., 1987). Additionally, the LCFA-inhibitors have in recent years shown poor performance on certain species including Palmer amaranth (Brabham et al., 2019). The combined reduction in efficacy from these common herbicides has made it difficult for growers to reach maximum yield potential in soybean.

Palmer amaranth is an annual broadleaf weed known for its competitive ability (Keeley et al., 1987). The competitive nature of Palmer amaranth, its ability to quickly adapt to selection pressure, and its extended period of germination make it difficult for soybean growers to control.

Palmer amaranth is a dioecious species, having separate male and female plants (Horak and Loughin, 2000), thus making this species strictly an outcrossing species. The genetic diversity of Palmer amaranth populations can be quite large, allowing for rapid herbicide resistance development.

Research has shown that Palmer amaranth at densities of 0.33, 3.33, and 10 plants m⁻¹ of row reduced soybean yield 17%, 64%, and 68%, respectively (Klingaman and Oliver 1994). Palmer amaranth produces more biomass and is more competitive than Common waterhemp (*Amaranthus rudis* Sauer) or Redroot pigweed (*Amaranthus retroflexus* L.) in soybean (Bensch et al., 2003). Globally this species has developed resistance to 8 modes of action (MOAs), with a population in Arkansas having confirmed multiple resistance to 5 MOAs (Heap, 2020).

Current herbicide programs that rely on the PPO-inhibitors, the newly launched synthetic auxins and glufosinate, are placing tremendous selection pressure on populations of Palmer amaranth where resistance to these herbicides has not developed. For over 50 years, PPO-inhibitors have been used in row crops and wide-spread use to control GR Palmer amaranth can cause rapid development of resistance. (Copeland et al., 2018). In 2015, the first confirmed global case of Palmer amaranth resistance to 2,4-D occurred in Kansas within a sorghum field (Kumar et al., 2019).

The latest attempt at a new chemistry for soybean growers is isoxaflutole (IFT) (5-cyclopropyl isoxazol-4-yl-2-mesyl-4-trifluoromethylphenyl ketone), a 4-Hydroxyphenylpyruvate Dioxygenase (HPPD) inhibiting herbicide (Pallett et al. 2001). Isoxaflutole was launched in 1998 for use as a PRE in small grains and corn (O'Brien et al. 2018). These crops are inherently tolerant to IFT, although tolerance across corn hybrids can vary (Simmons and Kells, 2003; O'Sullivan et al. 2001). In contrast, dicotyledonous crops and weeds are susceptible to IFT

(O'Brien et al. 2018). Injury from IFT begins in newly formed leaves. These leaves have a bleached appearance as IFT stops carotenoid synthesis and thus allows solar radiation to destroy chlorophyll (Pallett et al. 1998), leaving the plants white. Isoxaflutole is metabolized in plants and soil to its herbicidally active metabolite diketonitrile (DKN) [2-cyclopropyl-3-(2-mesy1-4-trifluoromethylphenyl)-3-oxopropanenitrile] (Pallett et al. 2001). Diketonitrile is metabolized into a biologically inactive benzoic acid derivative in plants, 2-mesy1-4-trifluoromethyl benzoic acid; however, this degradation step is slow in susceptible species, resulting in herbicidal selectivity (Alletto et al. 2012).

Recently the development of Credez GT27 soybeans (FG-72xLL55) by the BASF corporation has allowed for the potential implementation of IFT in soybean production. These cultivars are resistant to glyphosate, glufosinate and IFT. The specific mechanism of tolerance comes from the hppdPF W336 gene of *Pseudomonas fluorescens* strain A32 (ISAAA, 2020). This gene confers a modified p-HPPD enzyme that allows for tolerance to IFT. The 2mepsps gene from corn and the pat gene from *Streptomyces viridochromogenes* confer tolerances to glyphosate and glufosinate, respectively. Published research of crop tolerances and crop response to herbicide programs utilizing IFT and other herbicides in GT27 soybeans is limited. The purpose of this study is to determine the overall crop tolerance of these cultivars to herbicide programs utilizing IFT.

