ABSTRACT

INMAN, MATTHEW DARWIN. Weed Competition and Herbicide Use and Management in Flue-Cured Tobacco and Cotton. (Under the direction of Drs. Loren Fisher and Matthew Vann).

The commercialization of dicamba-tolerant crops has resulted in a widespread adoption of this technology along with the increased use of the herbicide dicamba. In an effort to sustain technology and delay further herbicide resistance, diligent stewardship of this technology must be pursued. Three studies were conducted to evaluate best management practices in using new dicamba technology. Two additional studies were conducted to investigate the impact of weed interference on flue-cured tobacco growth and also determining potential contributions of the herbicide rimsulfuron to current flue-cured tobacco weed management programs.

The first study evaluated the importance of timely postemergence (POST) herbicide applications on weed control and cotton yield. Weed control and yield were similar, regardless of the use of preemergence (PRE) herbicides, when POST applications were timely. However, if POST applications were delayed cotton yield suffered. The use of PRE herbicides created flexibility of POST application timings. Furthermore, there was no extra cost associated with treatments containing PRE herbicides, as yield and economic returns were similar or greater to the POST treatments without PRE herbicides.

The second study investigated changes in Palmer amaranth density and frequency of glyphosate-resistance (GR) and weed richness after eight years of glyphosate, dicamba, and residual herbicdes. Rapid increases in Palmer amaranth were observed with treatments containing glyphosate only. The biennial rotations of glyphosate and glyphosate plus dicamba slowed the increase in Palmer amaranth populations, however, were ineffective in reducing Palmer amaranth densities. Treatments that included PRE herbcides were more effective than not. By the end of the study there were no differences in Palmer amaranth density or frequency

of GR. Glyphosate plus dicamba was effective in reducing Palmer amaranth populations. Weed richness did not change over the course of the study. No tolerance to dicamba was observed from GR Palmer amaranth populations after 8 yr of the experiment.

The third study evaluated dicamba retention in sprayer tanks and also the potential impact to flue-cured tobacco. Significant dicamba residue was discovered even after three rinses. Dicamba formulation or cleaning agent had no impact on dicamba residue removal. Residue recovered could cause visual injury and yield loss any some cases on sensitive crops. Tobacco response to reduced rates of dicamba widely varied by environment. Exposure early in the season is more injurious compared to late season exposure. Injury was observed across all rates with the early application timing, with the lowest rate being 10,000th of a field labeled rate. The maximum yield reduction was 62% with a 55% reduction in value.

The fourth study determined the critical period of weed control for flue-cured tobacco. The CPWC was determined based on a 5% marketable yield loss. When combined across years, the CPWC was determined to be 3.5 to 7.8 WAT and 3 to 9.1 WAT in the broadleaf and grass weed species study and broadleaf species only, respectively. In both tests, tobacco quality and value decreased as the time duration of weed interference increased.

The fifth study evaluated flue-cured tobacco response to rimsulfuron. Furthermore, determine the best fit for rimsulfuron in a tobacco weed management program. Rimsulfuron applied postemergence over-the-top (POT) caused significant injury, however, injury was transient as no reduction in yield was observed. Weed control was similar to current herbicides used post-directed (PD) at layby. Due to the potential for injury, rimsulfuron is suggested for pre-transplant (PRE-T) or PD use.

Weed Competition and Herbicide Use and Management in Flue-Cured Tobacco and Cotton

by
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DEDICATION

This dissertation is dedicated to my loving wife Ashley and our two boys Braxton and Barrett. I also dedicate this to my family. I have received tremendous support from everyone over the course of my academic career and I will always be grateful.

BIOGRAPHY

Matthew Darwin Inman was raised in East Bend, NC and graduated from Forbush High School in 2002. In 2004, Matt graduated with an Associate in Applied Science Degree in Horticulture from Haywood Community College in Clyde, NC. From 2006 to 2010, Matt served active duty in the United States Marine Corps. In the fall of 2010, Matt started his Bachelor's Degree in Agronomy at North Carolina State University and completing this degree in the spring of 2013. In the summer of 2013, he was admitted into the graduate school of North Carolina State University where he began to pursue a Master's of Science degree in Crop Science under the direction of Drs. David Jordan and Katie Jennings. Upon completion, Matt started as an Extension and Research Associate in the tobacco agronomy program while pursuing a PhD under Drs. Loren Fisher and Matthew Vann.

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CHAPTER 1

Influence of Timing of Palmer Amaranth Control in Dicamba-Tolerant Cotton on Yield and Economic Return

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Abstract

Glyphosate-resistant (GR) Palmer amaranth continues to be challenging to control across the US cotton belt. Timely application of POST herbicides and herbicides applied at planting or during the season with residual activity are utilized routinely in cotton. Although glyphosate controls large Palmer amaranth that is not GR, herbicides such as glufosinate used in resistance management programs for GR Palmer amaranth must be applied when weeds are small. Dicamba has shown to complement both glyphosate and glufosinate systems in controlling GR and susceptible biotypes in tolerant cultivars. Two separate studies were conducted to determine Palmer amaranth control, cotton yield, and estimated economic net returns with various herbicide application timings 2, 3, 4, and 5 WAP with and without PRE herbicides. In general, Palmer amaranth was controlled 98% or greater when three or more herbicide applications were made regardless of timing and herbicide sequence. Two herbicide applications at 4 and 5 WAP, which included glyphosate plus dicamba, provided similar control compared to three applications by 8 WAP, however, yield was reduced 23% due to early season interference. The inclusion of a PRE herbicide program benefited treatments that missed early application timings. A single herbicide application of glyphosate plus dicamba, 5 WAP, provided 69% control of Palmer amaranth and when PRE herbicides were included control increased to 96%. There was no extra cost associated with treatments containing PRE herbicides, as yield and economic returns were similar or greater to the POST treatments without PRE herbicides.

Early season management of Palmer amaranth (*Amaranthus palmeri*) is critical in cotton production to maximize yield potential (Fast et al. 2009; MacRae et al. 2013; Norsworthy et al. 2016). Palmer amaranth continues to be one of the most challenging weed species to manage in cotton and other agronomic crops (Webster 2013). Implications of Palmer amaranth interference in cotton production have been well documented (Fast et al. 2009; MacRae et al. 2013; Morgan et al. 2001; Norsworthy et al. 2009; Rowland et al. 1999; Smith et al. 2000; Webster and Grey 2015). Vann et al. (2017a) reported cotton stunting ranging from 7 to 57% and lint yield reductions from 8 to 42% when the first postemergence (POST) herbicide applications were delayed 7 to 28 days; respectively. Furthermore, prolonged insufficient control of Palmer amaranth can lead to a rapid increase in Palmer amaranth populations and contribution of seed to the soil seedbank (Inman et al. 2016).

Resistance management has been at the forefront of weed management programs in cotton production since GR Palmer amaranth was first confirmed in 2005 (Culpepper et al. 2006). The use of soil-applied residual herbicides and timely POST applications, along with integrated management strategies, have become requirements in order to effectively manage GR Palmer amaranth and other resistant-weeds (Culpepper et al. 2010; Norsworthy et al. 2012; Sosnoskie et al. 2014). Postemergence herbicide options are limited for cotton growers. Since the transition from glyphosate-resistant (GR) cotton to glufosinate-tolerant cotton, glufosinate has been heavily relied upon (Barnett et al. 2013; Sosnoskie et al. 2014). Glufosinate has shown to be effective in controlling GR Palmer amaranth when timely applications are made (Barnett et al. 2013; Cahoon et al. 2015; Corbett et al. 2004). However, control is generally reduced when glufosinate is applied to Palmer amaranth taller than 8 cm (Coetzer et al. 2002; Culpepper et al. 2010). The rapid growth and competitive ability of Palmer amaranth (Ward et al. 2013) creates challenges for growers in making well-timed herbicide applications. In addition, at-plant residual herbicides may not control weeds adequately and may further decrease grower's flexibility in making timely POST applications.

The commercialization of dicamba-tolerant cotton has provided growers with an additional POST option in managing GR Palmer amaranth (Cahoon et al. 2015). Dicamba has shown to be an effective tank-mix partner in combinations of glyphosate or glufosinate for control of glyphosate-susceptible (GS) and GR Palmer amaranth (Cahoon et al. 2015; Johnson et al. 2010; Merchant et al. 2013; York et al. 2012). Depending on POST application timing and location, late-season Palmer amaranth control was increased at least 14% and 41% when dicamba was included compared to glufosinate and glyphosate alone; respectively (Cahoon et al. 2015). Regardless of rate, POST mixtures of glufosinate and dicamba were more effective at controlling 16- to 23-cm Palmer amaranth 12 days after application compared to glufosinate or dicamba alone (Vann et al. 2017b). Similar findings were reported by Merchant et al. (2013), where a 17 to 22% increase in control was observed with 20-cm tall Palmer amaranth when dicamba was included with glufosinate compared to glufosinate alone.

The performance of POST herbicide applications are related to rate, timing, and size of the weed at application. Failure of one of these fundamental components could potentially decrease weed control and increase selection pressure of the herbicides applied, contributing to herbicide resistance. With unpredictable weather conditions and larger farm sizes, timely herbicide applications are increasingly becoming a challenge. Furthermore, the potential increase in cost, due to the possibility of increased herbicides and trips to the field, may cause growers to be reluctant in following a well-timed spraying schedule. However, it has been shown that short-term and long-term benefits can be attained through weed management programs that offer greater diversity (Inman et al. 2017; Jordan et al. 2014). The objective for the first experiment was to compare different timings of POST herbicide applications of glufosinate and glyphosate plus dicamba on Palmer amaranth and annual grass control, cotton yield, and economic net returns without preemergence (PRE) herbicides. The objective for the second experiment was to follow the same POST timings and herbicides as experiment one, comparing Palmer amaranth control, cotton yield, and economic net returns with and without PRE herbicides.

Materials and Methods

Two separate experiments were established in North Carolina in multiple fields during 2015, 2016, and 2017 near Clayton (35.67ºN, 78.51ºW) and Rocky Mount (35.89ºN, 77.64ºW). Soils in Clayton were a Dothan loamy sand (fine-loamy, kaolinitic, thermic Plinthic Kandiudults) and Goldsboro sandy loam (fine-loamy, siliceous, subactive, thermic Aquic Paleudults) with 0.27 and 0.41% humic matter. Soils in Rocky Mount were a Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiudults) and an Aycock very fine sandy loam (fine-silty, siliceous, subactive, thermic Typic Paleudults) with 0.5% humic matter. Dyna-Gro® cotton ‘3385 B2XF’ (Crop Production Services, Loveland, CO) was planted in 2015. Cotton ‘DP 1522 B2XF’ (Monsanto, St Louis, MO) was planted in 2016. Cotton ‘DP 1538 B2XF’ (Monsanto, St Louis, MO) was planted in 2017. Cotton was planted in conventionally tilled, raised beds at a seeding of 14 seed m^{-1} of row. Plot sizes ranged from 3 to 4 rows (91-cm spacing) by 9 to 12 m; depending on location. Other than treatments imposed for the experiment, cotton was managed according to North Carolina Cooperative Extension Service recommendations (Edmisten et al., 2015).

In experiment one (POST only herbicides), treatments consisted of herbicides applied 2, 3, 4, and 5 weeks after planting (WAP); 3, 4, and 5 WAP; 4 and 5 WAP; and 5 WAP only. Additional treatments included herbicides applied 2 WAP only, 2 and 3 WAP; and 2, 3, and 4 WAP. Glufosinate was applied 2 and 3 WAP. At 4 and 5 WAP, glyphosate plus dicamba were applied. A non-treated control was also included. No PRE herbicides were applied at planting. In experiment 2 (PRE and POST herbicides), the same POST herbicide treatments as experiment one were followed with and without initial PRE herbicides applied immediately after planting. The PRE herbicide program consisted of fomesafen plus acetochlor plus diuron. A non-treated control was also included. Rates for all herbicides are located in Table 1. All herbicides were applied using CO_2-pressurized backpack sprayers equipped with TTI 110025 Turbo TeeJet® Induction nozzles (TeeJet Technologies, Wheaton, IL) delivering 140 L ha^{-1} at 165 kPa.

All environments had natural infestations of Palmer amaranth with a mixture of glyphosate-susceptible and GR Palmer amaranth; density was 75 plants m^2 or greater. Palmer amaranth heights and crop growth stage at each application timing can be found in Table 2. Most environments had dense populations of annual grass species ranging from 25 to 100 plants m^{-2}. Broadleaf signalgrass [(*Urochloa platyphylla* (Nash) R.D. Webster)], large crabgrass (*Digitaria sanguinalis* L.), Texas panicum (*Panicum texanum* Buckl.), and goosegrass [(*Eleusine indica* (L.) Gaertn.)] were the most dominant grass species. For both experiments, Palmer amaranth control was estimated visually using a 0 to 100 scale (Frans et al. 1986) weekly from 2 WAP to 8 WAP. Palmer amaranth density in each plot was determined by counting the number of plants from 1 m^{-2} in each plot. Palmer amaranth aboveground fresh biomass was collected from row middles in treated plots (17 to 23 m^2) within 3 weeks prior to harvest and 1 m^2 in the non-treated plots. In experiment 1, grass control, densities, and above ground biomass were recorded in the same manner as Palmer amaranth. In experiment 2, annual grass was controlled as needed with clethodim (Select Max, Valent USA, Walnut Creek, CA). All treated plots were mechanically harvested in mid-October to mid- November.

Estimated economic net return was calculated based on the North Carolina Cooperative Extension Service budget for cotton (Bullen, 2015) with a total production cost of 1,268.12 ha^{-1}, excluding herbicide cost. Herbicide cost was based on pricing from local chemical retailers and factored in total production cost. Ginning cost was based on seed cotton yield for each plot at a price of \$0.27 ha^{-1}. Economic return was calculated for three lint prices as the difference between the product of yield (45% lint at \$1.54 ha^{-1}, \$1.76 ha^{-1}, and \$1.98 ha^{-1} with 55% seed at \$0.30 ha^{-1}) and total production cost.

The experimental design was a randomized complete block with treatments replicated four times. The combination of location and year was considered an environment. Statistical analyses were performed using the PROC Mixed procedure in SAS (v. 9.4; SAS Institute, Cary, NC). Treatments were considered a fixed factor, and replication and environment were considered random factors. Treatment interactions containing replication or environment were set as random effects (Blouin et. al 2011). Significant treatment by environment interactions for Palmer amaranth control, lint yield, and economic returns were observed for both experiments. Similar trends were found when environments were analyzed individually; therefore, analyses were combined across environments. Furthermore, the treatment mean square was at least 3-fold greater than the treatment by environment interaction mean square, providing justification to combine results over environments. Type III statistics were used to test all fixed effects, and least square means were calculated based on $p \leq 0.05$ (Moore and Dixon 2015). Non-treated checks were excluded from all statistical analyses except for Palmer amaranth biomass.

Results and Discussion

Experiment 1 (POST only).

Regardless of herbicide sequence, Palmer amaranth was controlled 98% or greater 8WAP when herbicides were applied 3 times. When glyphosate and dicamba were applied twice (4 and 5 WAP), no difference in Palmer amaranth control was observed 8 WAP compared to treatments with at least three herbicide applications (Table 3). Control of Palmer amaranth declined to 75% when a single glyphosate plus dicamba application was delayed to 5 WAP. Vann et al. (2017a) reported 75% control of Palmer amaranth when the first POST application of glufosinate plus dicamba was delayed 5 WAP. Glufosinate alone applied 2 WAP and 2 and 3 WAP controlled Palmer amaranth 60 and 85% 8WAP; respectively. Although effective control was observed 14 days after treatment (DAT), longevity of herbicide applications at 2 WAP and 2 and 3 WAP only are not sufficient for season long control. Trends among treatments for annual grass control were similar to that of Palmer amaranth control (Table 3). Greatest control was observed when at least three herbicide applications were delivered or when glyphosate and dicamba was applied 4 and 5 WAP. A single application of glyphosate plus dicamba 5 WAP controlled annual grasses 85%, compared to glufosinate alone 2 WAP and 2 and 3 WAP 60 and 89%; respectively. Research has shown that, glyphosate is generally more effective on grass species compared to glufosinate; especially goosegrass (Corbett et al. 2004; Culpepper et al. 2000). Average aboveground biomass for non-treated checks were 21,500 kg ha^{-1} and 11,200 kg ha^{-1} for Palmer amaranth and annual grasses, respectively (data not shown). Treatments containing three or more herbicide applications reduced Palmer amaranth biomass at least 96% (data not shown). Annual grass biomass was reduced at least 93% with three or more herbicide applications or sequential applications of glyphosate plus dicamba at 4 and 5 WAP.

When at least three herbicide applications were made, no difference in cotton lint yields was observed regardless of timing sequence (Table 4). However, yields following three applications at 2, 3, and 4 WAP were lower compared with herbicides applied at 3, 4, and 5 WAP and 2, 3, 4, and 5 WAP, respectively. Buchanan and Burns (1970) suggested cotton should be maintained weed-free for approximately 8 WAP to protect maximum yields. Although weed control 8 WAP was similar between these applications timings, the lack of an herbicide beyond 4 WAP proves to be critical by the end of the season. Despite sequential applications of glyphosate plus dicamba (4 and 5 WAP) providing similar weed control compared to three or more herbicide applications, early season weed interference reduced lint yield 23 to 30% (Table 3). Vann et al. (2017a) reported 22.8% reduction in lint yield when the first POST application was delayed 14 days. Furthermore, Everman et al. (2007) reported a 72% reduction in lint yield when no early postemergence (EPOST) herbicide was used compared to glufosinate alone EPOST. Glufosinate alone at 2WAP provided the lowest yields. Similar yields were observed with glufosinate at 2 and 3 WAP and a single application of glyphosate plus dicamba 5 WAP (Table 4). Comparable to yield trends, economic net returns were highest when three or four herbicide applications were delivered. Although cotton lint yield was lower for sequential applications of glyphosate plus dicamba (4 and 5 WAP), reductions in herbicide cost allowed for similar returns compared to three and four applications (Table 4).

Experiment 2 (PRE and POST).

All POST herbicide sequences provided similar or greater control of Palmer amaranth when following PRE herbicides (Table 5). Palmer amaranth was controlled 99% or greater 8 WAP when three POST herbicide applications were made; with or without the use of PRE herbicides. Palmer amaranth control increased by 30, 27, 8 and 8% with POST timings 2 WAP only, 5 WAP only, 2 and 3 WAP, 4 and 5 WAP with PRE herbicides compared to no PRE; respectively. The PRE herbicide program alone provided 79% control of Palmer amaranth, similar to that of sequential POST herbicide applications at 2 and 3 WAP. The importance of PRE herbicides have been well documented in combating herbicide-resistant Palmer amaranth and reducing early season weed interference (Everman et al. 2009;Norsworthy et al. 2012; Whitaker et al. 2011).

Although not always significant, greater cotton lint yields were observed when the PRE herbicide program was used (Table 5). There were no differences in lint yields when at least three POST applications were applied; regardless of timing sequence or PRE herbicides. The PRE herbicide program alone resulted in similar yields to that of POST only application timings of 2 WAP, 2 and 3 WAP, and 5 WAP. The inclusion of PRE herbicides allowed for greater flexibility in the number of POST application timings. This was more evident with later POST application timings, 4 and 5 WAP and 5 WAP only, compared to early application timings, 2 WAP and 2 and 3 WAP. Everman et al. (2007) reported similar findings, showing cotton lint yields were comparable when PRE and postemergence-directed (PD) herbicide applications were made regardless of a mid-postemergence (MPOST) application.

Trends for economic returns among treatments were similar to that of cotton lint yield. Highest returns were observed with the 4 and 5 WAP and 5 WAP application timings when the PRE herbicide program was included (Table 6). Although greater cost was associated with including the PRE herbicide program, greater returns were observed when the PRE program was implemented with one and two application timings compared to no PRE. This was more evident when POST timings are delayed until 4 WAP, compared to timings at 2 WAP. This can be attributed to the differences in weed interference at critical growth stages (Buchanan and Burns

1970). There was no difference in economic returns when herbicides were applied at least three times irrespective of PRE herbicide treatment.

Total POST herbicide programs can be successful in some situations (Askew and Wilcut 1999; Burke et al. 2005; Culpepper and York 1998; Jordan et al. 1993). However, timely applications are critical when soil-applied herbicides are not included (Culpepper and York 1999; Vann et al. 2017a). Our data show excellent Palmer amaranth control was achieved when three or more timely POST applications were utilized. Similar weed control can be obtained with sequential applications of glyphosate plus dicamba at 4 and 5 WAP. However, cotton lint yields will suffer due to early season weed interference. Although effective at the time, this type of weed management program was a major contributor to the herbicide resistance issues growers' face today. Furthermore, soil weed seedbank dynamics should be included in weed management programs (Buhler et al. 1997; Norsworthy et al. 2018). Control of larger weeds are obtainable, however, weed seed contribution is unknown when herbicides do not fully control weeds. This research demonstrates that even when adequate weed control is obtained with larger weeds, early season weed interference can still adversely affect cotton yield. The use of PRE herbicides can offset missed early season weed control efforts; providing similar yields and economic returns compared to timely, early POST applications. Timely POST herbicide applications contribute to greater efficacy and provide greater crop protection, and while not quantified in this study, reduce weed seed contribution to the soil seedbank. Although no statistical difference was observed, there was no extra cost associated with the use of the PRE herbicide program compared to no PRE's. Yield was consistently greater across all POST treatments that included PRE's. Economic returns were also greater among treatments including PRE's, except for the four POST application program; where returns were similar

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Table 1. Herbicide active ingredient, trade name, application rate, and manufacturer.

Common name	Trade name	Rate (g ai or ae ha^{-1})	Manufacturer
Glufosinate	Liberty 280SL	660	Bayer CropScience, RTP, NC
Glyphosate	Roundup PowerMAX	946	Monsanto Company, St. Louis, MO
Dicamba	Xtendimax	560	Monsanto Company, St. Louis, MO
Fomesafen	Reflex	175	Syngenta Crop Protection, Greensboro, NC
Acetochlor	Warrant	840	Monsanto Company, St. Louis, MO
Diuron	Direx 4L	560	ADAMA, Raleigh, NC

Table 2. Cotton growth stage and Palmer amaranth height at application timing; averaged over environments.

