ABSTRACT

MAHONEY, DENIS JOSEPH. Biology and Management of Palmer amaranth in North Carolina. (Under the direction of Dr. David Jordan).

Palmer amaranth (*Amaranthus palmeri* S. Wats.) is one of the most challenging weeds to control in cropping systems in the US. Its robust growth, prolific seed production, and immense genetic diversity create short- and long-term management problems for producers. Overreliance on herbicides for control has led to the evolution of herbicide-resistant populations. Proper stewardship of existing and future management tools is needed to effectively control Palmer amaranth and mitigate the evolution of herbicide-resistant populations. Therefore, research trials were completed to gain a more holistic understanding of Palmer amaranth in NC including: 1) quantifying growth and seed production of this weed when competing with various row crops, 2) determining presence and distribution of herbicide-resistant populations in the NC Coastal Plain, and 3) developing management techniques to mitigate additions to the weed seedbank.

Research evaluating preemergence (PRE) and postemergence (POST) herbicide application efficacy revealed higher carrier volumes (281 and 562 L ha\(^{-1}\)) only provided 8 to 12\% higher PRE weed control compared to lower carrier volumes (70 and 140 L ha\(^{-1}\)). When followed by a POST herbicide application, the differences were less than 8\%, suggesting improved efficiencies may allow farmers to increase profitability and maintain weed control by using less water as the carrier. Resource constraints or climatic conditions may prevent the application or efficacy of PRE herbicides. Research evaluating POST herbicide application timing and frequency indicated at least two POST applications were needed to adequately control weeds while protecting soybean [*Glycine max* (L.) Merr.] yield and economic return. However, three to four sequential POST applications were needed to ensure 95 to 100\% weed control, which are more desirable control levels to manage and reduce weed seedbanks over
time. In another study, Palmer amaranth control was improved when planting increased soybean plant populations (546,000 to 778,000 plants ha\(^{-1}\)) compared to lower populations (268,000 plants ha\(^{-1}\)). Applying POST herbicides to these populations further increased control. Generally, an early POST herbicide application one week after planting (WAP) improved control compared to a late POST application three WAP. This was noted the following year in cotton (\textit{Gossypium hirsutum} L.) as lower Palmer amaranth densities were observed when an early POST application was made in soybean the previous season.

Research evaluating the growth and seed production of Palmer amaranth when emerging with and three WAP corn (\textit{Zea mays} L.), cotton, peanut (\textit{Arachis hypogaea} L.), and soybean was conducted to understand how the competing crop affects these parameters. When emerging with crops, Palmer amaranth reached 10 cm in height 17 to 18 days after planting suggesting timely POST herbicide applications are critical. Seed production for these plants was lowest in corn, followed by soybean, then peanut and cotton; however, seed production ranged from approximately 51,000 to over 500,000 seeds plant\(^{-1}\). When emerging three WAP, seed production was still immense in cotton and peanut (greater than 35,000 seeds plant\(^{-1}\)).

Resistance to glyphosate and thifensulfuron (acetolactate synthase inhibitor) was common (99 and 96%, respectively) in the 110 Palmer amaranth populations sampled from the NC Coastal Plain. However, more concerning were the survivors in populations following fomesafen (protoporphyrinogen oxidase inhibitor) and mesotrione (4-hydroxyphenylpyruvate dioxygenase inhibitor) applications. While the population-level data suggest field use rates of fomesafen will provide control, concerns about the survivors exist, especially when considering Palmer amaranth seed production. Many more populations survived the mesotrione application with confirmation of resistance by documenting heritability occurring in one population.
Increased stewardship is needed when considering the future release of HPPD-tolerant cotton and soybean. While no survivors were observed following glufosinate treatment, farm managers must be cognizant of timely applications in order to mitigate the resistance evolution and maintain efficacy long-term.
Biology and Management of Palmer Amaranth in North Carolina

by
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A dissertation submitted to the Graduate Faculty of
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Doctor of Philosophy

Crop Science

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DEDICATION

I would like to dedicate this work to my two wonderful kids who, without fully understanding, fueled, supported, and aided in the completion of this work. This research is also dedicated to my parents, Kevin and Tina Mahoney, whose support and encouragement was never ending. I am eternally grateful.
DENIS JOSEPH (DJ) MAHONEY was born and raised in Darlington, SC. He studied Turfgrass Management at Clemson University and following graduation in 2012, accepted a Research Assistant position under Dr. Travis Gannon and Dr. Matthew Polizzotto at North Carolina State University. He conducted research on arsenic movement from MSMA following application including a novel approach to quantify the effect of turfgrass management practices on arsenic distribution and species transformation. His work was presented at several Weed Science meetings and earned numerous awards. Following graduation in 2014, DJ accepted a job with DuPont Crop Protection as an Associate Investigator in the Herbicide Discovery Biology group. This experience highlighted some major issues regarding weed control and resistance the Crop Science community is facing. He then sought out a PhD program to research these issues and was offered a Research Assistant position with Dr. David Jordan. His work under Dr. Jordan focused on many aspects of Weed Science including chemical and cultural management techniques, weed ecology, physiology, and genetics. He looks forward to utilizing his skills and techniques in order to deliver innovative solutions to farmers worldwide.
ACKNOWLEDGMENTS