Materials and Methods

Experiments were conducted at the Central Crops Research Station near Clayton, NC during 2018 and 2019, and Bayer Environmental Science Research Station near Clayton, NC during 2018 and the BASF Research Facility near Pine Level, NC during 2019. Soil at location near Clayton, NC was a Norfolk sandy loam (Fine-loamy, kaolinitic, thermic Typic

Kandiuldults) with organic matter of 0.51% to 0.8% organic matter and pH 6.3 to 6.5. Soil at the Pine level site consisted of a well-drained Bonneau sand (Loamy, siliceous, subactive, thermic Arenic Paleudults) with 1.2% organic matter and pH of 6.7. Irrigation was used to supplement rainfall at the Pine Level site.

Soybean cultivar 'Credenz 4539GTLL' were planted using a 2-row cone planter to a depth of 1.9 cm. Plots were 4 rows spaced 91 cm apart by 7.6 m long. Planting dates can be found in Table 3.1. Treatments were arranged in a randomized complete block design with 4 replications. Herbicide treatments can be found in Table 3.2. Only the center 2 rows of each plot were treated. Rows 1 and 4 of each plot served as border to limit physical drift of herbicides and comparison for visual estimates of soybean injury.

Herbicide treatments consisted of 16 herbicide programs and nontreated check for comparison (Table 3.2). Preemergence herbicides were applied immediately following planting; POST herbicides were applied at V2-V4 growth stage. Herbicides were applied using a CO₂-pressurized backpack sprayer equipped with flat-fan nozzles (TeeJet XR8002VS, Spraying Systems Co. Wheaton, IL) delivering 140 L ha⁻¹ at 243 kPA. Acetochlor (Warrant Herbicide, Bayer CropScience LP St. Louis, MO) was applied PRE at 1261 g ai ha⁻¹ to the entire trial and hand weeding was used throughout the season to maintain experiments as weed-free. In addition, glyphosate at 1.26 kg ae ha⁻¹ plus glufosinate at 656 g ai ha⁻¹ was applied prior to canopy closure to control weeds that emerged after final crop response ratings. Insecticides were used as needed. At the end of the season, all plots received paraquat at 292 g ai ha⁻¹ to condition soybean for harvest.

Visual estimates of soybean injury were collected weekly beginning 1 week after emergence and ending 5 weeks after POST using a 0 to 100% scale, where 0 is no injury and 100

is complete crop death. Plots were harvested using an Almaco small plot combine and weighed with a HarvestMaster grain system to determine soybean yield.

Data were subjected to ANOVA using PROC GLM procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). Location and year were combined to create an environment parameter and identify interactions. Means were separated using a Tukey's HSD test, $P \leq 0.05$.

Results and Discussion

Soybean response to residual herbicides applied PRE was minimal 1 week after emergence (WAE) for all environments (Table 3.3). Injury from IFT, *S*-metolachlor and IFT plus *S*-metolachlor ranged from 0 to 2% and were less injurious than flumioxazin (8 to 10%) 1 WAE. Previous research has shown varying tolerance to flumioxazin PRE among soybean varieties with visual injury ranging from 1 to 11% (McNaughton et al., 2014). Crop injury from flumioxazin occurred just as the soybeans had emerged and once more just prior to POST treatments. This injury was transient and was limited to trifoliates that experienced soil splash from rain events (Figure 3.1). Isoxaflutole, *S*-metolachlor, and IFT plus *S*-metolachlor injury ranged from 0 to 6 % 2 WAE but remained less than flumioxazin (2 to 13 %) (Table 3.3). Varying environmental conditions resulted in locations that experienced weekly soil splashing onto trifoliates, causing necrosis and crop stunting in flumioxazin treated plots. Injury from IFT, *S*-metolachlor and IFT plus *S*-metolachlor was limited to very minor crop stunting. It has been noted that certain herbicides such as the chloroacetamides can reduce soybean growth; injurt mostly depends on soil moisture and rarely causes yield loss (Osborne et al., 1995)