Application timing	Cotton growth stage	Palmer amaranth heights	
		Maximum	Average
Wks after planting		———cm———	
2	Cot.-1 lf.	7	3.5
3	2-3 lf.	15	9
4	3-5lf.	30	18
5	6-8 lf.	61	38

Table 3. Palmer amaranth and annual grass control 8WAP as affected by POST only application timing.[a]

Herbicides and application timings (WAP)[b]					
2	3	4	5	Palmer amaranth control	Annual grass control[c]
				%	%
Glufosinate	Glufosinate	Glyphosate + dicamba	Glyphosate + dicamba	100 a	100 a
	Glufosinate	Glyphosate + dicamba	Glyphosate + dicamba	99 a	99 a
		Glyphosate + dicamba	Glyphosate + dicamba	92 ab	94 ab
			Glyphosate + dicamba	71 c	85 c
Glufosinate				58 d	60 d
Glufosinate	Glufosinate			82 bc	89 bc
Glufosinate	Glufosinate	Glyphosate + dicamba		98 a	97 a

[a] Means within a column followed by the same letter are not different according to Fisher’s Protected LSD test at $p \leq 0.05$. Data are pooled over environments. Non-treated control was not included in data analysis.
[b] Abbreviations: WAP, weeks after planting.
[c]Annual grass consisted of large crabgrass, Broadleaf signalgrass, Texas panicum, and goosegrass.

Table 4. Lint yield and economic net return as affected by POST application timing.[a]

Herbicides and application timings (WAP)[b]					Economic net return[c]		
					Cotton price $ kg^{-1}		
2	3	4	5	Lint yield	1.54	1.76	1.98
				kg ha^{-1}	$ ha^{-1}		
Glufosinate	Glufosinate	Glyphosate + dicamba	Glyphosate + dicamba	780 a	-151 a	21 a	193 a
	Glufosinate	Glyphosate + dicamba	Glyphosate + dicamba	740 a	-175 a	-12 a	151 ab
		Glyphosate + dicamba	Glyphosate + dicamba	545 bc	-456 ab	-337 ab	-217 bc
			Glyphosate + dicamba	300 de	-814 cd	-746 cd	-680 de
Glufosinate				135 e	-1,088 de	-1,058 de	-1,029 ef
Glufosinate	Glufosinate			470 cd	-582 bc	-478 bc	-375 cd
Glufosinate	Glufosinate	Glyphosate + dicamba		710 ab	-231 a	-75 a	82 ab
-	-	-	-	2 f	-1,265 e	-1,265 e	-1,265 f

[a] Means within a column followed by the same letter are not different according to Fisher's Protected LSD test at $p \leq 0.05$. Data are pooled over environments.
[b] Abbreviations: WAP, weeks after planting.
[c]Cotton price based on 45% lint and 55% cottonseed.

Table 5. Palmer amaranth control 8WAP and lint yield in response to POST application timing with and without PRE herbicides.[a]

POST Herbicides and application timings (WAP)				Palmer amaranth control		Lint yield	
2	3	4	5	No PRE	PRE	No PRE	PRE
				—%—		—kg ha^{-1}—	
Glufosinate	Glufosinate	Glyphosate + dicamba	Glyphosate + dicamba	100 a	100 a	770 a	780 a
	Glufosinate	Glyphosate + dicamba	Glyphosate + dicamba	99 ab	100 a	700 ab	820 a
		Glyphosate + dicamba	Glyphosate + dicamba	91 abc	99 ab	500 bc	770 a
			Glyphosate + dicamba	69 e	96 ab	150 de	685 ab
Glufosinate				55 f	85 cd	80 e	390 cd
Glufosinate	Glufosinate			81 bc	89 bcd	310 cd	370 cd
Glufosinate	Glufosinate	Glyphosate + dicamba		99 ab	100 a	670 ab	755 a
-	-	-	-	-	79 de	14 e	225 de

[a] Means within Palmer amaranth control or lint yield followed by the same letter are not different according to Fisher's Protected LSD test at $p \leq 0.05$. Data are pooled over environments. Non-treated for Palmer amaranth control was not included in data analysis.

Table 6. Influence of POST application timing with and without PRE herbicides on economic net return.[a]

Herbicides and application timings (WAP)				Economic net return — Cotton price $ kg^{-1}					
				1.54		1.76		1.98	
2	3	4	5	No PRE	PRE	No PRE	PRE	No PRE	PRE
				$ ha^{-1}					
Glufosinate	Glufosinate	Glyphosate + dicamba	Glyphosate + dicamba	-172 a	-184 ab	-3 a	-11 ab	166 a	161 ab
	Glufosinate	Glyphosate + dicamba	Glyphosate + dicamba	-244 ab	-88 a	-91 ab	92 a	63 ab	272 a
		Glyphosate + dicamba	Glyphosate + dicamba	-529 bc	-122 a	-420 bc	48 a	-310 bc	218 a
			Glyphosate + dicamba	-1,059 def	-226 ab	-1,026 def	-75 ab	-933 def	75 ab
Glufosinate				-1,174 ef	-715 cd	-1,156 ef	-630 cd	-1,137 ef	-544 cd
Glufosinate	Glufosinate			-847 cde	-788 cde	-779 cde	-707 cd	-710 cde	-625 cd
Glufosinate	Glufosinate	Glyphosate + dicamba		-293 ab	-194 ab	-146 ab	-27 ab	2 ab	139 ab
-	-	-	-	-1,245 f	-942 def	1,240 f	-892 de	1,240 f	-843 de

[a] Means within a followed by the same letter are not different according to Fisher's Protected LSD test at $p \leq 0.05$. Data are pooled over environments.

CHAPTER 2

Change in Weed Richness and Frequency of Glyphosate-Resistance after Eight Years of Glyphosate and Dicamba in Cotton

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Abstract

Research was established in 2011 to determine the impact of glyphosate and dicamba on glyphosate-resistant (GR) Palmer amaranth populations and the frequency of GR Palmer amaranth after 8 yr of use and to determine changes in weed richness over the course of the study. During the first four years, treatments included three sequential POST applications of glyphosate with or without pendimethalin plus diuron PRE; three sequential POST applications of glyphosate plus dicamba with and without these PRE herbicides; and a POST application of glyphosate plus dicamba plus acetochlor followed by one or two POST applications of glyphosate plus dicamba without PRE herbicides. Biennial rotations of POST applications of glyphosate only and glyphosate plus dicamba POST with and without PRE herbicides were also included. Glyphosate plus dicamba was applied to the entire test area for the final 4 years of the study. Following a rapid increase in Palmer amaranth density and frequency of the resistance during the first 4 years following glyphosate only, density decreased in a similar manner during the final 4 years when glyphosate plus dicamba was applied 2 or 3 times. Frequency of GR did not decrease from the maximum level observed after the first 4 years. Weed richness did not change over the course of the study. No difference in tolerance to dicamba was observed from GR Palmer amaranth populations after 8 yr of the experiment.

Dicamba-resistant cotton cultivars have been commercialized in the United States and the adoption of varieties with this trait has been widespread in cotton. To help aid in managing herbicide-resistant (HR) weeds, such as Palmer amaranth, dicamba is being included into glyphosate and glufosinate-based herbicide systems to manage evolved resistance to glyphosate and herbicides that inhibit acetolactate synthase (ALS) in resistance plants (Cahoon et al. 2015; Merchant et al. 2013; Meyer et al. 2015). The increased use of dicamba will increase selection pressure on dominant weed populations and potentially lead weed species shifts over time (Culpepper 2006; Shaner 2000). Diversifying effective herbicide programs including rotations in HR technology can slow the evolutionary process of HR (Kruger et al. 2009; Neve et al. 2011; Norsworthy et al. 2012). In addition, diversifying cropping systems, integrating cultural or mechanical weed management practices, and reducing overall herbicide use is required to reduce the selection pressure of any one selecting agent (Boerboom 1999). To maintain this technology and management tool, weed dynamics need to be understood after long-term exposure to dicamba.

Neve et al. (2011) suggested that the Palmer amaranth seedbank was the most important factor in governing occurrence of glyphosate resistance. The timing and type of production practices implemented directly affects the soil seedbank community (Smith 2006; Sosnoskie et al. 2006). Previous research has shown that under weed-free conditions Palmer amaranth seed was reduced 98% from the soil seedbank (Menges 1987). However, more than 18 million seed ha^{-1} still remained in the soil seedbank. Burnside et al. (1986) observed that just 1 yr without weed control following 5 yr of weed-free management was sufficient to replenish the soil weed seedbank to 90% of initial levels. A better understanding of soil weed seedbank dynamics can aid in further delaying HR.

Weed species shifts often occur due to newly introduced management tools that include both chemical and nonchemical controls (Culpepper et al. 2004; Tuesca et al. 2001; Cordeau et al. 2017). Storkey and Neve (2018) suggest reductions in weed management strategy is to blame for the decrease in weed diversity and ultimately the increase in herbicide resistance weeds. Furthermore, they show an increase in yield loss as weed richness is decreased. Hauser et al. (1974) found significant weed species shifts over time in response to crop changes and associated herbicides.

To expand on previous research (Inman et al. 2015) evaluating GR Palmer amaranth exposure to dicamba would be of great value in determining utility and maintaining sustainability of herbicide programs that include dicamba. The objective of this research was to further compare Palmer amaranth population dynamics and frequency of glyphosate resistance over 8 yr with herbicide programs that included glyphosate, dicamba, and residual herbicides. Also, compare changes in weed species composition over the course of the study.

Materials and Methods

The experiment was established in two separate fields during 2011 in North Carolina at the Upper Coastal Plain Research Station located near Rocky Mount (35.893N, 77.681W). Soil was a Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiudults) with 0.5% humic matter in field 1 and an Aycock very fine sandy loam (fine-silty, siliceous, subactive, thermic Typic Paleudults) with 0.5% humic matter in field 2. Plot sizes were six rows (91-cm spacing) by 15 m in field 1 and eight rows by 11 m field 2. Both fields were naturally infested with Palmer amaranth including both glyphosate-susceptible (GS) and ALS-susceptible Palmer amaranth and

GR and ALS-resistant Palmer amaranth. The frequency of ALS resistance was approximately 30% in these field with glyphosate resistance less than 10% when the experiment was initiated. Cotton was planted in conventionally tilled, raised beds at a seeding of 14 seed m^{-1} of row. Cotton in all years was planted in the 2nd or 3rd week of May. Palmer amaranth seeds were allowed to mature before disking fields twice in late September of each year at a slow speed to minimize soil and seed movement from plot to plot. Fields received two passes parallel to rows with a disc harrow and a field cultivator, followed by subsoiling and bedding on the same day of planting. Other than treatments imposed for the experiment, cotton was managed according to North Carolina Cooperative Extension Service recommendations (Edmisten et al., 2015).

Seven herbicide treatments were compared and herbicides were applied according to the manufacturer’s suggested use rate (Tables 1 and 2). Preemergence herbicides were applied immediately after planting, with POST herbicides applied 2 (POST-1), 4 (POST-2), and 6 (POST-3) wk after planting. Five of the treatments were maintained in the same plots for the duration of the experiment (2011 through 2018). These treatments included diuron plus pendimethalin PRE or no PRE herbicides followed by (fb) three POST applications of glyphosate or three POST applications of glyphosate plus dicamba and glyphosate plus dicamba plus acetochlor applied POST-1 fb glyphosate plus dicamba applied POST-2 and POST-3. Two additional herbicide programs included alternating years between glyphosate plus dicamba and glyphosate only at all three POST timings with or without diuron plus pendimethalin PRE in all 4 yr. Herbicides were applied using a CO_2-pressurized backpack sprayer equipped with flat-fan nozzles (AIXR 11002 TeeJetH Air Induction XR flat-spray nozzles, TeeJet Technologies, Wheaton, IL) calibrated to deliver 140 L ha^{-1} at 152 kPa.

During each year and prior to PRE herbicide applications from 2011-2014, weed density for each species were determined by collecting 10 soil cores (10.2 cm by 7.6 cm) for a total volume of 4,630 ml from each plot. Soil was placed in flats to a depth of 4 cm with a total surface area of 1,550 cm^2 and place in a greenhouse. The greenhouse was climate controlled for favorable conditions conducive for optimum weed seed germination (33 to 40 C at 80 to 90% relative humidity). Soil was irrigated with overhead sprinklers to promote germination of seed and adequate growth of seedlings. Approximately 3 wk after weed seedling establishment, density of emerged weeds was recorded. Plants were then treated with glyphosate potassium salt at 946 g ae ha^{-1} to determine the frequency of GR Palmer amaranth. The percentage of plants surviving glyphosate was determined in May of 2019, at the end of the study, plants surviving glyphosate were treated with dicamba at 560 g ae ha^{-1} to determine if there were any biotypes tolerant to dicamba after 8 yr of use. Seeds of a known dicamba-susceptible Palmer amaranth population were included as a control.

The experimental design was a randomized complete block and treatments were replicated four times in both fields. Statistical analyses were performed using the PROC Mixed procedure in SAS (v. 9.4; SAS Institute, Cary, NC). Treatments were considered a fixed factor, and replication and field were considered random factors. Treatment interactions containing replication or field were set as random effects (Blouin et. al 2011). Data for Palmer amaranth density from soil cores were converted to the common log before analysis. Data for these treatments were also subjected to regression procedures using the PROC REG (SAS Version 9.4; SAS Institute Inc., Cary, NC) procedure of SAS testing for linear, quadratic, and cubic functions for common log of Palmer amaranth density and frequency of glyphosate resistance vs. months after experiment initiation, as well as standard errors of each data point. Type III statistics were used to test all fixed effects, and least square means were calculated based on $P \leq 0.05$ (Moore and Dixon 2015).

Results and Discussion

Palmer amaranth population

The interaction of field by herbicide treatment for Palmer amaranth density in soil cores was not significant for any year during the course of the study, therefore, data were pooled for analysis (Table 3).

Greater detail on results from the first 4 yr of the experiment can be found elsewhere (Inman et al. 2015). There was no difference in Palmer amaranth density from soil cores in 2011 (Figure 1). However, after 4 yr of glyphosate only POST, Palmer amaranth density increased from 32 to 247 plants. The addition of PRE herbicides helped maintain lower Palmer amaranth densities when compared to glyphosate only POST. Treatments where glyphosate plus dicamba were included every year, Palmer amaranth densities were reduced over the course of the experiment. Alternating years of glyphosate and glyphosate plus dicamba resulted in a similar trend compared to glyphosate only with PRE herbicides. When PRE herbicides were used with the alternating years of glyphosate and glyphosate plus dicamba, lower Palmer amaranth densities were maintained compared to the absence of PRE herbicides. By the end of the experiment, there was no difference in Palmer amaranth densities across treatments. After 4 yr of timely glyphosate plus dicamba POST applications, a significant reduction in the soil weed-seed bank was observed. Modeling work by Diggle et al. (2003) predicted that herbicide tank mixtures could slow the rate of HR evolution more so compared to annual herbicide rotations.

Frequency of resistance

The interaction of field by herbicide treatment for the frequency of GR Palmer amaranth was not significant for any year during the course of the study, therefore, data were pooled for analysis (Table 3).

At experiment initiation, there was no difference in the frequency of GR Palmer amaranth ranging from 1 to 9% (Figure 2). After 1 yr, the frequency of GR Palmer amaranth was greatest in the glyphosate only treatment without PRE herbicides. After 4 yr, there was no difference in GR frequency among all treatments. Shergill et al. (2017) reported evolution of GR giant ragweed in a susceptible population after 4 yr of continuous glyphosate only use. In this study, the use of continuous glyphosate plus dicamba resulted in a reduction in Palmer amaranth and a lower contribution of GR seed to the soil seedbank. However, after 8 yr, no difference GR frequency was observed. This most likely resulted from GR pollen movement from adjacent plots and surrounding fields. It has been documented that GR Palmer amaranth pollen disperses up to 300 m under normal field conditions Sosnoskie et al. (2012). Furthermore, as stated by Jasieniuk et al. (1996), it is unlikely that resistance evolution is significantly delayed by the introduction of gene flow from susceptible biotypes to resistant populations. Even though care was taken to minimize weed seed movement across the fields, weed seed from surrounding weed management studies and likely contributed to the soil seed bank during field preparation each year. At the end if the study, there were no survivors from GR Palmer amaranth populations treated with a field rate (560 g ae ha^{-1}) of dicamba.

There are limitations of this experiment in determining the rate at which enhanced tolerance or evolved resistance to dicamba might be expected. Total plot area for the experiment was one ha when including both fields. Also, near complete control was obtained each yr with little to no contribution of seed to the seedbank from surviving plants. This would result in an unlikely increase in enhanced dicamba tolerance in the population if non-target site resistance is the primary mechanism to the herbicide in Palmer amaranth. The surface area of soils was used to estimate the number of plants that may have been exposed to the glyphosate plus dicamba over the 8 yr. The average density for each yr was summed over the 8 yr to calculate this estimate. Approximately 12 million plants may have been exposed to the glyphosate plus dicamba over this time period. This estimate also has limitations as it likely reflects only those weeds emerging from the additional flush in the field. There was likely considerably more seed in the soil cores that may have resulted in emergence if the procedure involved mixing soil in flats and allowing a second or third flush to emerge. If target site resistance is the primary mechanism of resistance, it would be expected that one or more individuals would be selected for in these fields if the frequency of dicamba resistance caused by a target site mutation was similar to that of other herbicides. Tehranchian et al. (2017) suggested that tolerance of Palmer amaranth to dicamba occurs after three generations of repeated exposure at sub-lethal rates under greenhouse conditions. The mechanism of action of dicamba in sensitive plants continues to be elusive. While the overall mode of action is known and is the result of unregulated auxin activity and over exposure of plant hormones such as abscisic acid (Grossman, 2009)

Weed richness

Weed diversity and densities of all species were observed and recorded from soil core grow outs immediately after planting each year (Table 5). No differences in weed richness were associated ($P = 0.5451$; $P = 0.2282$) with the interaction of year by treatment or the main effect of treatment, respectively. Previous research has shown greater species richness where mechanical disturbance was minimal (Sosnoskie et al. 2006). Other studies have shown weed densities are reduced when tillage intensity is increased (Anderson et al. 1998; Barberi and Lo Cascio 2001). Lack of differences are likely attributed to historical similarities of cropping systems and production practices in these fields. Conventional cotton, peanut, and soybean systems have traditionally been grown in these fields and fields are exposed to great soil disturbance from yearly tillage, disking, and bedding and, in general, similar herbicide regimes as well. Weed species richness ranged from 1 to 9 species across all years. Palmer amaranth was the dominant weed at the beginning of the experiment. Due to the selection pressure from the glyphosate only treatments, GR Palmer populations increased to higher levels and in some plots outcompeting other weed species. Overall, no consistent pattern of variation in species richness was observed across site years or treatments. There were no differences in survival of weed species other than Palmer amaranth when glyphosate was applied. Although beyond the scope of this research, these data suggest that selection of glyphosate resistance occurs more rapidly in Palmer amaranth than in the other species present in these fields. Evolved resistance to glyphosate in common ragweed and goosegrass has been reported (Heap, 2019).

This study further demonstrates the prolific growth and reproductive ability of Palmer amaranth when exposed to poor control strategies. The addition of PRE herbicides with glyphosate only decreased the rate of increase of Palmer amaranth, however, were ineffective in managing GS and GR Palmer amaranth over a 4 yr period. Similar trends were observed with biennial rotations of glyphosate and glyphosate plus dicamba over 4 yr. The addition of PRE herbicides were more effective than without, although were ineffective compared to dicamba being applied every year. Powles et al. (1997) showed that a mixture of two different herbicide MOA’s would delay herbicide resistance longer compared to rotating MOA. Over the course of the study, Palmer amaranth densities declined when dicamba was included during each year.

When glyphosate plus dicamba were applied to all plots for the last 4 yr, similar Palmer amaranth densities across all treatments were observed by the end of the experiment. Previous research has shown that dicamba controls GR Palmer amaranth (Cahoon et al. 2015; Merchant et al. 2013; Vann et al. 2017a; Vann et al. 2017b). However, this study shows the effectiveness of dicamba in decreasing dense populations of GR Palmer amaranth over the course of several yr. Furthermore, these data are consistent with other research that show the rapid increase in the frequency of GR when glyphosate is used alone (Culpepper et al. 2006; Shergill et al. 2017). As observed with the first 4 yr of this study, weed seed production is critical for rapid evolution of GR Palmer amaranth. To date, research has shown a lack of fitness penalties with GR Palmer amaranth compared to GS, therefore, there is little delay in the buildup of glyphosate-resistant individuals (Jasieniuk et al. 1996). These data show that growth in the frequency of GR in a population is not easily eliminated.

Although dicamba has shown to be an effective tool in managing GR Palmer amaranth in cotton over 8 continuous years, diligent stewardship of dicamba should be priority. Residual herbicides should be incorporated throughout the spraying season and POST only herbicide programs should be avoided. Dicamba resistance has been reported in Palmer amaranth after three generations of exposure to sub-lethal rates (Tehranchian et al. 2017). At the end of this study, no tolerance was found among any GR Palmer populations after 8 yr of dicamba. Very few, if any, weed escapes were allowed to grow and contribute to the soil weed seedbank. Diverse weed control and production practices are necessary in delaying the evolution of resistance (Norsworthy et al 2012).

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Table 1. Herbicide active ingredient, trade name, formulation, application rate, and manufacturer.[a]

Herbicide active ingredient	Trade name[a]	Formulation concentration	Application rate	Manufacturer
acetochlor	Warrant®	359 g ai L^{-1}	1260 g ai ha^{-1}	Monsanto Co., St. Louis, MO
dicamba diglycolamine salt	Clarity®	480 g ae L^{-1}	560 g ae ha^{-1}	BASF Ag Products, Research Triangle Park, NC
dicamba N, N-Bis-(3-aminopropyl)methylamine salt	Engenia®	600 g ae L^{-1}	560 g ae ha^{-1}	BASF Ag Products, Research Triangle Park, NC
diuron	Direx® 4L	480 g ai L^{-1}	840 g ai ha^{-1}	Makhtesshim Agan of North America, Raleigh, NC
glyphosate potassium salt	Roundup WeatherMAX®	540 g ae L^{-1}	946 g ae ha^{-1}	Monsanto Co.
pendimethalin	Prowl® H_2O	452 g ai L^{-1}	1065 g ai ha^{-1}	BASF Ag Products

[a] Clarity was used during 2011 to 2015. Engenia was used during 2016 to 18.