With the exception of flumioxazin PRE followed by (fb) fomesafen plus *S*-metolachlor plus glyphosate POST (5 to 44%) and IFT fb fomesafen plus *S*-metolachlor plus glufosinate POST (1 to 21%) soybean injury was 5% or less 1 week after POST (WAP) (Table 3.4). These

two herbicide treatments continued to be the most injurious the season. However, injury quickly declined after POST applications and only minor crop stunting was observed late in the season (Figure 1.3). Fomesafen injury was observed 2 days after application but was transient and limited to treated trifoliates (Figure 3.2). Injury from fomesafen also resulted in considerable stunting in 2019 (Figure 3.3); stunting was noticeable until 4 WAP. As with flumioxazin, soybean cultivars vary in tolerance to fomesafen (Belfry, Shropshire, & Sikkema, 2016).

Minimal increases in crop injury occurred from the tank mix of IFT plus *S*-metolachlor (Table 3.3). IFT injury could however be seen in HPPD-susceptible volunteer beans that emerged shortly after PRE application (Figure 3.4). Crop injury from PPO treatments peaked at 1 WAP, when stunting from PPO-inhibiting herbicides injury was most prevalent (Table 3.3). While injury and stunting were not permanent, early season crop stunting was noticeable and initial ratings 1 WAP showed injury greater than the accepted 15% industry standard for flumioxazin fb fomesafen plus *S*-metolachlor plus glyphosate (44%) and IFT fb fomesafen plus *S*-metolachlor plus glufosinate POST (21%) for certain environments. Environments that experienced the greatest injury consisted of sandy soils with heavy rainfalls shortly after applications. Cool temperatures, high moisture, and repeated exposure of the trifoliates to treated soil, may explain greater injury compared to previous research (Belfry, Shropshire, & Sikkema, 2016).

Injury from POST only herbicide treatments of glyphosate, glufosinate and glyphosate plus glufosinate ranged 0 to 2%. Injury from these treatments consisted of transient chlorosis of upper trifoliates from glyphosate and slight necrosis from glufosinate. Chlorosis was apparent 1 WAP, with symptomology quickly decreasing and disappearing 1 to 3 days later. Glyphosate is known to cause a reduction in chlorophyll in GR soybean by as much as 10%, as the metabolite,

aminomethylphosphonic acid (AMPA), is a known phytotoxin (Reddy, Rimando, and Duke, 2004).

The average soybean yield for all treatments, pooled across locations ranged from 3234 to 3732 kg ha⁻¹ (data not shown). No herbicide treatment, even those that cause some early season injury, affected soybean yield. Soybean yields did vary across environments (Table 3.7).

This research demonstrates GT27 soybeans are tolerant to IFT, current soybean herbicides, and IFT in combination with *S*-metolachlor. Isoxaflutole use on GT27 soybean is visually less injurious than flumioxazin. In addition, IFT alone and IFT combinations did not affect soybean yield. It can be concluded that IFT can be safely applied to GT27 soybeans. Tolerance of GT27 to PPO-inhibiting herbicides appear to fall within industry standards when environmental conditions are not conducive of soil splash. However, even in these less than ideal conditions there were no adverse effects on yield from these treatments.

This research has only investigated a few possible soybean herbicide programs and combinations with IFT. Further research should be conducted to evaluate other PRE and POST soybean herbicides and their effects on GT27 soybeans and effects when used in combination with IFT. Furthermore, because environment greatly influences soybean injury to many herbicides, additional locations across varying soil types, environments and production types should be further investigated.

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Figures and Tables

Table 3.1 Locations, planting, treatment, and harvest dates.

Location	Planting Date	PRE	POST	Harvest
Central Crops ^a 2018	24-May	24-May	14-Jun	18-Oct
Bayer ^b 2018	7-May	7-May	28-May	4-Oct
Central Crops 2019	5-Jun	5-Jun	26-Jun	19-Oct
BASF ^c 2019	14-May	14-May	4-Jun	11-Oct

^a Central Crops Research Station, near Clayton, NC

^b Bayer Environment Science Research Station, near Clayton, NC

^c BASF Pine Level Research Station, near Pine Level, NC

Table 3.2 Herbicides, rates and manufactures^a

Herbicide	Tradename	Rate g ai ha ⁻¹	Company	Location
Isoxaflutole	Alite 27	105.2	BASF Corporation	Research Triangle Park, NC
S-metolachlor	Dual II Magnum	1069	Syngenta Crop Protection, LLC	Greensboro, NC
Glyphosate	Roundup Power max	1263	Bayer CropScience LP	St. Louis, MO
Glufosinate	Liberty 280 SL	655	BASF Corporation	Research Triangle Park, NC
Flumioxazin	Valor SX	107.2	Valent U.S.A Corp	Walnut Creek, CA
Fomesafen+ S-metolachlor	Prefix	1483	Syngenta Crop Protection, LLC	Greensboro, NC

^a Specimen labels for each product, mailing addresses and web site addresses of each manufacture can be found at www.cdms.net

Table 3.3 Visual injury of soybean by environment and treatment^{ab}

Herbicide treatment		1 WAE ^e				2 WAE			
PRE ^c	POST ^d	CCRS 2018	Bayer 2018	CCRS20 19	BASF 2019	CCRS 2018	Bayer 2018	CCRS20 19	BASF 2019
Isoxaflutole	None	0 f	0 f	0 f	0 f	0 g	2 d-g	0 g	0 g
Isoxaflutole	Glyphosate	1 def	0 f	0 f	0 f	0 g	0 g	0 g	0 g
Isoxaflutole	Glufosinate	1 def	1 def	0 f	0 f	0 g	1 gef	0 g	0 g
Isoxaflutole	Glyphosate + Glufosinate	0 f	0 f	0 f	0 f	0 g	3 def	0 g	0 g
S-metolachlor	None	0 f	0 f	0 f	0 f	0 g	4 cd	1 gef	0 g
S-metolachlor	Glyphosate	0 f	1 def	1 def	0 f	0 g	0 g	1 gef	0 g
S-metolachlor	Glufosinate	0 f	1 def	0 f	0 f	0 g	0 g	0 g	0 g
S-metolachlor	Glyphosate + Glufosinate	1 def	2 cde	0 f	0 f	0 g	1 gef	0 g	0 g
Isoxaflutole + S-metolachlor	Glyphosate	1 def	3 c	0 f	0 f	0 g	2 d-g	0 g	0 g
Isoxaflutole + S-metolachlor	Glufosinate	1 def	1 def	1 def	0 f	0 g	3 def	1 gef	0 g
Isoxaflutole + S-metolachlor	Glyphosate + Glufosinate	0 f	2 cde	0 f	0 f	1 gef	6 c	0 g	1 gef
Flumioxazin	Fomesafen + S- metolachlor + Glyphosate	8 b	10 a	0 f	0 f	10 ab	9 b	2 d-g	13 a
Isoxaflutole	Fomesafen + S- metolachlor + Glufosinate	0 f	1 def	0 f	0 f	0 g	0g	0 g	0 g

^a Means of treatments using only PRE herbicides shown, POST herbicides labeled to show different treatments

^b Means within a rating period followed by the same letter are not different according to Tukey's HSD test at P=0.05