Table 2. PRE and POST herbicide treatments for years 2011-2014.[a]

Herbicide Treatments				
PRE	POST-1	POST-2	POST-3	Years
None	glyphosate	glyphosate	glyphosate	2011-2014
None	glyphosate plus dicamba	glyphosate plus dicamba	glyphosate plus dicamba	2011-2014
Pendimethalin plus diuron	glyphosate	glyphosate	glyphosate	2011-2014
Pendimethalin plus diuron	glyphosate plus dicamba	glyphosate plus dicamba	glyphosate plus dicamba	2011-2014
None	glyphosate plus dicamba plus acetochlor	glyphosate plus dicamba	glyphosate plus dicamba	2011-2014
None	glyphosate	glyphosate	glyphosate	2011 and 2013
	glyphosate plus dicamba	glyphosate plus dicamba	glyphosate plus dicamba	2012 and 2014
Pendimethalin plus diuron	glyphosate	glyphosate	glyphosate	2011 and 2013
	glyphosate plus dicamba	glyphosate plus dicamba	glyphosate plus dicamba	2012 and 2014

[a]All plots received glyphosate plus dicamba POST for remainder of study (2015 to 2018).

Table 3. Probability values for Palmer amaranth soil core density as influenced by herbicide treatment[a]

Source of variation	2011	2012	2013	2014	2015	2016	2017	2018	2019
Field	0.1562	0.7279	0.7441	0.5801	0.8781	0.2899	0.7440	0.2428	0.2563
Herbicide	0.6250	0.0116	<.0001	0.0005	0.0032	0.0002	0.0273	0.2631	0.4481
Field*Herbicide	0.1393	0.3466	0.8365	0.3559	0.7427	0.9665	0.3101	0.6970	0.2820

[a]Significance at $p \leq 0.05$ for means within a treatment factor.

Table 4. Probability values for the frequency of glyphosate-resistant (GR) Palmer amaranth as influenced by herbicide treatment[a]

Source of variation	2011	2012	2013	2014	2015[b]	2016	2017	2018	2019
Field	0.6781	0.2905	0.8022	0.5474	-	0.9116	0.8922	0.7939	0.3614
Herbicide	0.5601	0.0472	0.2091	0.3836	0.2258	0.8080	0.8399	0.8176	0.9684
Field*Herbicide	0.3528	0.1950	0.2686	0.6625	-	0.2386	0.1161	0.1450	0.2204

[a]Significance at $p \leq 0.05$ for means within a treatment factor.

[b]Frequency of GR Palmer amaranth data available for one field only in 2015.

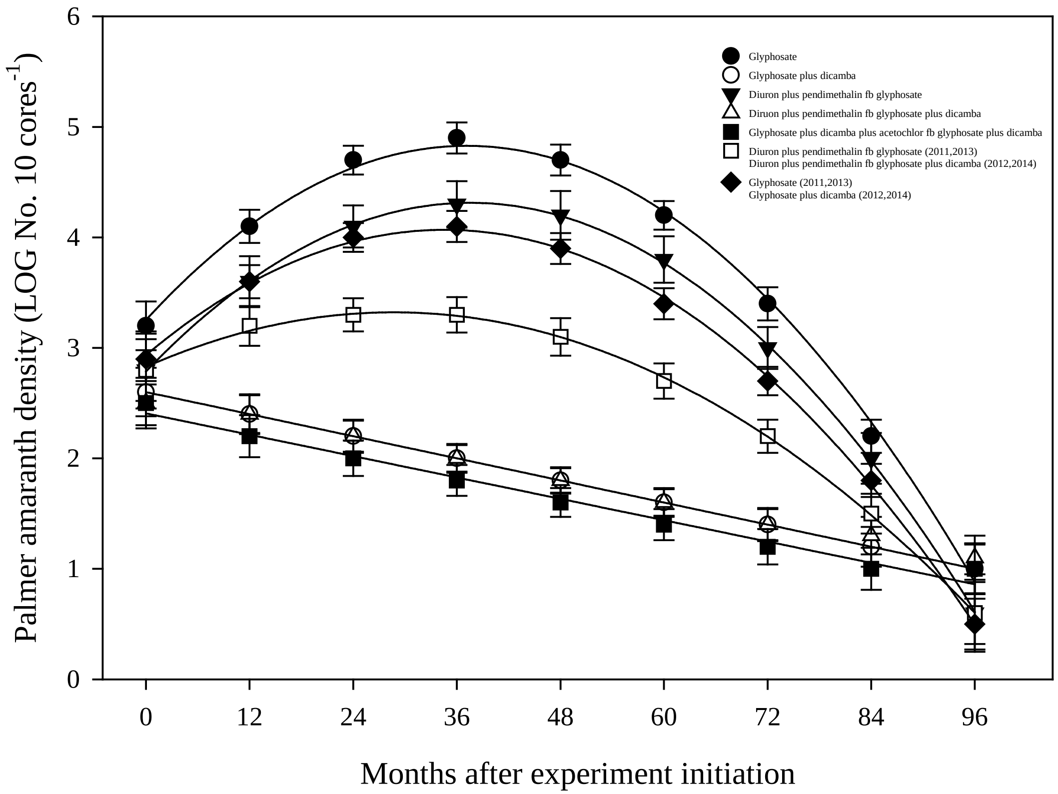

Figure 1. Density of Palmer amaranth from soil cores. Regression equations: glyphosate, $y = 3.2 + 0.89x – 0.0012x^2$; P = <.0001; $r^2 = 0.74$; glyphosate plus dicamba, $y = 2.63 – 0.017x$; P = <.0001; $r^2 = 0.22$; diuron plus pendemthalin fb glyphosate, $y = 2.77 + 0.083x – 0.0011x^2$; P = <.0001; $r^2 = 0.48$; diuron plus pendemthalin fb glyphosate plus dicamba, $y = 2.54 – 0.015x$; P = <.0001; $r^2 = 0.22$; glyphosate plus dicamba plus acetochlor fb glyphosate plus dicamba, $y = 2.45 – 0.017x$; P = <.0001; $r^2 = 0.20$; diuron plus pendemthalin fb glyphosate (2011, 2013), diuron plus pendemthalin fb glyphosate plus dicamba (2012, 2014), $y = 2.82 + 0.035x – 0.00061x^2$; P = <.0001; $r^2 = 0.46$; glyphosate (2011, 2013), glyphosate plus dicamba (2012, 2014), $y = 2.95 + 0.064x – 0.00094x^2$; P = <.0001; $r^2 = 0.66$. Error bars represent standard error for each data point.

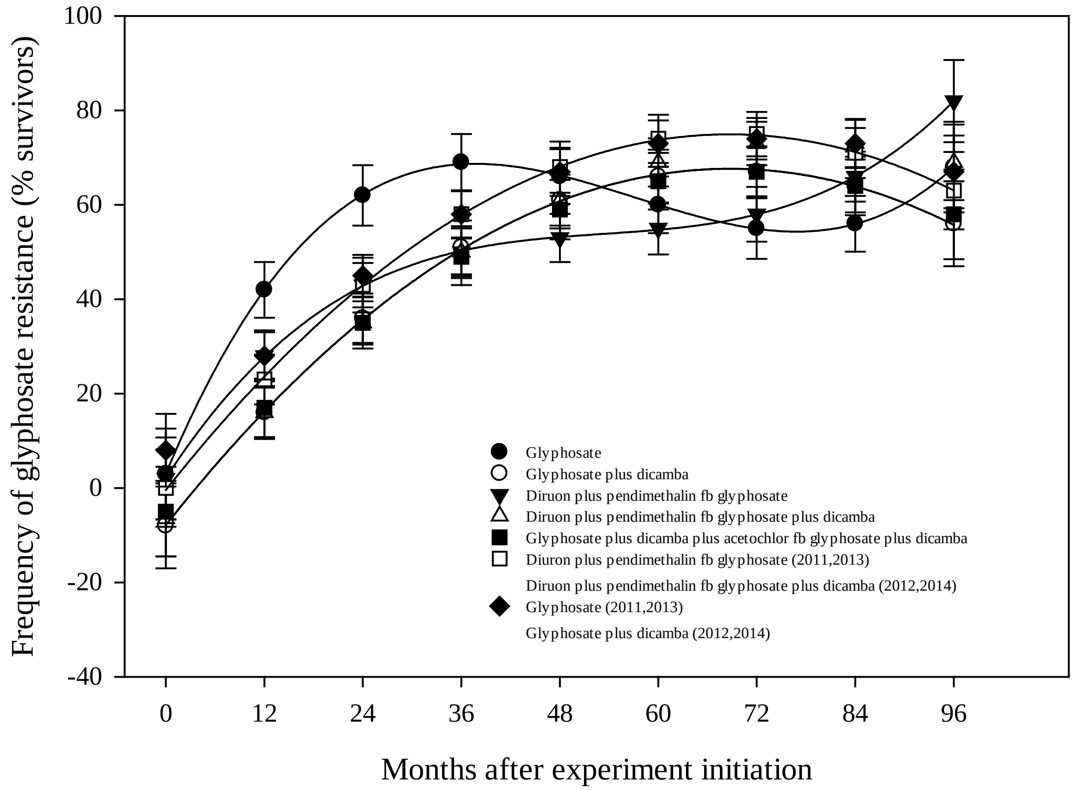

Figure 2. Frequency of glyphosate resistance of Palmer amaranth from soil cores. Regression equations: glyphosate, $y = 2.79 + 4.16x – 0.082x^2 + 0.00048x^3$; P = 0.0036; $r^2 = 0.33$; glyphosate plus dicamba, $y = -7.78 + 2.19x – 0.016x^2$; P = 0.0008; r^2= 0.40; diuron plus pendemthalin fb glyphosate, $y = 2.12 + 2.65x – 0.047x^2 + 0.00029x^3$; P = 0.0447; $r^2 = 0.43$; diuron plus pendemthalin fb glyphosate plus dicamba $y = -6.59 + 2.04x – 0.013x^2$; P = 0.0022; $r^2 = 0.51$; glyphosate plus dicamba plus acetochlor fb glyphosate plus dicamba, $y = -4.99 + 2.03x – 0.014x^2$; P = 0.0039; r^2= 0.36; diuron plus pendemthalin fb glyphosate (2011, 2013), diuron plus pendemthalin fb glyphosate plus dicamba (2012, 2014), $y = -0.75 + 2.21x – 0.016x^2$; P = 0.0002; r^2 = 0.45; glyphosate (2011, 2013), glyphosate plus dicamba (2012, 2014), $y = 7.66 + 1.85x – 0.012x^2$; P = 0.0017; r^2 = 0.43. Error bars represent standard error for each data point.

CHAPTER 3

Evaluation of Dicamba Retention in Spray Tanks and the Impact to Flue-Cured Tobacco

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Abstract

In recent years, there has been an increased use of dicamba due to the introduction of dicamba-tolerant cotton and soybean. This provides greater opportunity for off-target movement of dicamba. Flue-cured tobacco is known to be extremely sensitive to auxin herbicides; particularly dicamba. In addition to yield loss, residue from drift or equipment contamination can have severe repercussions for the marketability of the crop. Dicamba, and other auxin herbicides, are known to readily adhere to spray equipment. Studies were conducted to evaluate spray tank cleanout efficiency using various cleaning procedures. No difference in dicamba recovery was observed regardless of dicamba formulation and cleaning agent. Dicamba residue decreased with the number of rinses. There was no difference in dicamba residue recovered from the third rinse compared to residue from the tank after being refilled for subsequent tank use. Recovery ranged from 2 to 19% of the original mix rate among the three rinses. Field studies were also conducted to evaluate flue-cured tobacco response to reduced rates of dicamba ranging from 1/5th to 1/10,000th of a labeled rate. Injury and yield reductions varied by environment. In general, dicamba was more injurious when applied immediately after layby (7 WAT) compared to flowering (11 WAT). Greater injury resulted in greater yield reduction; however, plant response was largely determined by individual growing environment. Injury ranged from 0 to 100% depending on rate, application timing, and environment. The maximum yield reduction was 62% with a 55% reduction in value. Correlations show significant relationships between visual injury and yield and value reductions with Pearson values ranging from 0.24 to 0.63. These data can provide guidance to growers and stakeholders emphasizing the need for diligent stewardship when utilizing dicamba technology especially in proximity of sensitive crops.

In efforts to create new management options for herbicide-resistant weeds, dicamba-tolerant cotton and soybean have been developed. The adoption of this technology and the increased use of dicamba has greatly expanded the use pattern of this herbicide, resulting in major concerns of injury potential and damage of non-tolerant varieties and sensitive crops from off-target movement (Gordon et al. 2018). In general, dicamba can move off-target due to particle drift, volatilization, or through contamination from spray equipment (Behrens and Lueschen 1979; Egan et al. 2014; Mortensen et al. 2012). Many environmental factors, including temperature, humidity, and wind, can dictate the movement of spray droplets or volatility of an herbicide; including dicamba (Strachan et al. 2013). Auxin herbicides have shown to be difficult to remove from the plastic and rubber parts that make up commercial spray equipment (Steckel et al. 2005). While evaluating dicamba retention of different sprayer hose types, Cundiff et al. (2017) reported a 7 to 19% yield reduction in soybean depending on hose type. Boerboom (2004) reported 0.024 and 0.63% dicamba residue of the original mix rate from a spray tank and boom, respectively, following a standard cleanout procedure that included an ammonia water solution.

Low-doses of synthetic auxins have shown to be injurious to numerous crops including soybean (*Glycine max* L.) (Solomon and Bradley, 2014), cotton (*Gossypium hirsutum*) (Johnson et al. 2012), tomato (*Solanum lycopersicum*) (Bauerle MJ et al. 2015), and watermelon (*Citrullus lanatus*) (Culepper et al. 2018). Tobacco has shown sensitivity to auxin herbicides from foliar and soil exposure. Typical symptomology of tobacco is described as downward cupping of leaves, epinastic leaf growth, reduced leaf expansion with loss of apical dominance and abortion of meristem in severe cases (Fung et al. 1973; Sheets and Worsham 1991). In a simulated drift study, Lewis et al. (2011) reported visual injury in flue-cured tobacco of up to 32% 12 weeks after transplanting (WAT) from 0.31 g ae ha^{-1} (1/1,000th labeled rate) of aminocyclopyrachlor.

Klingman and Guedez (1967) reported no reduction in yield at one location and a 57% reduction in yield at another when picloram was applied pre-transplant incorporated (PTI) at 1.2 g ai ha^{-1}. Although minimal, visual symptoms were observed when 0.025 g ha^{-1} of picloram and 6.4 g ha^{-1} of dicamba were applied PTI (Sheets and Worsham, 1991). Depending on environment, visual injury 2 weeks after application (WAA) ranged from <5 to 40% and 10 to 95% when dicamba was applied 6 WAT at 0.6 and 11 g ae ha^{-1} (Johnson, 2011).

North Carolina is the number one producer of flue-cured tobacco (*Nicotiana tabacum L.*) in the United States. In 2017, North Carolina growers harvested 65,990 ha generating over $720 million in production value, making it the most economically important crop in the state (NCDA stats, 2018). In tobacco producing regions, soybean and cotton are regularly grown and often used as rotational crops. In 2018, approximately 55 and 56% of soybean and cotton varieties planted in North Carolina were dicamba-tolerant varieties, respectively (RA Vann, personal communication; USDA, 2018). With trends in adoption rates associated with new technology, it is likely dicamba-tolerant varieties will further increase in coming years (Mortensen et al. 2012). Depending on the part of the state and tobacco planting dates, dicamba applications in cotton and soybean can occur during all aspects of tobacco plant development. The increased use of dicamba has great potential to affect the growth, development, and marketability of tobacco grown in close proximity to fields where soybean and cotton are simultaneously growing. Tobacco exhibiting visual injury symptoms from non-labeled pesticides can be rendered non-marketable by the tobacco industry. Furthermore, tobacco-farming operations using dicamba for dicamba-tolerant soybean and cotton varieties are at greater risk for equipment contamination. However, little data exists on the exposure of flue-cured tobacco to off-target dicamba applications. The objective of this study was to document the contamination potential of dicamba in a spray tank following various rinse procedures. In addition, evaluate the response of flue-cured tobacco to a single, foliar exposure event of dicamba applied early or late in the growing season.

Materials and Methods

Sprayer tank cleanout procedure

Tank cleanouts were performed in 2016, 2017, and 2018 to compare tank cleanout procedures and efficiency after the addition of the suggested use rate of three formulations of dicamba. Two and a half gallon polyethylene vessels (ULINE, Braselton, GA) consistent with commercial sprayer equipment were utilized to evaluate each herbicide cleanout procedure. Dicamba products used were dimethylamine salt (Rifle, Loveland Products Inc., Greeley, Colorado), diglycolamine salt (Xtendimax, Monsanto Company, St. Louis, MO, and *N*, *N*-Bis-(aminopropyl) methylamine salt (Engenia, BASF, Research Triangle Park, NC). Cleaning agents consisted of water only, ammonia, a commercial tank cleaner (All Clear, Loveland Products, Greeley, CO), or no rinse. At the experiment initiation, each spray tank was contaminated with 560 g ai ha^{-1} of dicamba as a 140 L ha^{-1} solution in a 7.57 L mix, agitated for approximately 15 seconds, and allowed to sit for 24 hours. After the incubation period, a 20 ml sample was collected from each tank to ensure contamination rates were similar. Each spray tank then underwent a triple rinse cleanout procedure with water. Tank cleaners were added with the second rinse cycle among treatments including ammonia or tank cleaner. Rinse times were approximately 15 seconds each and rinse volume was 10% of the original tank mix. Following each rinse, a 20 mL sample was collected and analyzed to quantify dicamba residue. Simulating sprayer equipment use and cleanout, or lack thereof, following each herbicide, each tank was filled with water to a volume of 7.57 L when a sample was collected after the triple rinse procedure. Dicamba residue was quantified using high performance liquid chromatography–diode array detector (HPLC-DAD) instrumentation (Agilent-1260 Infinity; Agilent Technologies, Inc., Wilmington, DE) by the Pesticide and Trace Element Environmental Fate and Behavior Lab at North Carolina State University. All reagents and solvents used for extraction and residue analysis were HPLC-grade. Dicamba residue sample preparation analysis was conducted as described by Fogarty et al. (1994). Prior to injection samples were centrifuged for 15 min at 3,500 rpm and then filtered using 0.45 µm nylon membrane. Analyte concentrations were quantified using peak area measurements (OpenLAB CDS CHemStation, version C.01.04; Agilent Technologies Inc., Wilmington, DE), and concentrations above the calibration curve were diluted and re-injected for analysis. Limits of quantification and detection were 0.156 and 0.05 mg L^{-1} (ppm), respectively. Standard solutions were included with each injection. Fifteen treatments were evaluated for each herbicide. Treatments included: no rinse, one rinse, two rinse, three rinse, and a refill with each of the three cleaning agents. Three replications for each treatment were used in the experiment, with two runs per herbicide.

Field experiment

Field studies were conducted in North Carolina at Oxford (36.3115N, -78.6155W), Kinston (35.3019N, -77.5729W), and Whiteville (34.4153N, -78.7892W) in 2018. Soil types were a Helena sandy loam (fine, mixed, semi-active, thermic Aquic Hapludults), a Norfolk sandy loam (fine-loamy, kaolinitic, thermic Typic Kandiudults), and a Wagram loamy fine sand (loamy, kaolinitic, thermic Arenic Kandiudults) at those respective sites. Soil pH at all field sites ranged from 5.8 to 6.2 with < 1% organic matter. Standard field preparation was performed prior to planting each year. Test sites were plowed, disked, and bedded approximately three weeks prior to transplanting. Tobacco was transplanted on May 14, April 30, and April 24 at Oxford, Kinston, and Whiteville, respectively. Individual plots contained three rows, each 3.4 m wide by 13.7 m long in Kinston and 3.6 m wide by 13.7 m at the other two locations. The center row of each plot was treated and used for data collection and harvest. Planting density in all environments was 14,820 plants ha^{-1}. The flue-cured tobacco cultivar NC 196 (Gold Leaf Seed Company, Hartsville, SC) was produced at the all locations. Tobacco at all sites was produced according to North Carolina Cooperative Extension Service recommendations throughout the duration of the study (Fisher, 2018).

Dicamba was applied at 0.056, 0.112, 0.224, 0.56, 1.12, 2.24, 5.6, 28, 112 g ae ha^{-1}. Rates coincide with 1/5th to 1/10,000th of a labeled rate (560 g ae ha^{-1}) and were derived from the tank cleanout data. A non-treated check was also included. All herbicide rates were applied POST-over-the-top at 7 or 11 WAT. Application timings were chosen to represent potential timings of POST applications of dicamba in dicamba-tolerant soybean or cotton. All herbicide applications were applied using CO_2-pressurized backpack sprayers equipped with TTI 110025 Turbo TeeJet® Induction nozzles (TeeJet Technologies, Wheaton, IL) delivering 140 L ha^{-1} at 165 kPa.

The experimental design was a randomized complete block design with a factorial arrangement with three or four replications, depending on growing environment. Visual estimates of crop injury were recorded at 7, 14, and 24 DAA (days after application) for each application on a 0 to 100% scale (0%, no visible injury; 100%, complete plant death) derived from Lewis et al. (2011). Plots were harvested four times in each growing environment and leaves were cured in a forced-air bulk-curing barn. Cured-leaf was then weighed to quantify yield and assigned a USDA government grade. Each government grade is associated with a numerical grade index value ranging from 1 to 100, which describes leaf maturity and ripeness (Bowman et al. 1988) as well as an associated financial value that reflects modern price indices (Fisher et al. 2018). Fifty-gram composite cured leaf samples were also collected from each treatment for analysis of percentage total alkaloids and percentage reducing sugars using the methods outlined by Davis (1976).

Data for both the tank cleanout and field studies were Treatments were considered a fixed factor, and replication and environment were considered random factors when appropriate. Treatment interactions containing replication or environment were set as random effects. Treatment means were reported using least square means. Means were separated using Fisher's Protected LSD at $P \leq 0.05$. Field study data were further evaluated using linear and non-linear regression models to determine relationships between herbicides rates and application timings. The linear regression analysis were chosen to describe variable response and a correlation analysis was conducted to determine relationship between tobacco injury and yield components.

Results and Discussion

Sprayer tank cleanout

No significant interactions for dicamba formulation, number of rinses, or cleaning agent were observed when evaluating sprayer tank cleanout efficiency (Table 1). Additionally, the main effect of formulation and cleaning agent did not modify dicamba retention in a sprayer tank. Therefore, data were analyzed across formulation and cleaning agent and the main effect of rinse number is presented.

Dicamba was recovered from all three rinses as well as from the refill treatment (Table 2). Dicamba retention decreased with each rinse, however, there was no difference between the third rinse and subsequent tank use. A single rinse removed approximately 80% of the original concentration. In contrast, Osborne et al. (2015) reported in all but three samples of dicamba and 2,4-D, 90 to 95% of the pre-rinse solution was removed from a single rinse. However, by the third rinse > 95% of dicamba was removed relative to the initial concentration; similar to results of this study. Boerboom (2004) reported recovery of 0.021% of the original concentration of dicamba in subsequent tank use following standard cleanout procedure. In our study, dicamba recovered from a similar treatment was found to be 0.006% of the original concentration. Differences across studies show the extreme variability that can occur during cleanout procedures and among various equipment type. Recovered amounts were minute; however, small amounts have shown to be injurious to sensitive crops (Bauerle MJ et al. 2015; Culepper et al. 2018; Johnson et al. 2012; Solomon and Bradley, 2014).

Field experiment

The interaction of environment by rate by timing was significant (P = <.0001) for visual injury 7, 14 and 24 DAA. Additionally, the rate by timing interaction was significant for all environments (P = <.0001); therefore, this interaction is presented for each individual environment. Significant linear regressions were noted for all evaluation dates for both application timings across all three environments (Figures 1, 2, 3).

At Oxford, visual injury ratings ranged from 4 to 98% and 0 to 40% with early and late applications, respectively (Figure 1). On average, the early application was more injurious across all rates when compared to the late application. Rates of 1.45 LOG g ae ha^{-1} ($1/20^{th}$X) and higher applied early caused severe growth reduction and complete reduction of lateral leaf expansion in the upper stem region. By 24 DAA, visual injury increased to approximately 22% in the lowest evaluated rate (1/10,000thX). With the late application, visual injury was $\leq$ 33% in the highest rate across the three evaluation dates. Minimal differences were observed with rates of -0.65 LOG g ae ha^{-1} (1/2,500thX) and lower when applied at the late application timing. At Kinston, visual injury ranged from 0 to 100% and 0 to 51% with the early and late application timings, respectively (Figure 2). The lowest rate, -1.25 LOG g ae ha^{-1} (1/10,000thX), produced 13% visual injury with the early application. The highest two rates (1.45 and 2.05 LOG g ae ha^{-1}) resulted in severe growth reduction, abortion of the meristem, and plant death in some cases. Rates higher than 0.05 LOG g ae ha^{-1} (1/500thX) were required to produce > 10% visual injury within the late application. Across all three evaluation dates, minimal differences were noted within each rate with the late application. This can be attributed to the overall advanced maturity of the crop at the time of the late application. At Whiteville, injury ranged from 0 to 97% and 0 to 66% with the early and late application, respectively (Figure 3). In contrast to the other environments, greatest injury symptoms from the early application were achieved 14 DAA compared to 24DAA across all rates. Growing conditions were optimal in this environment, resulting in rapidly growing plants. Abortion of the meristem was noted with rates of 1.45 LOG g ae ha^{-1} (1/20thX) and higher. Differences in visual injury across environments are not uncommon. Johnson (2011) reported visual injury on tobacco ranging from 10 to 90% within the same rate of dicamba across four environments.

A significant environment by rate by timing interaction (P = 0.0155) was observed for percent yield reduction. At Oxford and Kinston, percent yield reductions were influenced by the rate and timing of application interaction (P = 0.0185 and <.0001), respectively. At Whiteville, the rate by timing interaction was not significant (P = 0.4460) and percent yield reductions were only influenced by the main effect of rate (P = 0.0276).

At Oxford, significant linear regressions were noted for both application timings (Figure 4), with all dicamba rates causing significant yield reductions. The highest rate caused a 47 and 28% yield reduction when applied at the early and late timings, respectively. Rates of -0.65 LOG g ae ha^{-1} (1/2,500thX) and lower provided similar reductions (15 and 19%) in yield across both application timings. There was a 16% difference between the highest and lowest rate in yield reductions with the later application timing. At Kinston, a significant linear regression was noted for the early application (Figure 5). A 62% yield reduction was observed with the highest rate in this environment. Yield reductions were less than 10% with rates of -0.95 and -1.25 LOG g ae ha^{-1} (1/5,000th and 1/10,000thX). On average, there was an approximate 23% yield reduction across all rates with the late application; with no differences among rates. At Whiteville, percent yield reduction was not affected by timing of dicamba application (Figure 6). Significant yield reductions were observed across all rates ranging from 11 to 28%. There were no differences with rates lower than -0.65 LOG g ae ha^{-1} (1/2,500thX). Favorable growing conditions at this environment allowed for plant recovery compared to the other environments. Although injury trends were similar with higher rates across environments, there was wide variability in how the observed injury translated into yield reductions.

The interaction of environment by rate by timing was significant (P = 0.0019) for percent value reduction. Although the rate and timing interaction was significant (P = 0.0153) at Oxford, trends among application timing were similar (Figure 7). For both timings, as herbicide rate increased, percent value reduction increased. Maximum value reductions were 40 and 36% for the early and late timing, respectively. There was no difference between timings among any rate.

Similarly the interaction of rate and timing (P = 0.0007) influenced value reduction at Kinston (Figure 8). With the early application, value reductions ranged from 11 to 59% linearly increasing as herbicide rate increased. Similar to yield, there were no differences in value reductions across rates with the late application. At this environment, plants matured quicker compared to the other environments due to significant wind and rain events. Plants were near or at physiological maturity and plant growth had ceased prior to the late application. At Whiteville, neither the interaction (P = 0.3909) or main effects of herbicide rate (P = 0.0640) or application timing (P = 0.7508) were significant. However, a significant linear regression was noted for value reduction (Figure 9). Value reductions ranged from 12 to 34%. Previous research shows no difference in value when dicamba was applied three to seven days prior to harvest at 224 and 448 g ai ha^{-1} (Seltmann et al. 1989).

Cured-leaf quality was not affected by the interaction (P = 0.2774) or main effects of herbicide rate (P = 0.2974) and application timing (P = 0.1512) (data not shown). Johnson (2011) reported a reduction in quality in two of four environments with the highest rate of dicamba (140 g ae ha^{-1}) only. Seltmann et al. (1989) reported no reductions in cured-leaf quality when dicamba was applied to plants three to seven days before harvest at 224 or 448 g ae ha^{-1}. Total alkaloids were slightly increased with the early application compared to the late application at Whiteville and Oxford (Table 3). No difference in total alkaloids were observed at Kinston. Previous work has shown an increase in nicotine content when an auxin herbicide was applied to tobacco compared to the non-treated (LR Fisher, personal communication). However, many factors can influence nicotine production including environmental conditions, management practices, and overall plant stress (Bush, 1999). These data show maximum nicotine production can be obtained in more favorable environments. Reducing sugars were not altered regardless of herbicide rate, timing or environment.

The correlation analysis confirmed significance between visual injury and percent yield reduction (Table 4). Pearson’s correlation coefficients ranged from 0.28 to 0.63 depending on application timing and injury assessment timing. Visual injury with the early application timing was shown to be a stronger indicator for potential yield loss when compared to the late application; however, this prediction indicator is moderate at best. The relationship between visual injury and leaf quality was significant for the early application; however, low coefficient values show this to be an inconsistent trend. Value reduction in response to visual injury was significant for both application timings. Similar to yield, visual injury from the early application is a moderate to low indicator of value reduction. Trends with reducing sugars show a negative impact with the early application timing; however, correlation values were poor. There was no significant relationship with total alkaloids and visual injury at either application timing. In previous research, Johnson et al. (2011) observed similar relationships between early injury symptoms from dicamba and yield and quality of flue-cured tobacco.

Results from these studies show the importance of spray equipment cleaning efficiency when using dicamba. Regardless of dicamba formulation and additional cleaning agents, rinse frequency is the most important factor in removing dicamba residue from a spray tank. Furthermore, it can be argued that these data are a best case scenario as the spray tank was the only equipment evaluated. It is documented that herbicide residue can stick or become trapped in a variety of places within a sprayer system, with the potential of the plumbing (hoses, pumps, screens, etc.) being the most difficult to clean due to the lack of access (Cundiff et al. 2017; Whitford et al. 2015). Also, water was used as a subsequent tank use refill as opposed to an herbicide such as glyphosate; which is very efficient at removing residue from internal spray parts (Steckel et al. 2005). These data show the sensitivity of flue-cured tobacco to dicamba. Overall, visual injury and plant response varied greatly across environments. This is common among other crops as variability in response to auxin herbicides are highly dependent upon many environmental factors (Egan et al. 2014; Leon et al. 2014). In general, the early timing was more injurious compared to the later application timing, which was not surprising as plants were near physiological maturity at the later application timing. Research has shown that the maturity of leaf tissue can greatly affect the sensitivity and response of a tobacco plant exposed to an auxin herbicide (White and Hemphill, 1972). Furthermore, differences in plant stresses and management factors can greatly affect leaf maturity and ripening and the timing of harvest in tobacco. Due to the variable response of plants exposed to auxin herbicides, predictions of yield and value reductions are inconsistent. Yield reductions were not always apparent; however, visual symptoms are cause for a crop to be rendered unmarketable therefore it is a total loss. However, this information can aid in decision making for growers and advisors following an herbicide exposure event. To address other herbicide technology concerns, further research is needed to evaluate flue-cured tobacco response to 2,4-D. Furthermore, evaluation of crop response across a greater range of tobacco growing areas would be of value.

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Table 1. Probability and F values for tank cleanout study.*

Source of variation	F statistic	P > F
Formulation	1.2	0.2816
Rinse	12,091.5	<.0001
Cleaning agent	0.2	0.7863
Rinse × cleaning agent	0.1	0.9987
Formulation × rinse	1.7	0.0910
Formulation × cleaning agent	0.8	0.5288
Formulation × rinse × cleaning agent	0.7	0.8318

Table 2. Percent dicamba recovered from spray tanks by rinse and subsequent tank use following a labeled use rate.[a]

	% of initial concentration
Concentrate	95 a
First rinse	19 b
Second rinse	4 c
Third rinse	0.2 d
Refill[b]	0.006 d

[a]Means followed by the same letter are not significantly different at $p \leq 0.05$. Data are pooled across formulations and cleaning agents.
[b]Tank was refilled with water to simulate subsequent tank use.

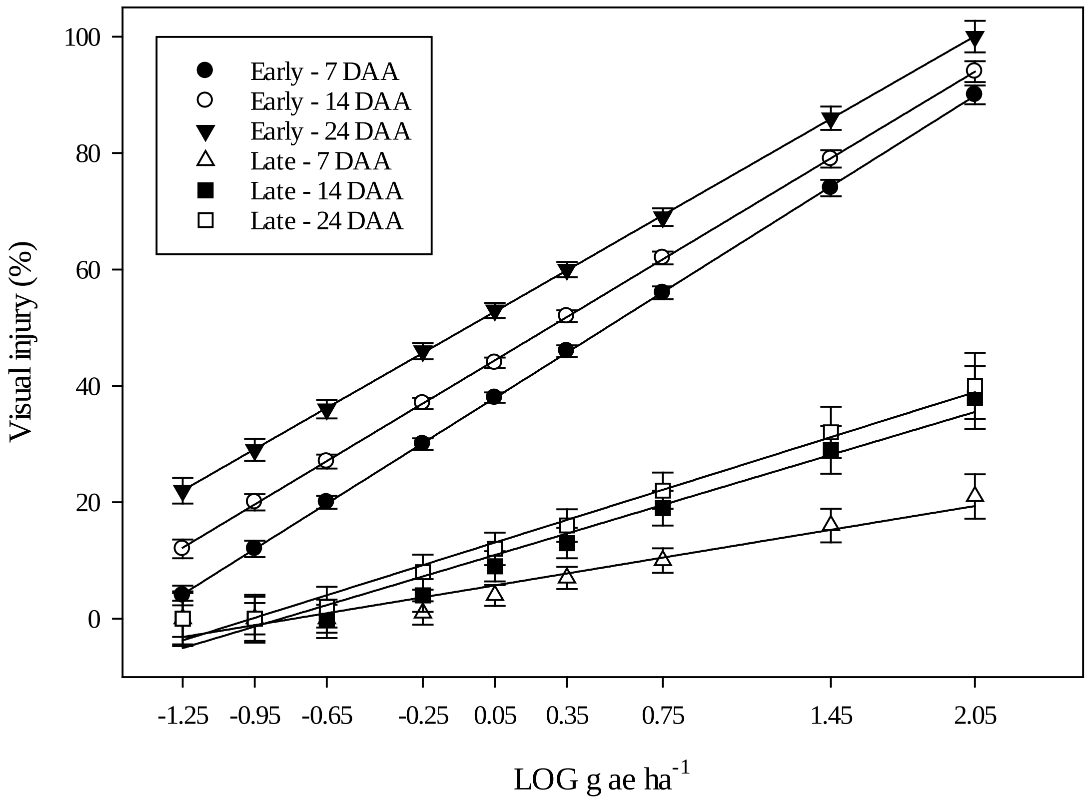

Figure 1. Tobacco injury 7, 14, and 24 DAA at Oxford, NC in response to dicamba rate and application timing. Regression expressions: Early application: 7 DAA, $y = 36.78 + 25.95x$; P = <.0001; r^2 =0.96; 14 DAA, $y = 43.25 + 24.62x$; P = <.0001; r^2 =0.95; 24 DAA, $y = 51.44 + 23.57x$; P = <.0001; r^2 =0.91; Late application: 7 DAA, $y = 3.49 + 8.73x$; P = <.0001; r^2 0.40; 14 DAA, $y = 7.81 + 14.83x$; P = <.0001; r^2 0.50; 24 DAA, $y = 11.29 + 14.09x$; P = <.0001; r^2 0.45. Error bars represent 95% CI.

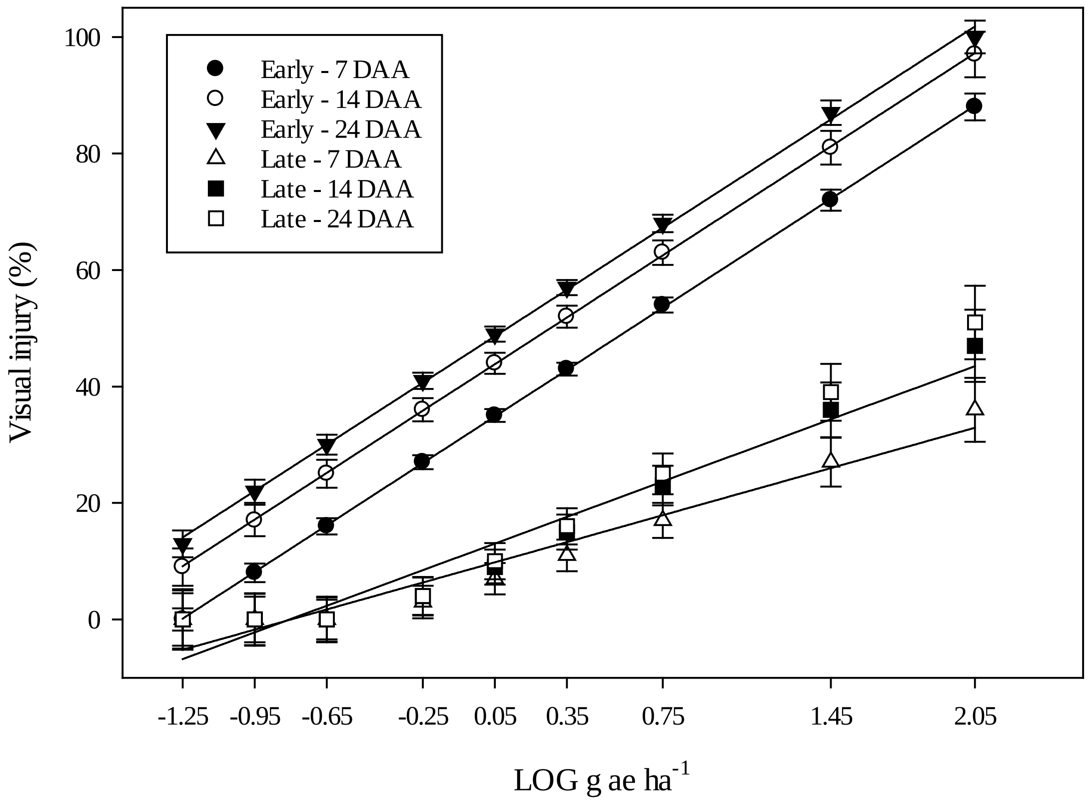

Figure 2. Tobacco injury 7, 14, and 24 DAA at Kinston, NC in response to dicamba rate and application timing. Regression expressions: Early application: 7 DAA, y = 33.77 + 26.64x; P = <.0001; r^2 =0.94; 14 DAA, y = 42.68 + 26.64x; P = <.0001; r^2 =0.86; 24 DAA, y = 47.67 + 27.35x; P = <.0001; r^2 =0.93; Late application: 7 DAA, y = 6.18 + 14.59x; P = <.0001; r^2 0.48; 14 DAA, y = 8.38 + 19.06x; P = <.0001; r^2 0.56; 24 DAA, y = 9.22 + 20.61x; P = <.0001; r^2 0.58. Error bars represent 95% CI.

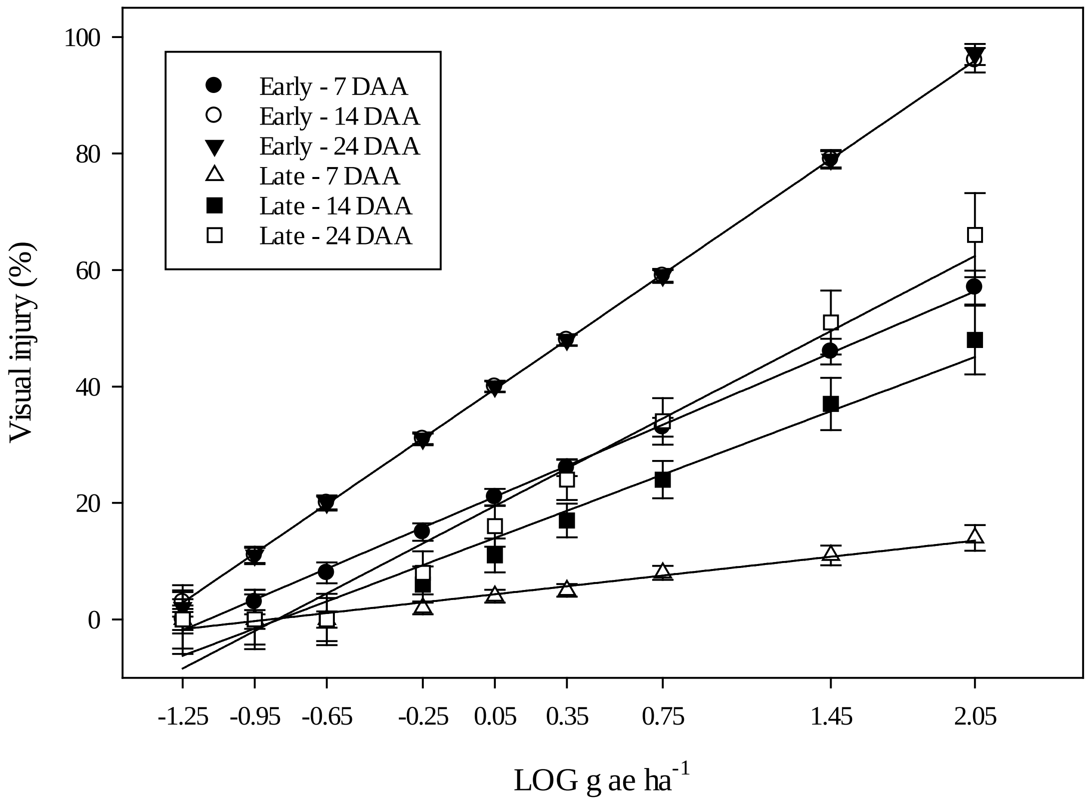

Figure 3. Tobacco injury 7, 14, and 24 DAA at Whiteville, NC in response to dicamba rate and application timing. Regression expressions: Early application: 7 DAA, y = 19.81 + 18.06x; P = <.0001; r^2 =0.87; 14 DAA, y = 38.25 + 28.22x; P = <.0001; r^2 =0.97; 24 DAA, y = 37.84 + 28.64x; P = <.0001; r^2 =0.98; Late application: 7 DAA, y = 3.62 + 5.16x; P = <.0001; r^2 0.49; 14 DAA, y = 10.19 + 18.36x; P = <.0001; r^2 0.64; 24 DAA, y = 14.75 + 25.07x; P = <.0001; r^2 0.69. Error bars represent 95% CI.