^c Abbreviation: PRE, preemergence herbicide treatment

^d Abbreviation: POST, postemergence herbicide treatment

^e Abbreviation: WAE, weeks after emergence

Table 3.4 Visual injury of soybean by environment and treatment^a

Herbicide treatment		1 WAP ^d				2 WAP			
PRE ^b	POST ^c	CCRS 2018	Bayer 2018	CCRS 2019	BASF 2019	CCRS 2018	Bayer 2018	CCRS 2019	BASF 2019
None	Glyphosate	0 g	1 fg	0 g	0 g	0 e	0 e	1 cde	0 e
None	Glufosinate	0 g	0 g	1 fg	1 fg	0 e	0 e	0 e	0 e
None	Glyphosate + Glufosinate	1 fg	0 g	0 g	1 fg	0 e	0 e	0 e	1 cde
Isoxaflutole	None	0 g	1 fg	0 g	1 fg	0 e	0 e	0 e	0 e
Isoxaflutole	Glyphosate	0 g	0 g	0 g	2 fg	0 e	0 e	0 e	1 cde
Isoxaflutole	Glufosinate	0 g	1 fg	0 g	1 fg	0 e	1 cde	1 cde	0 e
Isoxaflutole	Glyphosate + Glufosinate	0 g	2 fg	0 g	1 fg	0 e	0 e	1 cde	1 cde
<i>S</i> -metolachlor	None	0 g	2 fg	0 g	3 fg	0 e	0 e	1 cde	0 e
<i>S</i> -metolachlor	Glyphosate	0 g	0 g	1 fg	0 g	0 e	2 cde	0 e	0 e
<i>S</i> -metolachlor	Glufosinate	0 g	0 g	0 g	3 fg	0 e	0 e	0 e	0 e
<i>S</i> -metolachlor	Glyphosate + Glufosinate	0 g	0 g	0 g	2 fg	0 e	0 e	0 e	0 e
Isoxaflutole + <i>S</i> -metolachlor	Glyphosate	1 fg	2 fg	2 fg	1 fg	0 e	3 cd	1 cde	1 cde
Isoxaflutole + <i>S</i> -metolachlor	Glufosinate	0 g	4 efg	0 g	2 fg	0 e	2 cde	1 cde	1 cde
Isoxaflutole + <i>S</i> -metolachlor	Glyphosate + Glufosinate	1 fg	5 d-g	0 g	3 fg	0 e	0 e	2 cde	1 cde
Flumioxazin	Fomesafen + <i>S</i> - metolachlor + Glyphosate	5 d-g	7 c-f	11 cd	44 a	1 cde	1 cde	3 cd	14 a
Isoxaflutole	Fomesafen + <i>S</i> - metolachlor + Glufosinate	1 fg	9 cde	12c	21 b	0 e	3 cd	4 c	9 b

^a Means within a rating period followed by the same letter are not different according to Tukey's HSD test at P=0.05

^b Abbreviation: PRE, preemergence herbicide treatment

^c Abbreviation: POST, postemergence herbicide treatment

^d Abbreviation: WAP, weeks after POST

Table 3.5 Visual injury of soybean by environment and treatment^a

Herbicide treatment		3 WAP ^d				4 WAP			
PRE ^b	POST ^c	CCRS 2018	Bayer 2018	CCRS 2019	BASF 2019	CCRS 2018	Bayer 2018	CCRS 2019	BASF 2019
None	Glyphosate	0 c	0 c	0 c	0 c	0 c	0 c	0 c	1 c
None	Glufosinate	0 c	0 c	0 c	0 c	0 c	0 c	0 c	1 c
None	Glyphosate + Glufosinate	0 c	0 c	0 c	0 c	0 c	0 c	0 c	1 c
Isoxaflutole	None	0 c	0 c	0 c	1 c	0 c	0 c	0 c	0 c
Isoxaflutole	Glyphosate	0 c	0 c	0 c	1 c	0 c	0 c	0 c	1 c
Isoxaflutole	Glufosinate	0 c	0 c	0 c	2 c	0 c	0 c	0 c	2 c
Isoxaflutole	Glyphosate + Glufosinate	0 c	0 c	0 c	0 c	0 c	0 c	0 c	1 c
S-metolachlor	None	0 c	1 c	0 c	0 c	0 c	0 c	0 c	0 c
S-metolachlor	Glyphosate	0 c	0 c	1 c	0 c	0 c	0 c	0 c	0 c
S-metolachlor	Glufosinate	0 c	0 c	0 c	0 c	0 c	0 c	0 c	0 c
S-metolachlor	Glyphosate + Glufosinate	0 c	0 c	0 c	0 c	0 c	0 c	0 c	0 c
Isoxaflutole + S-metolachlor	Glyphosate	0 c	0 c	2 c	1 c	0 c	0 c	0 c	1 c
Isoxaflutole + S-metolachlor	Glufosinate	0 c	0 c	1 c	2 c	0 c	0 c	0 c	1 c
Isoxaflutole + S-metolachlor	Glyphosate + Glufosinate	0 c	1 c	0 c	1 c	0 c	1 c	0 c	1 c
Flumioxazin	Fomesafen + S- metolachlor + Glyphosate	0 c	2 c	1 c	14 a	0 c	1 c	0 c	6 a
Isoxaflutole	Fomesafen + S- metolachlor + Glufosinate	0 c	2 c	1 c	9 b	0 c	1 c	0 c	4 b