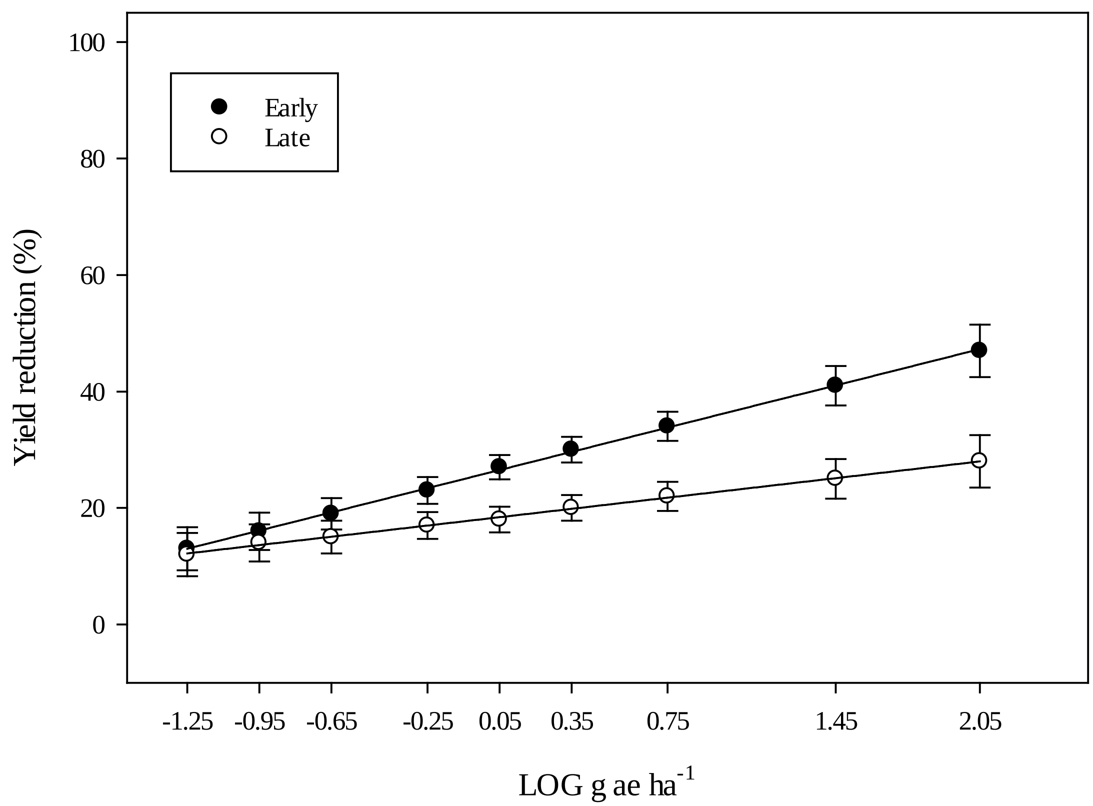

Figure 4. Percent tobacco yield reduction at Oxford, NC in response to dicamba rate and application timing. Regression expressions: Early application: $y = 26.05 + 10.31x$; P = <.0001; r^2 =0.41; Late application: $y = 18.05 + 4.68x$; P = 0.0341; r^2 0.13. Error bars represent 95% CI. Non-treated control total yield: 4,460 and 4,210 kg ha^{-1}, early and late applications, respectively.

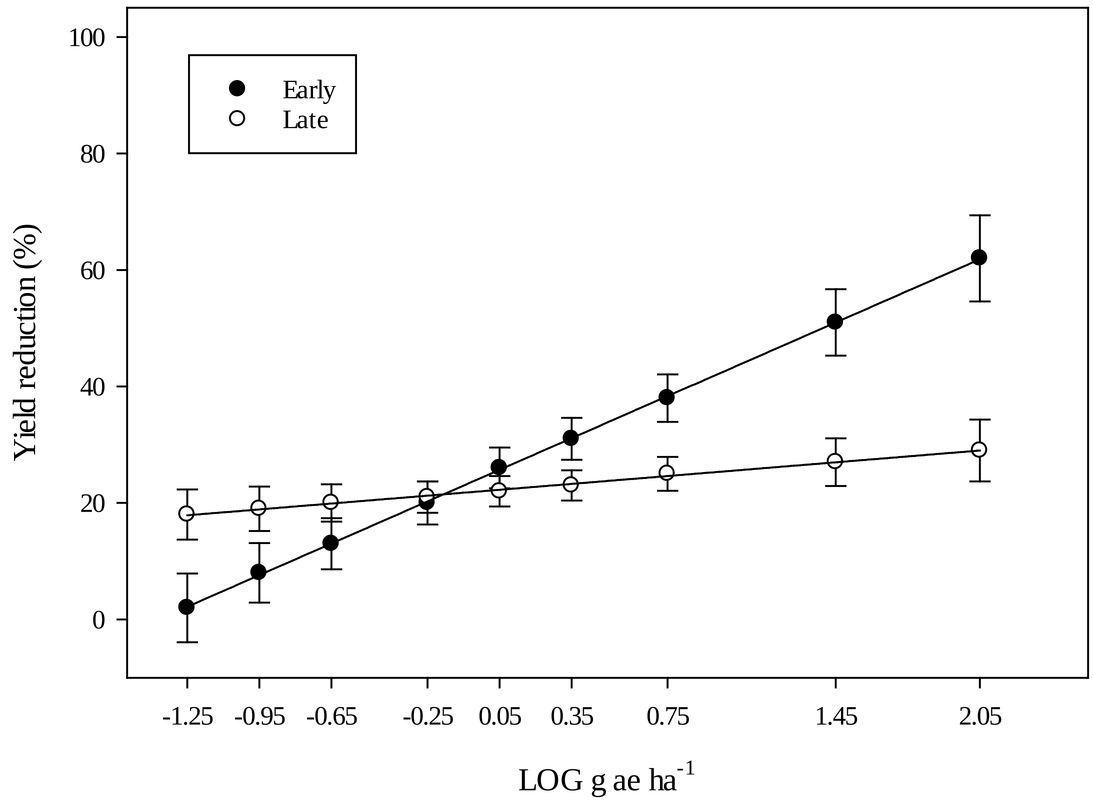

Figure 5. Percent tobacco yield reduction at Kinston, NC in response to dicamba rate and application timing. Regression expressions: Early application: y = 24.72 + 18x; P = <.0001; r^2 =0.45; Late application: y = 22.22 + 3.36x; P = 0.1860; r^2 0.05. Error bars represent 95% CI. Non-treated control total yield: 2,530 and 2,815 kg ha^{-1}, early and late applications, respectively.

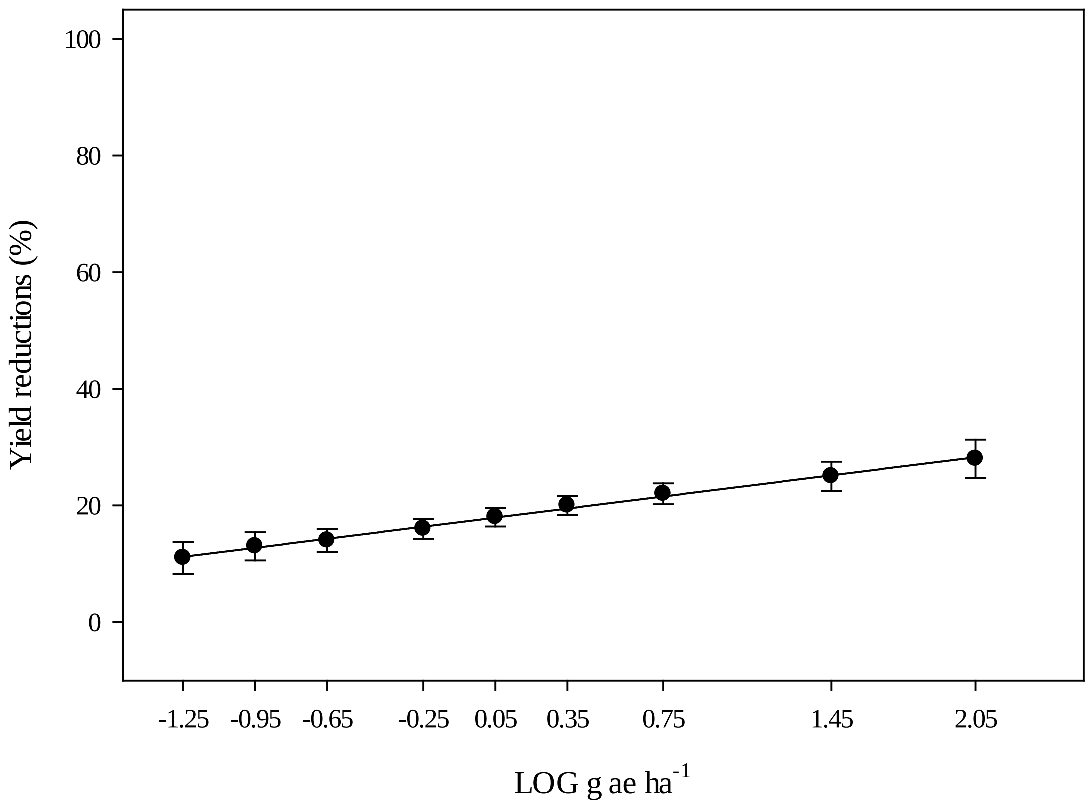

Figure 6. Percent tobacco yield reduction at Whiteville, NC in response to dicamba rate. Regression expression: $y = 17.77 + 5.19x$; P = 0.0018; $r^2 = 0.17$. Error bars represent 95% CI. Non-treated control total yield: 4,320 kg ha^{-1}. Data are pooled over application timing.

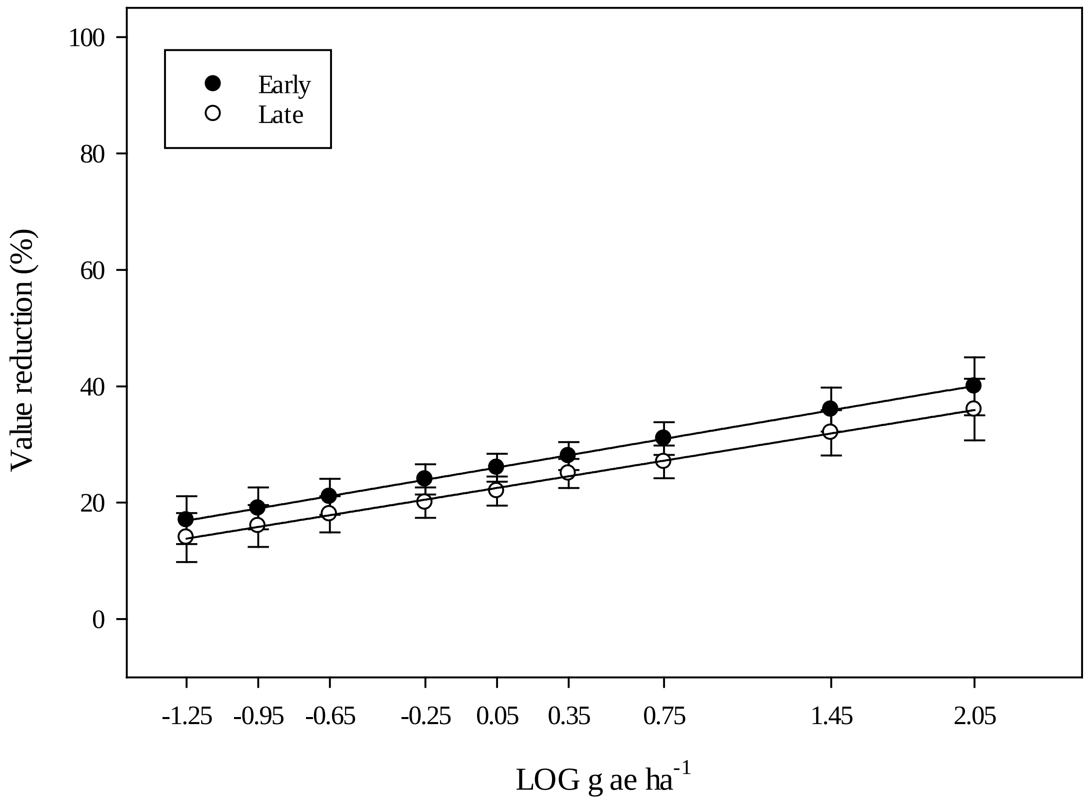

Figure 7. Percent tobacco value reduction at Oxford, NC in response to dicamba rate and application timing. Regression expressions: Early application, y = 25.97 + 6.92x; P = 0.0060; r^2 =0.20; Late application, y = 22.15 + 6.66x; P = 0.0090; r^2 =0.18. Error bars represent 95% CI. Non-treated control value: \$16,050 and \$16,405 ha^{-1}, early and late applications, respectively.

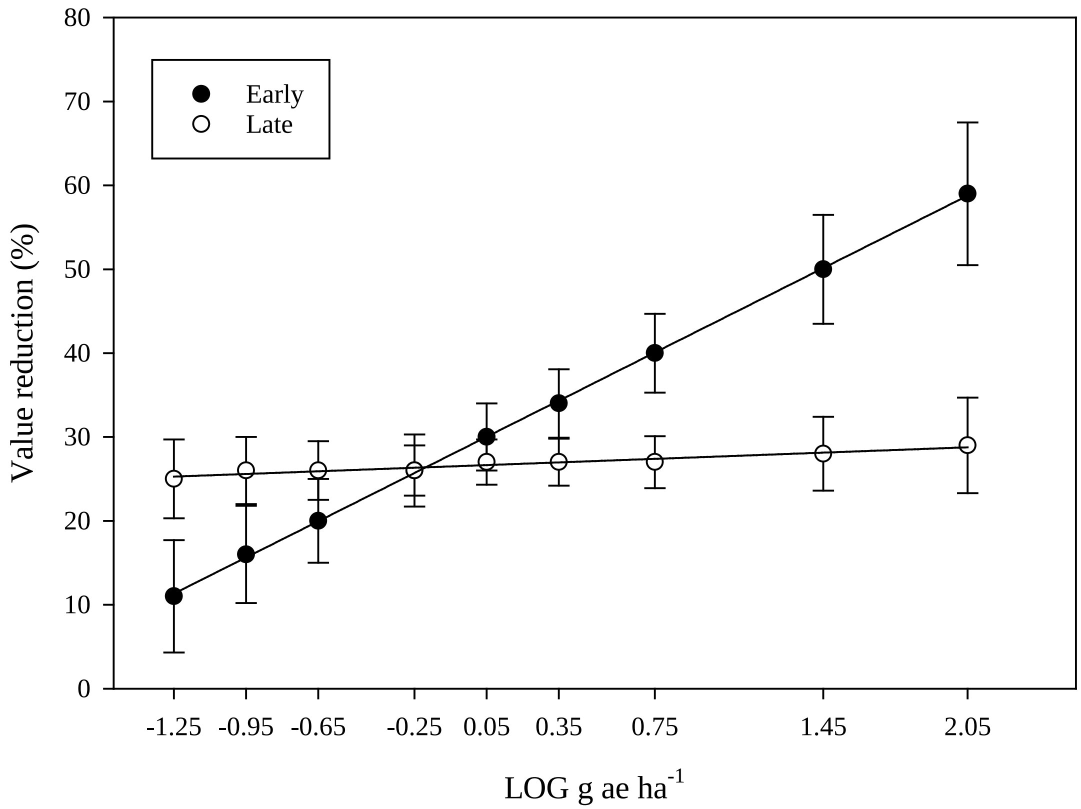

Figure 8. Percent tobacco value reduction at Kinston, NC in response to dicamba rate and application timing. Regression expressions: Early application: y = 29.26 + 14.46x; P = 0.0008; r^2 =0.29; Late application: y = 26.67 + 1.09x; P = 0.6853; r^2 =0.01. Error bars represent 95% CI. Non-treated control value: \$8,650 and \$9,840 ha^{-1}, early and late applications, respectively.

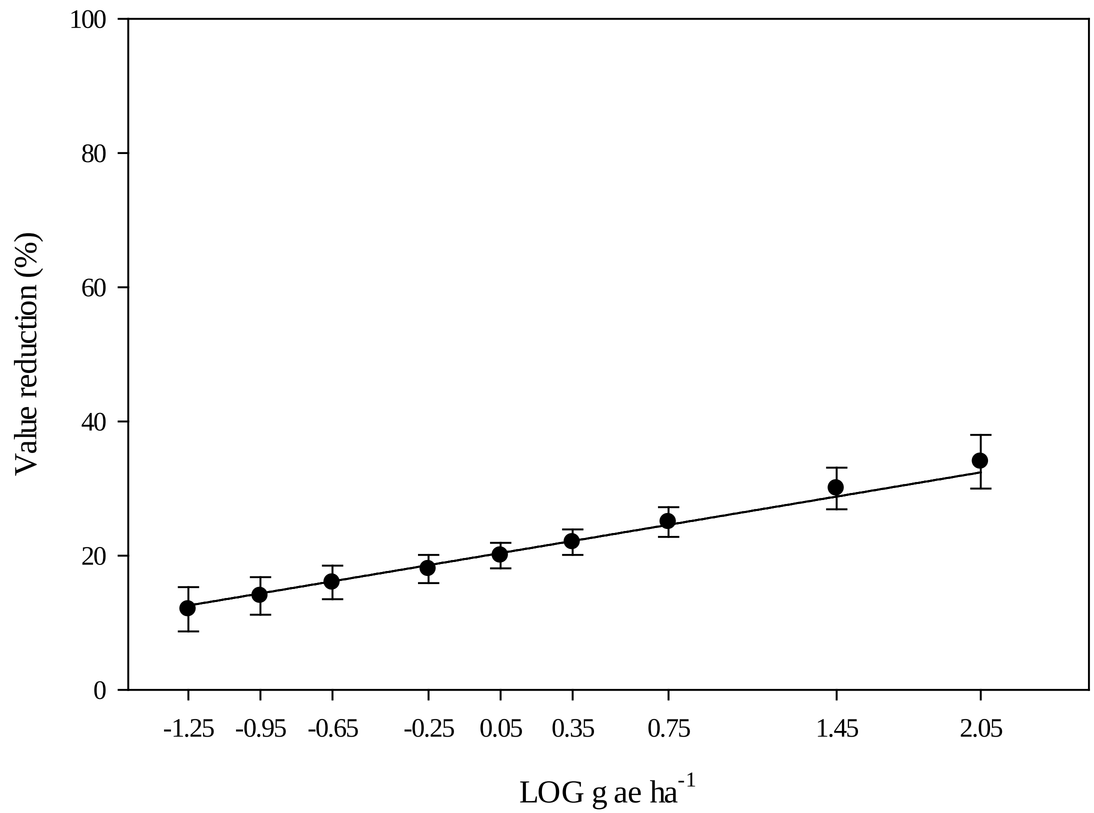

Figure 9. Percent tobacco value reduction at Whiteville, NC in response to dicamba rate. Regression expression: $y = 20.03 + 6.67x$; P = 0.0009; $r^2 = 0.19$. Error bars represent 95% CI. Non-treated control value: \$16,125 ha^{-1}. Data are pooled over application timing.

Table 3. Percent total alkaloids in response to dicamba application timing.[a]

Application timing	Oxford	Whiteville	Kinston
		%	
7 WAT	2.79 a	2.89 a	2.19 a
11 WAT	2.60 b	2.59 b	2.22 a

[a]Means followed by the same letter are not significantly different at $p \leq 0.05$. Data are pooled across dicamba rate.

Table 4. Pearson correlations for tobacco quality, yield reduction (%), value reduction (%), total alkaloids, and reducing sugars in response to visual injury (%) 7, 14, and 24 days after application (DAA) by application timing.

Variable	Visual injury (%) DAA					
	7		14		24	
Early application	P-value	Regression Coefficient	P-value	Regression Coefficient	P-value	Regression Coefficient
Quality	.0011	-0.30	0.0029	-0.28	0.0077	-0.25
Yield reduction (%)	<.0001	0.63	<.0001	0.56	<.0001	0.55
Value reduction (%)	<.0001	0.51	<.0001	0.47	<.0001	0.45
Total alkaloids	0.4673	0.07	0.1179	0.15	0.0712	0.18
Reducing sugars	0.0210	-0.22	0.0460	-0.19	0.0136	-0.24
Late application						
Quality	0.2048	0.12	0.0763	0.17	0.0999	0.16
Yield reduction (%)	0.0034	0.28	0.0007	0.32	0.0006	0.32
Value reduction (%)	0.0116	0.24	0.0083	0.25	0.0067	0.28
Total alkaloids	0.1720	-0.13	0.3504	-0.09	0.5281	-0.06
Reducing sugars	0.1073	-0.15	0.0844	-0.17	0.0540	-0.18

CHAPTER 4

Critical Period of Weed Control in Flue-Cured Tobacco

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Abstract

Two separate field studies were conducted to in North Carolina to determine the critical period of weed control (CPWC) in flue-cured tobacco. Natural populations of broadleaf and grass weed species were used in one study and natural populations of broadleaf weed species only in a second study were allowed to compete with flue-cured tobacco. In weed-removal treatments, weeds were allowed to compete with tobacco for 2, 4, 6, 8, or 10 weeks after transplanting (WAT) and then kept weed-free for the remainder of the season. In weed establishment treatments, tobacco was maintained weed-free by hand-removal for 2, 4, 6, 8, or 10 WAT after which weeds were allowed to compete for the remainder of the season. Weedy and weed-free checks were also included in the study. The CPWC was determined based on a 5% marketable yield loss. When combined across years, the CPWC was determined to be 3.5 to 7.8 WAT and 3 to 9.1 WAT in the broadleaf and grass weed species study and broadleaf species only, respectively. In both tests, tobacco quality and value decreased as the time duration of weed interference increased. There are many limitations to suggesting a precise time-period to weed management due to variability across environments, cultural practices, and crop-weed species specifics. However, this research can be utilized to provide weed management information and aid in decision making to flue-cured tobacco growers and show the importance of season-long weed control.

Flue-cured tobacco accounts for approximately 83,000 ha and an excess of $900 million in total revenue (USDA, NASS 2018). North Carolina accounts for 78% of the production area in the United States and consistently generates more than $700 million in farm gate revenue; making it the most economically important crop in the state (NCDA stats, 2018). Weed interference reduces yield, quality, and value of tobacco through direct competition for sunlight, water, and nutrients, (Peedin 1999). Weeds also serve as hosts for insects and diseases that are common in a tobacco cropping system.

In North Carolina, Palmer amaranth (*Amaranthus palmeri* S. Wats), yellow nutsedge (*Cyperus esculentus* L.), Large crabgrass (*Difitaria sanguinalis* L.), common ragweed (*Ambrosia artemisifolia*), and morningglory species (*Ipomoea spp*.) are among the most common and troublesome weed species in tobacco production (Webster, 2013). Due to limited herbicide options, weed management in tobacco is relied heavily upon cultivation (for the first six weeks) and hand-weeding along with cultural control methods (Vann et al. 2019). The timing of these weed control practices are critical as equipment and labor costs increase with each trip to the field. Weed interference at harvest can physically damage plants reducing yield and quality. Bailey (2013) reported a 5% reduction in dark tobacco yield from leaf damage and loss during hand harvesting due to morningglory species. Furthermore, weed seed and plant vegetation can contaminate harvested leaf, further reducing quality and value.

The critical period for weed control (CPWC) is defined as the period of time that weeds must be controlled to prevent yield or quality loss (Bailey 2013; Knezevic et al. 2002). This time period represents the time interval between two separately measured weed-crop competition components. The beginning of the CPWC is determined by the timing of weed-removal which is defined as the maximum time duration that a weed can grow and interfere with the crop before an unacceptable yield loss has occurred. The end of the CPWC is determined by the weed-free or weed establishment period and is defined the minimum length of time that weed emergence must be prevented to ensure weed growth does not reduce crop yield (Knezevic et al. 2002).

Theoretically, weed control outside of the CPWC is not justified as yields would be similar. The CPWC can be influenced by many factors including environmental conditions, management strategies, cultural practices, as well as crop-weed species specifics (Knezevic et al. 2002; McGowen et al. 2018; Swanton et al. 2010; Williams 2006). It is thought that the CPWC for tobacco would be shorter compared to direct seeded crops due to tobacco being transplanted and its large stature. Few studies have evaluated the timing of weeding and its impact on flue-cured tobacco yield parameters. The CPWC for tomato has been determined under various transplanting practices with diverse results. In plasticulture tomato production systems, tomato should be maintained free of eastern black nightshade (Solanum ptychanthum Dunal) 4 to 7 WAT to avoid greater than 20% yield loss (Buckelew et al.2006). Weaver and Tan (1983) reported the CPWC of transplanted tomato to be 4 to 5 WAT when exposed to natural populations of mixed weed species. In field-seeded processing tomato, the critical period was 7 to 9 wk after seeding in natural populations of mixed weed species (Weaver 1984). In peanut, Everman et al. (2008) found the CPWC to be 4.3 to 9 WAT for grass species and 2.6 to 8 WAT for broadleaf species. The objective of this study was to determine the CPWC in flue-cured tobacco in the presence of broadleaf and grass weed species and broadleaf species only.

Materials and Methods

Two separate field studies were conducted in North Carolina at the Upper Coastal Plains Research Station near Rocky Mt. (35.8923N, -77.6796W). Soil type was Goldsboro fine sandy loam (fine-loamy, silicious, subactive, thermic Aquic Paleudults) at Rocky Mt. Soil pH ranged from 5.8 to 6.2 with <1% organic matter**.** Standard field preparation was performed prior to transplanting each year. Test sites were plowed, disked, and bedded approximately two weeks prior to transplanting. The flue-cured tobacco cultivar NC 196 (Gold Leaf Seed Company, Hartsville, SC) was transplanted at a density of 14,820 plants ha^{-1} for all studies and years. Tobacco was transplanted on April 19, April 18, and April 30 in 2016 2017, and 2018, respectively. Individual plots were three rows wide, 4.9 M wide by 13.7 long with the center row of each plot used for data collection and harvest. No herbicides were applied and no cultivation was performed on test sites to allow for maximum weed interference. For the broadleaf weed only study, annual grasses were controlled with sethoxydim (Poast, BASF Corporation RTP, NC) and hand-weeding as needed. Other than no herbicides and cultivation, tobacco was produced according to North Carolina Cooperative Extension Service recommendations (Fisher, 2016).

Treatments were arranged in a randomized complete block design with three or four replications; depending on year. In removal treatments (REM), weeds were allowed to compete with tobacco for 2, 4, 6, 8, or 10 WAT. Plots were then kept weed-free for the remainder of the season. In the establishment treatments (EST), plots were maintained free of weeds by hand-weeding until 2, 4, 6, 8, or 10 WAT; after which Palmer amaranth were allowed to establish and compete with the tobacco for the remainder of the season through harvest. Additionally, season-long weedy and weed-free controls were included. Natural populations of broadleaf and grass weed species were present in both studies (Table 1).

Plots were harvested four times in each growing environment and leaves were cured in a forced-air bulk curing barn. Cured-leaf was then weighed to quantify yield and assigned a USDA government grade. Each government grade is associated with a numerical grade index value ranging from 1 to 100, which describes leaf maturity and ripeness (Bowman et al., 1988) as well as an associated financial value that reflects modern price indices (Fisher et al., 2019). Fifty-gram composite cured leaf samples were also collected from each treatment for analysis of percentage total alkaloids and percentage reducing sugars using the methods outlined by Davis (1976). A yield loss estimate is an arbitrary measurement and the range of percentage of yield loss depends on the economics of weed management or the risk that the farming operation is willing to take (Knezevic et al. 2002). In previous studies, a 5% acceptable level of yield loss was used to measure the CPWC in different crops such as peanut (*Arachis hypogaea* L.), sweet corn (*Zea mays* L.), and tomato (*Solanum lycopersicum*) (Chaudhari et al. 2016; Everman et al. 2008; Williams 2006). Thus, we assumed that a 5% yield loss would be acceptable to tobacco growers and therefore used it for the calculations in this study.

All data were checked for normality and homogeneity of variance by plotting residuals prior to statistical analyses. Data were subjected to ANOVA using the PROC Mixed procedure in SAS (v. 9.4; SAS Institute, Cary, NC). Nonlinear models were fit to best describe relative marketable yield as a function of removal and establishment treatments. A three-parameter logistic model (Equation 1) was fitted to describe the effect of removal treatments on marketable flue-cured tobacco yield (Knezevic et al. 2002). The three parameters were A, B, and X_0, where A is the yield asymptote, X_0 is the inflection point (WAP), B is the estimate of the duration of change. A three-parameter Gompertz model (Equation 2) was fitted to demonstrate the effect of establishment treatments on the marketable tobacco yield (Knezevic et al. 2002). The three parameters were A, B, and X_0, where A is the yield asymptote, X_0 is the inflection point (WAP), B is the estimate of the duration of change. Data regressions were accomplished using SigmaPlot 14 (SYSTAT Software; Chicago, IL). The parameter estimates of logistic and Gompertz models are listed in Table 2.

$$Y = A/(1+\exp[-(time-X_0)/B]) \quad [1]$$

$$Y = A \times \exp(-\exp(-(time-X_0)/B)) \quad [2]$$

Results and Discussion

Broadleaf and grass species study

The year by treatment interaction was significant for percent marketable tobacco yield for both removal and establishment studies; therefore data are presented by year. Pooled data are also presented. Increasing durations of weed interference reduced tobacco yields in both years. Season-long competition of a mixed weed species population reduced tobacco yield by 62 to 97% during 2016 and 2017, respectively (Figures 1 and 2). In North Carolina, Wilson (1995) reported a 77% yield reduction in flue-cured tobacco when weeds were not controlled. Similar findings have been reported in other crops, where significant yield reductions have been observed from season-long weed interference (Chaudhari et al. 2016; Everman et al. 2008; Seem et al. 2003). The CPWC required to maintain a 5% yield loss or less widely varied by year. The CPWC was estimated to be 4.9 to 6.2 and 3.3 to 9 WAT in 2016 and 2017; respectively (Figures 1 and 2). When data are pooled over years, the suggested CPWC is estimated to be 3.5 to

7.8WAT (Figure 3). Lolas (1986) reported, in burley and Oriental tobacco, yield was not reduced until weed interference remained longer than 3 WAT when exposed to mixed populations of broadleaf and grass weed species.

No significant interactions for year for removal or establishment treatments for tobacco heights were observed; therefore data were combined across years (Table 2). By 6 WAT, no difference in plant height was observed in removal or establishment treatments compared to the season-long weed-free or weedy; respectively. The removal of weeds 2 WAT treatment provided similar plant heights compared to the season-long weed-free treatment, 8 WAT. In the establishment study, all establishment treatments had greater plant heights compared to the season-long weedy plots 8 WAT.

The interaction of tobacco quality and year was significant, however, when analyzed separately the trends were similar; therefore data were pooled for analysis. Interactions associated with tobacco value, total alkaloids, and reducing sugars was not significant and data are pooled over years (Table. 3). In this study, 6 WAT showed to be the threshold for tobacco quality. There was no reduction in quality when weed interference was delayed beyond 6 WAT. When weeds were allowed to compete before 6 WAT and were not removed until after 6 WAT, significant reductions in tobacco leaf quality were observed. A 10% reduction in flue-cured tobacco quality was reported under season-long weedy conditions (Wilson, 1995). Prolonged weed interference, drastically reduced tobacco value. Although there was no differences in quality, tobacco value was greater when weeds were removed 4 WAT compared to 6 WAT. When weed-removal was delayed until 6 WAT, value was reduced 23% compared to the season-long weed-free. In the establishment study, there were no differences in value between plots that were weed-free for 8 WAT compared to 10 WAT. When weeds were established at 2 and 4

WAT, tobacco had similar values compared to the season-long weedy treatment. There were no differences in total alkaloids when weed interference was delayed until after 6 WAT. Tobacco had lower reducing sugars when weed-removal was delayed until after 10 WAT, while all weed establishment treatments had higher reducing sugars compared to the season-long weed free plots.

Broadleaf species only study

The year by treatment interaction was significant for percent marketable tobacco yield for both removal and establishment studies; therefore data are presented by year. Pooled data are also presented. Season-long weed interference reduced tobacco yields at least 90% in both years (Figures 1 and 2). This is attributed to a major reduction in plant growth and development while also harvesting of some plots were prevented due to weed pressure. The CPWC required to maintain a 5% yield loss or less widely varied by year. The CPWC was estimated to be 3.5 to 9.6 and 2.6 to 8.1 WAT in 2016 and 2017; respectively (Figures 4 and 5). When data are pooled over years, the suggested CPWC is estimated to be 3 to 9.1 WAT (Figure 6). Medlen (1978) reported no reduction in yield of flue-cured tobacco when maintained free of common ragweed for 2 WAT. In contrast, Ian et al. (2013) found the CPWC for flue-cured tobacco in Zimbabwe to occur between 4 and 9 WAT in the presence of predominantly broadleaf weed species.

No significant interactions for year for removal or establishment treatments for tobacco heights were observed; therefore, data were combined across years (Table 4). By 6 WAT, no difference in plant height was observed in removal or establishment treatments compared to the season-long weed-free or weedy; respectively. There were no differnces in plant heights, 8 WAT, if weeds were removed 6 WAT or before. No differences in plant heights were observed with any treatment, 8 WAT, in the establishment study.

There were no significant interactions of year and quality, value, total alkaloids, or reducing sugars, therefore, data are pooled over years (Table 5). Tobacco quality was not reduced when weed-removal occurred 4 WAT or before. Thereafter, quality was reduced significantly as weed interference duration increased. There was no difference in quality when tobacco was weed-free for 6 WAT or later. Tobacco in plots that allowed weeds to establish at 2 and 4 WAT had no difference in quality compared to plots with season-long weed interference. Tobacco value was reduced 70% when weed-removal was delayed until 6 WAT compared to the season-long weed-free. Value was similar when weeds were removed at or before 4 WAT. In the establishment study, as the weed-free period increased tobacco value increased. There was a 59% difference in tobacco value in plots where weeds began establishment at 8 WAT compared to 10 WAT. Total alkaloids were similar when weeds were removed 4 WAT or before and kept weed-free until 6 WAT. Reducing sugars were not affected if weed-removal occurred by 8 WAT. Similar to total alkaloids, reducing sugars were similar when plots were kept weed-free for 6 WAT.

Although the weed pressure in these studies were unrealistic compared to most tobacco growers fields and the lack of cultivation impeded plant growth, these results can further contribute to the development of an integrated weed management plan for flue-cured tobacco growers. It is most common for growers to apply herbicide pre-transplant and rely on cultivation (until 6 WAT) and then hand-weeding for the remainder of the season. In an unpublished survey of North Carolina tobacco growers, less than one-third of growers use a herbicide at lay-by and approximately 60% said weed pressure is greatest during harvest. The dramatic reductions in yield, quality, and value demonstrates the importance of weed control measures in managing early-season weed competition as well as late-season interference. The CPWC is an estimation of the most critical time to employ weed control practices as to reduce yield loss in the most moderate manner. Practical implications of this study is to encourage growers to invest in late-season weed management practices along with current (early-season) production weed control strategies. Furthermore, with the limited herbicide options available in tobacco production, emphasis should be towards reducing weed seed production as a means to decrease the soil weed seed-bank and to delay herbicide resistance.

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Table 1. Dominant weed species and average densities for both the broadleaf and grass species study and the broadleaf only study at Rocky Mt.[a]

Weed species	Weed density (plants m^{-2})	
	Broadleaf and grass study	Broadleaf only study
Palmer amaranth (*Amaranthus palmeri* S. Watts.)	31	60
Common ragweed (*Ambrosia artemisiifolia* L.)	3	8
Common lambsquarters (*Chenopodium album* L.)	14	10
Eclipta (*Eclipta prostrata* (L.) L.)	2	3
Pitted morninglory (*Ipomoea lacunosa* L.)	11	7
Yellow nutsedge (*Cyperus esculentus* L.)	11	11
Texas millet (*Urochloa texana* (Buckl.) R.D. Webster;	35	-
Large crabgrass (*Digitaria sanguinalis* (L.) Scop.)	90	-
Broadleaf signalgrass (*Urochloa platyphylla* (Munro ex C. Wright) R.D. Webster	50	-

Table 2. Parameter estimates for the three-parameter logistic model and Gompertz model for relative (% weed-free check) marketable flue-cured tobacco yield as a function of time (weeks after planting, WAT).

	Logistic[a]				Gompertz[b]			
Study	A	B	X_0	R^2	A	B	X_0	R^2
Broadleaf and grass species								
2016	100.03	5.07	8.72	0.99	111.93	3.29	0.25	0.99
2017	101.27	5.07	5.51	0.99	100.58	1.68	4.16	0.99
Pooled	97.13	4.77	7.47	0.98	118.26	3.71	2.23	0.98
Broadleaf only								
2017	102.78	5.07	5.71	0.99	126.71	2.92	5.95	0.99
2018	97.26	5.01	5.38	0.97	100.29	1.05	5.13	0.99
Pooled	96.03	6.79	5.41	0.99	103.91	1.61	5.27	0.99

[a] Three parameter logistic equation: $Y = A/(1+\exp[-(\text{time}-X_0)/B])$, where A is the yield asymptote, B is the duration of change, and X_0 is the point of inflection (WAP).
[b]Gompertz equation: $Y = A \text{ x } \exp(-\exp(-(\text{time}-X_0)/B))$, where A is the yield asymptote, B is the duration of change, and X_0 is the point of inflection (WAP).

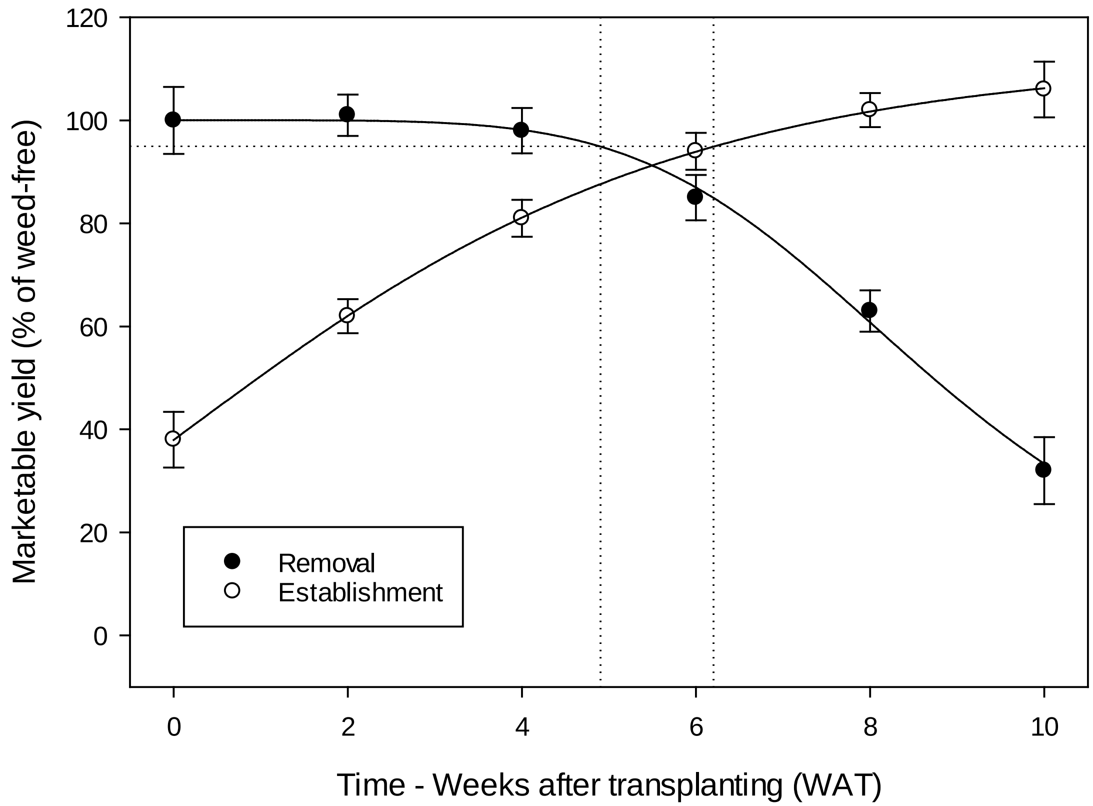

Figure 1. The influence of weed removal and establishment timing on marketable yield (% weed-free check) of flue-cured tobacco in the presence of a mixed population of broadleaf and grass weed species in 2016. Points represent observed mean ± SE. Solid lines represent predicted values.

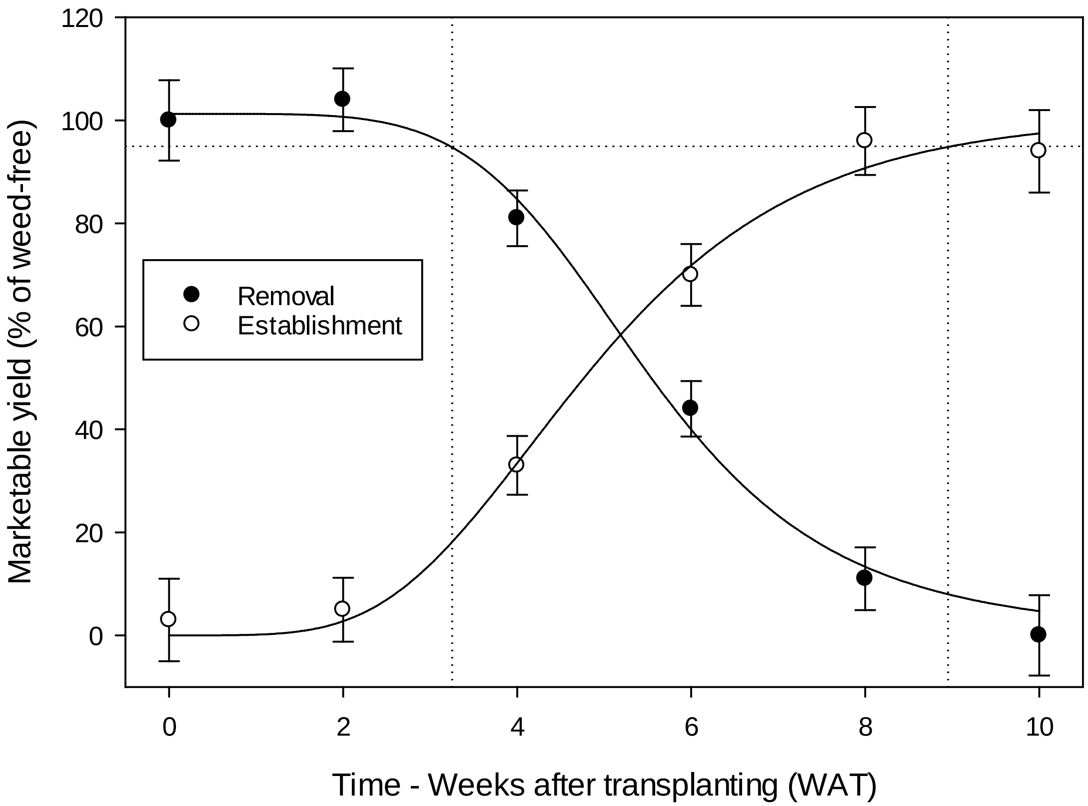

Figure 2. The influence of weed removal and establishment timing on marketable yield (% weed-free check) of flue-cured tobacco in the presence of a mixed population of broadleaf and grass weed species in 2017. Points represent observed mean ± SE. Solid lines represent predicted values.

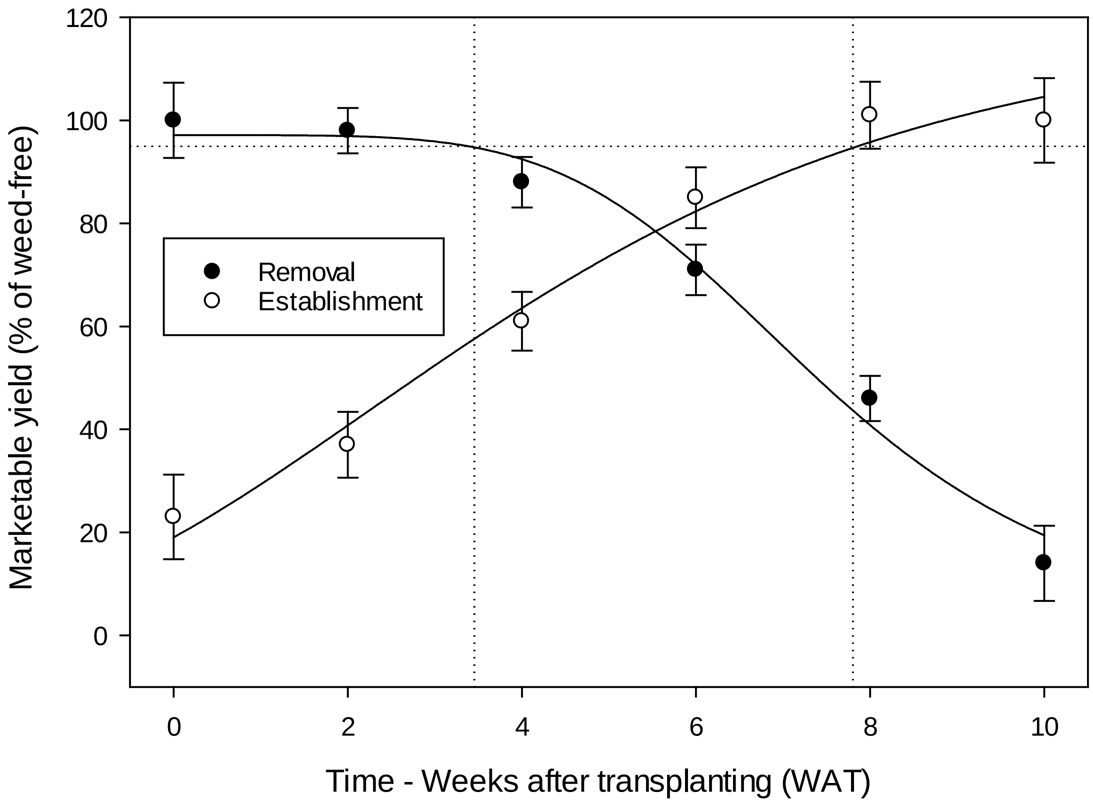

Figure 3. The influence of weed removal and establishment timing on marketable yield (% weed-free check) of flue-cured tobacco in the presence of a mixed population of broadleaf and grass weed species. Points represent observed mean ± SE. Solid lines represent predicted values. Data are pooled over years.