^a Means within a rating period followed by the same letter are not different according to Tukey's HSD test at P=0.05

^b Abbreviation: PRE, preemergence herbicide treatment

^c Abbreviation: POST, postemergence herbicide treatment

^d Abbreviation: WAP, weeks after POST

Table 3.6 Visual injury of soybean by environment and treatment^a

Herbicide treatment		5 WAP ^d			
PRE ^b	POST ^c	CCRS 2018	Bayer 2018	CCRS 2019	BASF 2019
None	Glyphosate	0 c	0 c	0 c	0 c
None	Glufosinate	0 c	0 c	0 c	0 c
None	Glyphosate + Glufosinate	0 c	0 c	0 c	0 c
Isoxaflutole	None	0 c	0 c	0 c	0 c
Isoxaflutole	Glyphosate	0 c	0 c	0 c	0 c
Isoxaflutole	Glufosinate	0 c	0 c	0 c	0 c
Isoxaflutole	Glyphosate + Glufosinate	0 c	0 c	0 c	0 c
<i>S</i> -metolachlor	None	0 c	0 c	0 c	0 c
<i>S</i> -metolachlor	Glyphosate	0 c	0 c	0 c	0 c
<i>S</i> -metolachlor	Glufosinate	0 c	0 c	0 c	0 c
<i>S</i> -metolachlor	Glyphosate + Glufosinate	0 c	0 c	0 c	0 c
Isoxaflutole + <i>S</i> -metolachlor	Glyphosate	0 c	0 c	0 c	0 c
Isoxaflutole + <i>S</i> -metolachlor	Glufosinate	0 c	0 c	0 c	0 c
Isoxaflutole + <i>S</i> -metolachlor	Glyphosate + Glufosinate	0 c	0 c	0 c	0 c
Flumioxazin	Fomesafen + <i>S</i> -metolachlor + Glyphosate	0 c	0 c	0 c	4a
Isoxaflutole	Fomesafen + <i>S</i> -metolachlor + Glufosinate	0 c	0 c	0 c	1 b

^a Means within a rating period followed by the same letter are not different according to Tukey's HSD test at P=0.05

^b Abbreviation: PRE, preemergence herbicide treatment

^c Abbreviation: POST, postemergence herbicide treatment

^d Abbreviation: WAP, weeks after POST

Table 3.7 Yields by environment^{ab}

Environment	Yield
	Kg ha ⁻¹
Central Crops Research Station 2018	3535 b
Bayer Environmental Science Research Station 2018	4715 a
Central Crops Research Station 2019	2882 c
Pinelevel Research Farm 2019	2646 c

^a Significance between yield only occurred between environments.

^b Means within a column followed by the same letter are not different according to Tukey's HSD test at P=0.05

Figure 3.1 Crop Injury from Flumioxazin^a



^a Injury from flumioxazin occurred immediately following soybean emergence, then again following 1 rain event prior to POST treatments.

Figure 3.2 Fomesafen Crop Injury^a



^a Transient injury from fomesafen on treated trifoliate

Figure 3.3 Crop Stunting from Fomesafen injury^a



^a Fomesafen stunting 2 WAP

Figure 3.4 Volunteer Soybean injury from Isoxaflutole^a



^a HPPD- susceptible volunteer Soybean that emerged shortly after PRE application of isoxaflutole