Table 3. Effect of weed removal and establishment treatments 6 and 8 weeks after transplanting (WAT) on tobacco plant height in response to broadleaf and grass weed species. Data are pooled over years.[a]

	Plant heights			
WAT	Removal		Establishment	
	6 WAT	8 WAT	6 WAT	8 WAT
	cm			
0	38 a	58 a	36 a	43 b
2	36 a	55 a	40 a	60 a
4	25 a	42 b	40 a	62 a
6	30 a	39 b	36 a	60 a
8	29 a	40 b	38 a	59 a
10	26 a	38 b	45 a	65 a

[a]Means within column followed by the same letter are not significantly different according to Fisher's protected LSD test at $P \leq 0.05$

Table 4. Flue-cured tobacco quality, value, total alkaloids and reducing sugars as influenced by weed removal and establishment treatments with broadleaf and grass weed species.[a]

Treatment (WAT)[c]	Quality	Value	Total alkaloids	Reducing sugars
	0-100	$ ha^{-1}	%	%
Removal				
0	77 a	9,890 ab	2.14 a	19.9 a
2	76 a	9,855 ab	2.18 a	18.4 a
4	79 a	10,865 a	1.97 a	21.9 a
6	78 a	7,605 b	1.93 a	21.9 a
8	53 b	3,950 c	1.24 b	18.6 a
10	43 b	2,855 c	0.76 c	11.6 b
Establishment				
0	45 c	4,745 d	0.92 d	9.7 b
2	66 b	6,135 cd	1.66 c	20.2 a
4	66 b	6,770 cd	1.91 bc	17.5 a
6	75a	8,440 bc	2.18 ab	16.7 a
8	74 a	9,365 ab	2.23 ab	16.9 a
10	73 a	11,430 a	2.35 a	17.1 a

a Data pooled over 2016 and 2017.

b Means within column and within establishment or removal followed by the same letter are not significantly different according to Fisher's protected LSD test at $P \leq 0.05$.

[c]Abbreviations: WAT, weeks after transplanting.

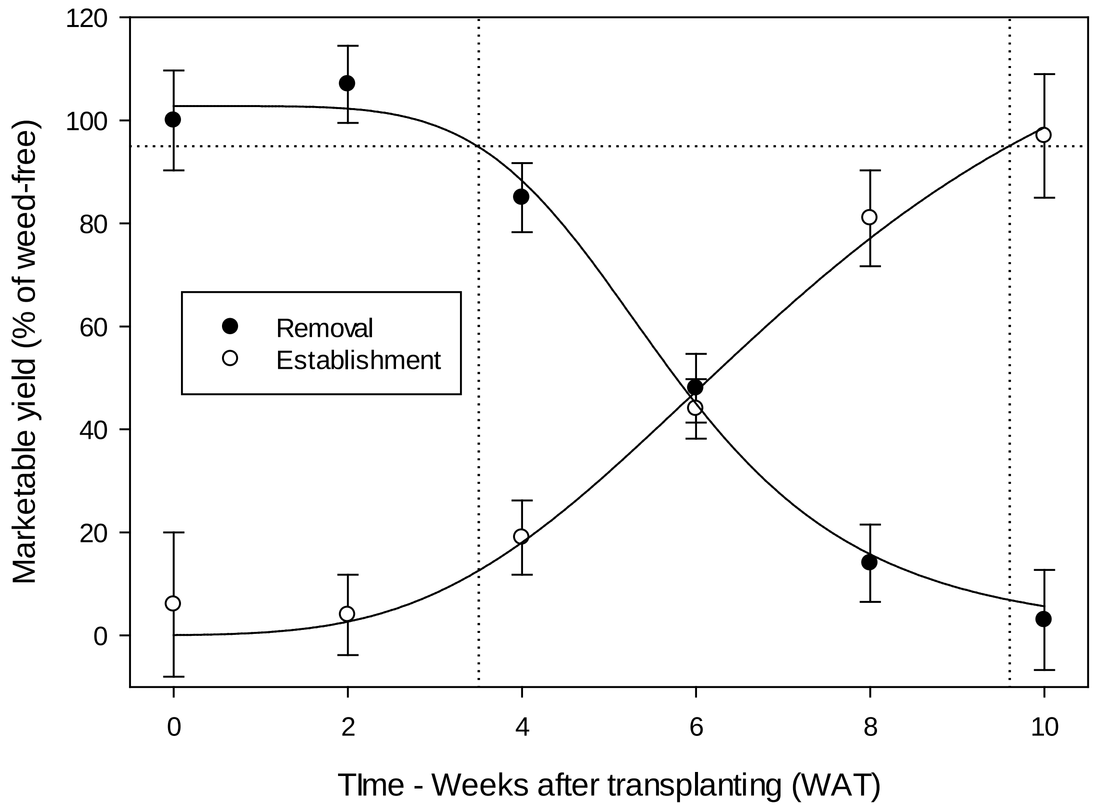

Figure 4. The influence of weed removal and establishment timing on marketable yield (% weed-free check) of flue-cured tobacco in the presence of a mixed population of broadleaf weed species only in 2017. Points represent observed mean ± SE. Solid lines represent predicted values.

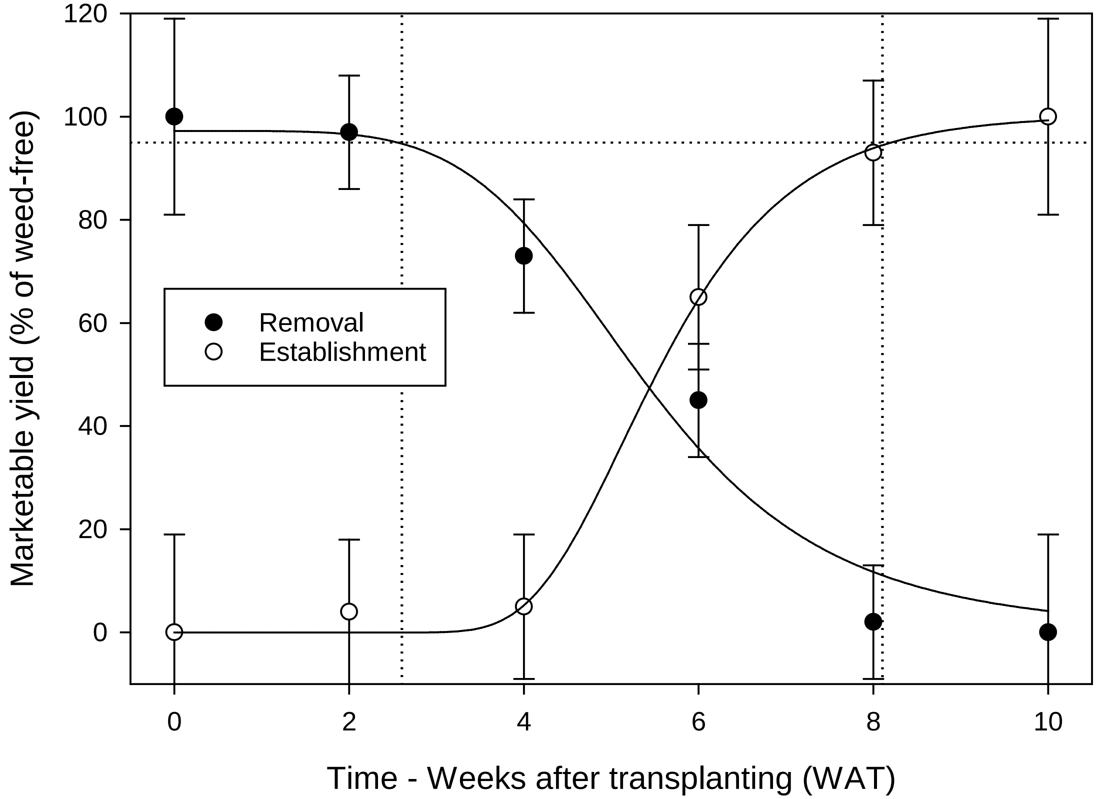

Figure 5. The influence of weed removal and establishment timing on marketable yield (% weed-free check) of flue-cured tobacco in the presence of a mixed population of broadleaf weed species only in 2018. Points represent observed mean ± SE. Solid lines represent predicted values.

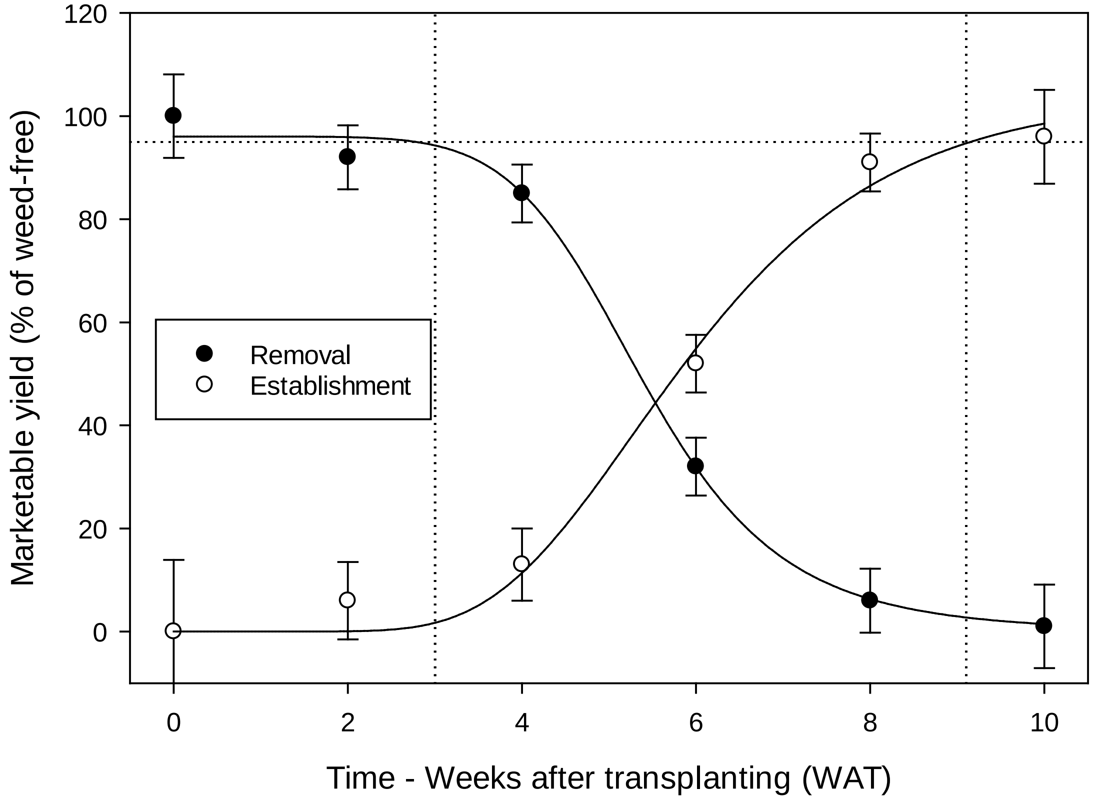

Figure 6. The influence of weed removal and establishment timing on marketable yield (% weed-free check) of flue-cured tobacco in the presence of a mixed population of broadleaf weed species only. Points represent observed mean ± SE. Solid lines represent predicted values. Data are pooled over 2017 and 2018.

Table 5. Effect of weed removal and establishment treatments 6 and 8 weeks after transplanting (WAT) on tobacco plant height in response to broadleaf only weed species. Data are pooled over years.

WAT	Plant heights: Removal		Plant heights: Establishment	
	6 WAT	8 WAT	6 WAT	8 WAT
	cm			
0	14 a	56 a	15 a	59 a
2	12 a	54 a	16 a	62 a
4	12 a	50 ab	18 a	62 a
6	11 a	50 ab	15 a	63 a
8	10 a	47 b	14 a	67 a
10	10 a	38 c	14 a	74 a

[a]Means within column followed by the same letter are not significantly different according to Fisher's protected LSD test at $P \leq 0.05$

Table 6. Flue-cured tobacco quality, value, total alkaloids and reducing sugars as influenced by weed removal and establishment treatments with broadleaf weed species only.[a]

Treatment (WAT)[c]	Quality	Value	Total alkaloids	Reducing sugars
	0-100	$ ha^{-1}	%	%
Removal				
0	67 a	5,840 a	1.16 a	14.1 a
2	60 a	5,115 a	1.21 a	14.5 a
4	59 a	4,190 a	1.25 a	14.3 a
6	29 b	1,785 b	0.32 b	11.5 a
8	20 bc	333 b	0.18 b	10.6 a
10	10 c	71 b	0.07 b	1.9 b
Establishment				
0	11 b	594 c	0.51 b	5.4 c
2	30 b	780 c	0.59 b	7.3 bc
4	28 b	932 bc	0.79 b	8.6 bc
6	68 a	1,485 bc	2.12 a	14.3 ab
8	57 a	2,010 b	1.97 a	15.7 a
10	70 a	4,940 a	1.81 a	18.9 a

a Data pooled over 2017 and 2018.
b Means within column and within establishment or removal followed by the same letter are not significantly different according to Fisher's protected LSD test at $P \leq 0.05$.
[c]Abbreviations: WAT, weeks after transplanting.

CHAPTER 5

Evaluation of Rimsulfuron Timing and Rate in Flue-Cured Tobacco

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Abstract

Field studies were conducted from 2016 through 2018 to evaluate flue-cured tobacco response to rimsulfuron application timing and rate in flue-cured tobacco. Additionally, weed control was evaluated in combination with current recommended herbicide programs for flue-cured tobacco production. Visual injury ranged from 6 to 33% across environments when rimsulfuron was applied postemergence over-the-top (POT) 3 weeks after transplanting. By 6 WAT no visual injury was observed and there were no differences in yield, quality, or value of cured-leaf when compared to tobacco receiving only clomazone plus sulfentrazone pre-transplant (PRE-T). No injury was observed for any PRE-T or post-directed (PD) at lay-by application containing rimsulfuron. When applied PD, rimsulfuron performed similar to pendimethlain and napropamide for Palmer amaranth, broadleaf signalgrass, large crabgrass, and goosegrass control, while providing slightly greater control of yellow nutsedge. These results indicate rimsulfuron did not adversely affect yield components of flue-cured tobacco. However, due to potential early season injury, POT applications of rimsulfuron will not be recommended. The addition of rimsufluron, PRE-T and PD, would provide flue-cured tobacco growers with additional options for weed control.

Flue-cured tobacco accounts for approximately 83,000 ha and an excess of $900 million in total revenue (USDA, NASS 2018). North Carolina accounts for 78% of the production area in the United States and consistently generates more than $700 million in farm gate revenue; making it the most economically important crop in the state (NCDA stats, 2018). It is critical for tobacco growers to produce clean, high quality leaf free of foreign material including weed seed (CORESTA, 2005). Weed seed contamination of cured-leaf can have severe consequences for growers including rejection at the point of sale or decreased marketing potential in foreign markets.

Flue-cured tobacco production acreage is relatively small in comparison to other major agronomic crops in North Carolina, such as corn, cotton, and soybean. Currently, there are only seven herbicides registered for use in tobacco production in North Carolina (Fisher 2016). Because of this, tobacco growers generally achieve sufficient weed control through an integrated approach of chemical, mechanical/physical, and cultural practices (Vann et al. 2019; Worsham, 1970). Soil-applied herbicides, applied prior to transplanting, are most commonly used while cultivation supplements weed control efforts through the first six weeks of the season (lay-by) (Vann et al. 2019). Only two herbicides with residual activity, pendimethalin and napropamide, are labeled for lay-by application. Late-season herbicide applications are limited to carfentrazone only after-first-harvest to limit vegetation exposure and leaf injury.

Control of weeds that escape residual herbicides or cultivation early in the season could reduce late-season weed interference and contamination of harvested leaf. Previous research has shown potential for ALS-inhibiting herbicides in a tobacco weed control program. Porterfield et al. (2005) found excellent flue-cured tobacco response and similar yields to trifloxysulfuron (3.75 and 7.5 g ai ha^{-1}) when applied PRE-T. No difference in dark tobacco yield was observed when trifloxysulfuron and halosulfuron were applied post-directed (PD) or postemergence over-the-top (POT) (Bailey, 2007). Hagood and Komm (1987) showed that flue-cured tobacco yields were similar to non-treated when imazaquin was applied PRE-T or early-POST at rates up to 0.56 kg ha^{-1}. Rimsulfuron is an acetolacte synthase-inhibiting (ALS) herbicide for PRE and POST control of numerous weed species (Robinson et al. 1996). Rimsulfuron is currently labeled in agronomic, vegetable, and fruit crops; including Solanaceous crops such as tomato (*Solanum lycopersicum L.)* and potato (*Solanum tuberosum L.)*. Seventy percent of flue-cured tobacco growers in North Carolina use clomazone plus sulfentrazone PRE-T (unpublished data). A greater selection of herbicides and modes of action would be of great benefit for tobacco growers in North Carolina. The objectives of this research were to determine flue-cured tobacco tolerance to rimsulfuron rate and timing and to determine the contributions of rimsulfuron to weed management programs currently used in tobacco production.

Material and Methods

Herbicides and rates used in both experiment 1 and 2 can be found in Table 1.

Experiment 1

Field studies were conducted in North Carolina across four environments in Oxford (36.3275N, -78.6582W) and Meadow (35.3190N, -78.3810W) in 2016 and Kinston (35.3039N, -77.5814W) and Whiteville (34.4089N, -78.7906W) in 2017. Soil types were a Helena sandy loam (fine, mixed, semi-active, thermic Aquic Hapludults), a Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiudults), a Norfolk sandy loam (fine-loamy, kaolinitic, thermic Typic Kandiudults), and a Norfolk sandy loam (fine-loamy, siliceous, thermic Typic Paleudults) at

Oxford, Meadow, Kinston, and Whiteville; respectively. Soil pH at all field sites ranged from 5.8 to 6.2 with <1% organic matter. The treatment list can be found in Table 2. Pre-transplant applications were applied within 3 to 7 days prior to transplanting and POT applications were applied 3 weeks after transplanting (WAT). Herbicide applications were made using CO_2-pressurized backpack sprayer calibrated to deliver 187 L ha^{-1} at 140 kPa for both PRE-T and POT applications. The spray boom was equipped with TeeJet TTI 110025 flat spray nozzles (Spraying Systems Co., Wheaton, IL). A mix population of annual grasses were the most dominant weed species in all environments with densities ranging from 3 to 25 m^2. Broadleaf signalgrass [*Urochloa platyphylla* (Nash) R.D. Webster], large crabgrass (*Digitaria sanguinalis* L.), and goosegrass [*Eleusine indica* (L.) Gaertn.] were the dominant species.

Experiment 2

Two field studies were conducted in North Carolina in 2018 in Kinston (35.3005N, -77.5746W) and Rocky Mt. (35.8923N, -77.6796W). Soil types were Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiudults) in Kinston and Goldsboro fine sandy loam (fine-loamy, silicious, subactive, thermic Aquic Paleudults) at Rocky Mt. Soil pH at both sites ranged from 5.8 to 6.2 with <1% organic matter. Eight treatments included clomazone alone and clomazone plus sulfentrazone PRE-T. Both PRE-T treatments were followed by rimsulfuron, pendimethlalin, napropamide PD or no herbicide. Rates can be found in table 1. Pre-transplant applications were applied approximately 24 hours prior to transplanting and PD applications were applied at lay-by; approximately 6 WAT. Pre-transplant applications were applied in the same manner using the same equipment as experiment 1. Post-directed applications were made using CO_2-pressurized backpack sprayer calibrated to deliver 187 L ha^{-1} at 140 kPa with a single nozzle spray boom equipped with a TeeJet TK-VS2 flood nozzle (TeeJet Technologies, Wheaton, IL). Palmer amaranth (*Amaranthus palmeri*), yellow nutsedge (*Cyperus esculentus* L.), large crabgrass, Broadleaf signalgrass, and goosegrass were the dominant weed species. Weed densities were as follows: Palmer amaranth - 2 and 24 m^2, yellow nutsedge - 12 and 21 m^2, grasses - 17 and 54 m^2 at Kinston and Rocky Mt., respectively.

For both experiments, treatments were arranged in a randomized complete block design with three or four replications; depending on location. Standard field preparation was performed prior to planting each year. All treatments were compared against the grower standard of clomazone plus sulfentrazone PRE-T. Test sites were plowed, disked, and bedded approximately two weeks prior to transplanting. Tobacco in 2016 was transplanted April 22 and May 11 in Meadow and Oxford, respectively. In 2017, tobacco was transplanted in Kinston and Whiteville on April 11 and April 18; respectively. In 2018, tobacco was transplanted on April 30 at both Kinston and Rocky Mt. Individual plots contained four treated rows, each 4.5 m wide by 13.7 m long in Kinston and 4.9 m wide by 13.7 m at all other locations. The center two rows of each plot were used for data collection and harvest. Planting density in all environments was 14,820 plants ha^{-1}. The flue-cured tobacco cultivar NC 196 (Gold Leaf Seed Company, Hartsville, SC) was produced at the all locations; except Meadow, NC where K326 (Gold Leaf Seed Company, Hartsville, SC) was grown. Other than herbicide treatments, tobacco at all sites was produced according to North Carolina Cooperative Extension Service recommendations (Fisher, 2016).

In both experiments, visual estimates of percent crop injury were recorded bi-weekly. Crop injury was evaluated using a 0 to 100% scale where 0 = no plant injury and 100 = plant death (Frans et al. 1986). Weed control was evaluated after-first-harvest approximately 12 WAT. Tobacco plant heights from soil surface to the highest point of the top of the growing point was recorded 6WAT in experiment 1 only. Plots were harvested four times in each growing environment and leaves were cured in a forced-air bulk curing barn. Cured-leaf was then weighed to quantify yield and assigned a USDA government grade. Each government grade is associated with a numerical grade index value ranging from 1 to 100, which describes leaf maturity and ripeness (Bowman et al., 1988) as well as an associated financial value that reflects modern price indices (Fisher et al., 2016). Fifty-gram composite cured leaf samples were also collected from each treatment for analysis of percentage total alkaloids and percentage reducing sugars using the methods outlined by Davis (1976).

Data for both experiments were checked for normality and homogeneity of variance by plotting residuals prior to statistical analyses. All data were subjected to ANOVA using the PROC Mixed procedure in SAS (v. 9.4; SAS Institute, Cary, NC). Treatments were considered a fixed factor, and replication and environment were considered random factors. Treatment interactions containing replication or environment were set as random effects. Treatment means were reported using least square means. Means were separated using Fisher's Protected LSD at $P \leq 0.05$. Non-treated checks were excluded from all statistical analyses.

Results and Discussion

Experiment 1

No visual injury was observed from any PRE-T treatment, regardless of herbicide combination or rate (data not shown). Visual injury ranged from 6 to 33% 1 wk after application (WAA) from POT herbicide applications (Table 3). Within POT injury data, there was a significant rate by environment interaction ($P = <.0001$); therefore data are presented by environment (Table 3). Injury observed included yellowing, leaf mottling and distortion similar to that described in previous research using trifloxysulfuron POT (Bailey, 2007). Visual injury across both rates was greater at Meadow compared to Oxford in 2016. This can be attributed to the earlier transplanting timing and influence of cooler temperatures on slow plant growth following the POT application at this environment. In 2017, greater injury was observed with 34 g ha^{-1} compared to the 17g ha^{-1} at both field sites. Regardless of rate and environment, by 3 WAA no visual injury was observed (data not shown).

Data for plant heights are available for Oxford 2016 and Whiteville 2017 only. There was no difference in plant heights across treatments; however, within POT applications, differences were observed among rates. There was a significant environment by rate interaction ($P = 0.0408$) for plant heights 6 WAT; therefore, data are presented by environment (Table 3). At Oxford 2016, 34 g ai ha^{-1} of rimsulfuron reduced plant heights 25% compared to 17g ai ha^{-1} when applied POT. Reductions in plant heights have been reported when ALS-inhibiting herbicides are applied POT to tobacco (Bailey, 2007; Bailey and Pearce, 2014). In contrast, no differences among POT application rate were observed at Whiteville 2017.

Yield data for Meadow 2016 is not available. The environment by treatment interaction was not significant ($P = 0.1087$) for total yield, therefore data were analyzed across environments. There was no difference among treatments between individual harvests or total yield (Table 4). Early season injury and stunting observed with POT applications was transient and had no lasting effect on plant development. Similar findings have been reported in tobacco when ALS-inhibiting herbicides are applied POT (Bailey, 2007). Furthermore, no difference was observed among treatments for cured-leaf quality, value, and leaf chemistry (Table 5 and 6), which further demonstrates the transient nature of the injury observed.

There was a significant environment by treatment interaction (P = <.0001) for grass control, therefore data are presented by environment. No difference in weed control among treatments was observed at Oxford 2016 or Whiteville 2017 (Table 7). Lack of differences at these environments can be attributed to low weed pressure. At Kinston in 2017, treatments containing clomazone provided greater grass control compared to treatments without clomazone (Table 6). Regardless of whether rimsulfuron was applied PRE-T or POT, there was no difference in grass control compared to clomazone plus sulfentrazone alone PRE-T. Greater grass control was observed when rimsulfuron was applied POT 3 WAT compared to tank-mixed with sulfentrazone alone PRE-T. When data were analyzed across rates, there were no differences in weed control between the two rates of rimsulfuron (data not shown).

Experiment 2

There was no injury observed from any herbicide treatment, PRE-T or PD applications (data not shown). The environment by treatment interaction was significant for total yield (P = 0.0002), cured-leaf quality (P = 0.0016), and gross value (P = <.0001); therefore, data were analyzed by environment. There was not a significant environment by treatment interaction for total alkaloids (P = 0.8505), however, the interaction for reducing sugars (P = 0.0198) was significant; therefore leaf chemistry was analyzed by environment.

There was no difference among treatments between individual harvests or total yield at Kinston 2018 (Table 8). At Rocky Mt. 2018, there was significant differences among treatments for total yield (Table 9). Treatments consisting of clomazone and sulfentrazone PRE-T provided greater yields compared clomazone alone. These results are not surprising as sulfentrazone controls many weed species that dominate most tobacco productions systems, such as Palmer amaranth and yellow nutsedge (Fisher and Smith, 2001). Although not significant, treatments including a PD herbicide application 6 WAP provided greater yields compared to no PD application. There was no yield difference among herbicides applied PD. Significant differences were also observed with individual harvest's 1 and 3 (Table 8). In general, trends were similar to that of total yield. Pre-transplant treatments containing clomazone and sulfentrazone had greater yields across harvests compared to clomazone alone.

At Kinston 2018, there was no difference among herbicide treatments for cured-leaf quality, gross value, or leaf chemistry (Table 10 and 11). Similar to trends with yield, at Rocky Mt. 2018, both cured-leaf quality and gross value were greatest among treatments containing sulfentrazone and clomazone (Table 12). In general, more favorable leaf chemistry was observed with treatments providing greater weed control (Table 13). Differences in reducing sugars followed similar trends. Increased weed control contributes to greater yields, an increase in cured-leaf quality, more desirable leaf chemistry and overall greater value of the crop (Hauser and Miles 1975; Collins and Hawks 1993).

The environment by treatment interaction was significant for Palmer amaranth ($P = 0.0218$) and grass control ($P = 0.0171$); therefore, data were analyzed by environment. The environment by treatment interaction was not significant for yellow nutsedge control ($P = 0.1354$); therefore, data were pooled across environments. Trends for Palmer amaranth and grass control at both environments were similar, however, weed pressure was much higher at Rocky Mt. 2018 compared to Kinston 2018. In both environments, greatest Palmer amaranth control was achieved with treatments containing clomazone and sulfentrazone compared to clomazone alone (Table 13). There was no difference in control with treatments containing clomazone and sulfentrazone regardless of a lay-by herbicide. However, in most cases greater control was observed when a lay-by herbicide was applied with clomazone only treatments. In general, treatments consisting of a lay-by herbicide provided greater control of grasses compared no lay-by herbicide applied, regardless of PRE-T herbicides. For both environments, greatest control of yellow nutsedge was observed with treatments consisting of clomazone and sulfentrazone PRE-T followed by a lay-by herbicide. Similar control was also observed with clomazone only PRE-T followed by rimsulfuron at lay-by. Since being labeled, sulfentrazone has been the primary herbicide to control Palmer amaranth and yellow nutsedge in tobacco production systems (Fisher and Smith 2001; Bailey et al. 2013).

These data indicate flue-cured tobacco is tolerant to rimsulfuron applied PRE-T at rates of 17 or 34 g ai ha^{-1}. In environments where grass pressure is low, rimsulfuron has shown to be an alternative to clomazone PRE-T. Despite early season injury from over-the-top applications of rimsulfuron, no lasting effects were observed across post-harvest parameters. However, due to excessive visual injury symptoms in some situations, PD applications would be most acceptable in a flue-cured tobacco production system. Rimsulfuron applied PD at lay-by provided similar weed control and in some cases greater control than currently labeled herbicides. The addition of a lay-by herbicide did not always increase yield, however, the potential for increased weed control later in the season can help prevent harvest losses due to weed interference and foreign material in cured-leaf. Overall, rimsulfuron could provide broader options for weed control in flue-cured tobacco.

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Table 1. Herbicides and rates used in experiments 1 and 2.

Common name	Trade name	Rate (g ai ha^{-1})		Manufacturer
		Experiment 1	Experiment 2	
Clomazone	Command 3ME	840	840	FMC Corporation Agricultural Products Group
Sulfentrazone	Spartan 4F	175	175	FMC Corporation Agricultural Products Group
Rimsulfuron	Matrix SG	17 or 34	34	Dupont Crop Protection
Pendimethalin	Prowl H_2O	-	1,064	Prowl H_2O, BASF Corporation
Napropamide	Devrinol 2-XT	-	840	United Phosphorus, Inc

Table 2. Pre-transplant (PRE-T) and postemergence over-the-top (POT) herbicide combinations for experiment 1 in 2016 and 2017.[a]

PRE-T	POT	Rimsulfuron rate[c]
		g ai ha^{-1}
Clomazone + sulfentrazone	-	-
Clomazone + sulfentrazone	rimsulfuron	17
Clomazone + sulfentrazone	rimsulfuron	34
Clomazone + sulfentrazone + rimsulfuron	-	17
Clomazone + sulfentrazone + rimsulfuron	-	34
Sulfentrazone + rimsulfuron	-	17
Sulfentrazone + rimsulfuron	-	34
Sulfentrazone	rimsulfuron	17
Sulfentrazone	rimsulfuron	34

[a] Abbreviations: +, tank mix.
[b] Clomazone and sulfentrazone was applied 840 and 175 g ai ha^{-1} in all treatments; respectively.
[c] Rimsulfuron rates listed are for both PRE-T and POT applications.

Table 3. Percent crop injury 1 wk after postemergence over-the-top (POT) application and plant heights 6 weeks after transplanting as influenced by herbicide rate of POT application in experiment 1.[a]

	Visual injury				Plant heights[c]	
	2016		2017		2016	2017
Rimsulfuron rate[b]	Oxford	Meadow	Whiteville	Kinston	Oxford	Whiteville
g ai ha^{-1}	%				cm	
17	6 a	25 a	9 b	7 b	33 a	16 a
34	7 a	33 a	18 a	13 a	25 b	16 a

[a] Means followed by the same letter are not significantly different according to Tukey-Kramer at $p \leq 0.05$.
[b] Data pooled over herbicide combinations.
[c] Data available for environments presented only.

Table 4. Flue-cured tobacco yield in experiment 1 in response to herbicide treatment at Oxford 2016, and Kinston and Whiteville 2017.[a]

Treatment[b]	Rimsulfuron rate	Cured tobacco yield				
		Harvest 1	Harvest 2	Harvest 3	Harvest 4	Total
	g ai ha^{-1}	——kg ha^{-1}——				
Clomazone + sulfentrazone	-	655 a	695 a	925 a	1,440 a	3,715 a
Clomazone + sulfentrazone fb rimsulfuron	17	705 a	680 a	965 a	1,450 a	3,800 a
Clomazone + sulfentrazone fb rimsulfuron	34	645 a	790 a	995 a	1,385 a	3,815 a
Clomazone + sulfentrazone + rimsulfuron	17	680 a	720 a	1,025 a	1,555 a	3,980 a
Clomazone + sulfentrazone + rimsulfuron	34	745 a	720 a	1,055 a	1,350 a	3,870 a
Sulfentrazone + rimsulfuron	17	645 a	655 a	880 a	1,300 a	3,480 a
Sulfentrazone + rimsulfuron	34	635 a	650 a	890 a	1,140 a	3,315 a
Sulfentrazone fb rimsulfuron	17	715 a	685 a	885 a	1,245 a	3,530 a
Sulfentrazone fb rimsulfuron	34	700 a	730 a	945 a	1,330 a	3,705 a

[a] Means followed by the same letter are not significantly different according to Tukey-Kramer at $p \leq 0.05$.
[b] Abbreviations: +, tank mix; fb, followed by application 3 weeks after transplanting.

Table 5. Flue-cured tobacco quality grade index and gross value in experiment 1 in response to herbicide treatment Oxford 2016, and Kinston and Whiteville 2017.[a]

Treatment	Rimsulfuron rate	Quality index	Gross value
	g ai ha^{-1}	0-100	\$ ha^{-1}
Clomazone + sulfentrazone	-	67 a	10,940 a
Clomazone + sulfentrazone fb rimsulfuron	17	65 a	10,940 a
Clomazone + sulfentrazone fb rimsulfuron	34	70 a	12,240 a
Clomazone + sulfentrazone + rimsulfuron	17	68 a	12,145 a
Clomazone + sulfentrazone + rimsulfuron	34	69 a	12,055 a
Sulfentrazone + rimsulfuron	17	71 a	11,375 a
Sulfentrazone + rimsulfuron	34	74 a	11,145 a
Sulfentrazone fb rimsulfuron	17	73 a	11,705 a
Sulfentrazone fb rimsulfuron	34	68 a	11,395 a

[a] Means followed by the same letter are not significantly different according to Tukey-Kramer at $p \leq 0.05$.
[b] Abbreviations: +, tank mix; fb, followed by application 3 weeks after transplanting.

Table 6. Flue-cured tobacco leaf chemistry (total alkaloids and reducing sugars) in experiment 1 in response to herbicide treatment at Oxford 2016, and Kinston and Whiteville 2017.[a]

		Cured-leaf chemistry	
Treatment	Rimsulfuron rate	Total Alkaloids	Reducing Sugars
	g ai ha^{-1}	%	
Clomazone + sulfentrazone	-	2.75 a	12.25 a
Clomazone + sulfentrazone fb rimsulfuron	17	2.65 a	14.21 a
Clomazone + sulfentrazone fb rimsulfuron	34	2.95 a	12.77 a
Clomazone + sulfentrazone + rimsulfuron	17	2.67 a	12.84 a
Clomazone + sulfentrazone + rimsulfuron	34	2.59 a	14.73 a
Sulfentrazone + rimsulfuron	17	2.65 a	13.32 a
Sulfentrazone + rimsulfuron	34	2.57 a	14.21 a
Sulfentrazone fb rimsulfuron	17	2.53 a	14.08 a
Sulfentrazone fb rimsulfuron	34	2.82 a	13.46 a

[a] Means followed by the same letter are not significantly different according to Tukey-Kramer at $p \leq 0.05$.
[b] Abbreviations: +, tank mix; fb, followed by application 3 weeks after transplanting.

Table 7. Percent annual grass control 12 WAP in experiment 1 as influenced by herbicide treatment by environment.[a, b]

Treatment	rimsulfuron rate	Oxford 2016	Whiteville 2017	Kinston 2017
	g ai ha^{-1}		%	
Clomazone + sulfentrazone	-	91 a	95 a	93 ab
Clomazone + sulfentrazone fb rimsulfuron	17	91 a	94 a	96 a
Clomazone + sulfentrazone fb rimsulfuron	34	91 a	95 a	96 a
Clomazone + sulfentrazone + rimsulfuron	17	91 a	95 a	94 ab
Clomazone + sulfentrazone + rimsulfuron	34	94 a	94 a	93 ab
Sulfentrazone + rimsulfuron	17	89 a	94 a	75 d
Sulfentrazone + rimsulfuron	34	93 a	95 a	78 d
Sulfentrazone fb rimsulfuron	17	88 a	94 a	85 c
Sulfentrazone fb rimsulfuron	34	92 a	93 a	89 bc

[a]Means followed by the same letter are not significantly different according to Tukey-Kramer at $p \leq 0.05$. The non-treated control was not included in data analysis.

[b] Annual grass consisted of large crabgrass, goosegrass, and Broadleaf signalgrass.

Table 8. Flue-cured tobacco yield in experiment 2 in response to herbicide treatment at Kinston 2018.[a]

Treatment[b]	Cured tobacco yield				
	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Total
	kg ha^{-1}				
Clomazone + sulfentrazone	535 a	580 a	715 a	760 a	2,590 a
Clomazone + sulfentrazone fb rimsulfuron	570 a	465 a	680 a	535 a	2,480 a
Clomazone + sulfentrazone fb pendimethalin	675 a	630 a	805 a	720 a	2,830 a
Clomazone + sulfentrazone fb napropamide	580 a	680 a	800 a	730 a	2,790 a
Clomazone	540 a	590 a	785 a	715 a	2,630 a
Clomazone fb rimsulfuron	770 a	690 a	705 a	700 a	2,865 a
Clomazone fb pendimethalin	740 a	715 a	680 a	695 a	2,830 a
Clomazone fb napropamide	590 a	765 a	760 a	625 a	2,740 a

[a] Means followed by the same letter are not significantly different according to Tukey-Kramer at $p \leq 0.05$.
[b] Abbreviations: +, tank mix; fb, followed by application 6 weeks after transplanting.

Table 9. Flue-cured tobacco yield in experiment 2 in response to herbicide treatment at Rocky Mt. 2018.[a]

	Cured tobacco yield				
Treatment[b]	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Total
	—kg ha^{-1}—				
Clomazone + sulfentrazone	835 abc	505 a	520 a	300 a	2,160 ab
Clomazone + sulfentrazone fb rimsulfuron	1,080 ab	605 a	595 a	270 a	2,550 a
Clomazone + sulfentrazone fb pendimethalin	745 abc	560 a	550 a	605 a	2,460 a
Clomazone + sulfentrazone fb napropamide	1,110 a	765 a	580 a	275 a	2,730 a
Clomazone	340 c	170 a	190 b	165 a	865 c
Clomazone fb rimsulfuron	600 abc	385 a	190 b	215 a	1,390 bc
Clomazone fb pendimethalin	530 bc	330 a	185 b	275 a	1,320 c
Clomazone fb napropamide	510 c	390 a	390 ab	250 a	1,540 bc

[a] Means followed by the same letter are not significantly different according to Tukey-Kramer at $p \leq 0.05$.
[b] Abbreviations: +, tank mix; fb, followed by application 6 weeks after transplanting.

Table 10. Flue-cured tobacco quality grade index and gross value in experiment 2 in response to herbicide treatment at Kinston 2018.[a]

Treatment	Quality index	Gross value
	0-100	$ ha^{-1}
Clomazone + sulfentrazone	67 a	7,675 a
Clomazone + sulfentrazone fb rimsulfuron	74 a	7,310 a
Clomazone + sulfentrazone fb pendimethalin	66 a	7,670 a
Clomazone + sulfentrazone fb napropamide	64 a	7,485 a
Clomazone	67 a	7,580 a
Clomazone fb rimsulfuron	71 a	8,860 a
Clomazone fb pendimethalin	73 a	9,005 a
Clomazone fb napropamide	67 a	7,810 a

[a] Means followed by the same letter are not significantly different according to Tukey-Kramer at $p \leq 0.05$.
[b] Abbreviations: +, tank mix; fb, followed by application 6 weeks after transplanting.

Table 11. Flue-cured tobacco leaf chemistry (total alkaloids and reducing sugars) in experiment 2 in response to herbicide treatment at Kinston 2018.[a]

	Leaf chemistry	
Treatment	Total alkaloids	Reducing sugars
	%	
Clomazone + sulfentrazone	2.41 a	20.24 a
Clomazone + sulfentrazone fb rimsulfuron	2.25 a	19.85 a
Clomazone + sulfentrazone fb pendimethalin	2.34 a	20.17 a
Clomazone + sulfentrazone fb napropamide	2.27 a	21.95 a
Clomazone	2.18 a	21.12 a
Clomazone fb rimsulfuron	2.43 a	20.63 a
Clomazone fb pendimethalin	2.25 a	21.60 a
Clomazone fb napropamide	2.38 a	19.21 a

[a] Means followed by the same letter are not significantly different according to Tukey-Kramer at $p \leq 0.05$.
[b] Abbreviations: +, tank mix; fb, followed by application 6 weeks after transplanting.

Table 12. Flue-cured tobacco quality grade index and gross value in experiment 2 in response to herbicide treatment at Rocky Mt. 2018.[a]

Treatment	Quality index	Gross value
	0-100	$ ha^{-1}
Clomazone + sulfentrazone	76 a	7,420 a
Clomazone + sulfentrazone fb rimsulfuron	71 ab	8,390 a
Clomazone + sulfentrazone fb pendimethalin	71 ab	8,180 a
Clomazone + sulfentrazone fb napropamide	59 bc	7,540 a
Clomazone	55 c	2,285 b
Clomazone fb rimsulfuron	45 c	3,260 b
Clomazone fb pendimethalin	53 c	3,220 b
Clomazone fb napropamide	47 c	3,725 b

[a] Means followed by the same letter are not significantly different according to Tukey-Kramer at $p \leq 0.05$.
[b] Abbreviations: +, tank mix; fb, followed by application 6 weeks after transplanting.

Table 13. Flue-cured tobacco leaf chemistry (total alkaloids and reducing sugars) in experiment 2 in response to herbicide treatment at Rocky Mt. 2018.[a]

	Leaf chemistry	
Treatment	Total alkaloids	Reducing sugars
	%	
Clomazone + sulfentrazone	2.72 a	6.17 ab
Clomazone + sulfentrazone fb rimsulfuron	2.67 a	8.02 a
Clomazone + sulfentrazone fb pendimethalin	2.59 a	5.97 ab
Clomazone + sulfentrazone fb napropamide	2.58 a	6.22 ab
Clomazone	2.00 a	3.80 c
Clomazone fb rimsulfuron	2.33 a	6.03 ab
Clomazone fb pendimethalin	2.11 a	5.92 abc
Clomazone fb napropamide	2.34 a	4.96 bc

[a] Means followed by the same letter are not significantly different according to Tukey-Kramer at $p \leq 0.05$.
[b] Abbreviations: +, tank mix; fb, followed by application 6 weeks after transplanting.

Table 14. Palmer amaranth, yellow nutsedge, and annual grass control 12 weeks after planting for experiment 2 in response to herbicide treatment at Kinston and Rocky Mt. 2018.[a]

	Palmer amaranth		Annual grass[c]		Yellow nutsedge
Treatment[b]	Kinston	Rocky Mt.	Kinston	Rocky Mt.	Kinston/Rocky Mt.
	———————————	———————————	%	———————————	———————————
Clomazone + sulfentrazone	100 a	91 a	90 c	88 b	88 bc
Clomazone + sulfentrazone fb rimsulfuron	100 a	95 a	99 a	96 a	98 a
Clomazone + sulfentrazone fb pendimethalin	100 a	96 a	98 ab	96 a	96 ab
Clomazone + sulfentrazone fb napropamide	98 a	92 a	98 ab	93 ab	96 ab
Clomazone	28 d	20 c	93 bc	87 b	24 d
Clomazone fb rimsulfuron	46 bc	47 b	99 a	96 a	93 abc
Clomazone fb pendimethalin	51 b	45 b	94 abc	96 a	86 c
Clomazone fb napropamide	37 cd	30 bc	95 abc	96 a	89 bc

[a] Means followed by the same letter are not significantly different according to Tukey-Kramer at $p \leq 0.05$. Data are pooled over environments.
[b] Abbreviations: +, tank mix; fb, followed by application 6 weeks after transplanting.
[c] Annual grass consisted of large crabgrass, goosegrass, and Broadleaf signalgrass.