I am very grateful to so many people that deserve recognition for their help along this journey. In reality, this section could be just as long as my dissertation itself. Dr. Jordan, thank you for taking a chance on the “turf guy with a kid” when I reached out to you in 2016. Your guidance and thoughtful attentiveness have allowed me to explore and accomplish things in a relatively short period of time. I have learned much along the way that will stick with me for a lifetime. Dr. Vann, I sincerely appreciate you allowing me to take over your space as my projects began swallowing more and more territory and for always lending a hand directly or ensuring I always had a second when needed. Dr. Leon, your thought-provoking questions and stimulating conversations improved my work and understanding of science exponentially. Dr. Burgos, your vast understanding of plant physiology and resistance mechanisms has not only challenged me but helped guide my research which led me to a deeper understanding of both subjects. Dr. Jennings, your lending hand and thoughtful inputs improved the science behind this work drastically. Thank you all for everything over the past three years.

To my kids, thank you for your sacrifices made. I know you are too young to fully understand the breadth of those sacrifices, but they are many. All of the late nights, conference times, weekends at home so I could write have been tough, but you always have a smile and an enthusiastic energy. To Amanda, thank you for your nudge those years back to help me make the leap of faith back to school. To Krista, your help and encouragement over the past year have been so appreciated and truly immeasurable. You were always willing to lend a hand in every situation, even when that meant thinning plants until dark. To my parents and siblings, your love and support have been a constant throughout this whole process and have allowed me to achieve the goals I originally had in mind for many years.
I would be remiss to not thank others around me who have helped along this journey. Many thanks are due to Drew Hare who battled alongside me in the field and greenhouse to ensure my work was completed. I certainly would not have been able to tackle all my work had it not been for his help. I am ever in debt to Kim Howell and Jared Smith without whom, my genetics work would have been exponentially lengthier and more difficult. Your patience with me as I fumbled through the work was astounding and you were always willing to lend an ear or a hand whenever I needed. To Dr. Charlie Cahoon who answered all my questions and provided great insight along the way. Your willingness to provide help as I became shorthanded was remarkable and I will be ever appreciative. To the Tobacco and Cotton crews, many thanks for lending a hand and being willing to jump into some not-so-fun work with me. A big thank you to Drew, Ray, and Eric for all the conversations and lent hands along the way. Lastly, and certainly not least, thank you to my great friends Andy and Lexie who always had the upmost confidence I would finish and continuous encouragement along the way. I am truly blessed, humbled, and indebted to all of you that have helped me along my journey. Thank you.
# TABLE OF CONTENTS

LIST OF TABLES ........................................................................................................................................ vii
LIST OF FIGURES ....................................................................................................................................... ix

**Chapter 1: Evolved Resistance to Herbicides in Palmer Amaranth**
**Accessions Collected in the North Carolina Coastal Plain** ................................................................. 1
  Abstract ...................................................................................................................................................... 1
  Introduction ............................................................................................................................................... 3
  Materials and Methods ............................................................................................................................ 4
  Results and Discussion ............................................................................................................................. 11
  References ............................................................................................................................................... 29

**Chapter 2: The Influence of Soybean Population and Postemergence Herbicide Application Timing on In- and Subsequent-Season Weed Control and Economic Returns** ................................................................................................................. 34
  Abstract .................................................................................................................................................. 35
  Introduction ............................................................................................................................................ 36
  Materials and Methods ........................................................................................................................... 38
  Results and Discussion ........................................................................................................................... 41
  References ............................................................................................................................................... 48

**Chapter 3: Palmer Amaranth (Amaranthus palmeri) Growth and Seed Production When in Competition with Row Crops in North Carolina** ............................................................................................................................. 58
  Abstract .................................................................................................................................................. 59
  Introduction ............................................................................................................................................ 60
  Materials and Methods ........................................................................................................................... 62
  Results and Discussion ........................................................................................................................... 65
  Research Implications ............................................................................................................................ 72
  References ............................................................................................................................................... 75

**Chapter 4: The Influence of Postemergence Herbicide Timing and Frequency on Weed Control and Soybean Yield** ................................................................................................................................. 96
  Abstract .................................................................................................................................................. 96
  Weed Interference and Control in Soybean ............................................................................................. 98
  Experimental Location and Planting ....................................................................................................... 100
  Experimental design ............................................................................................................................... 101
  Weed Control, Yield, and Economic Returns ......................................................................................... 105
  Research Implications ............................................................................................................................ 108
  References ............................................................................................................................................... 110

**Chapter 5: The Effect of Nozzle Selection and Carrier Volume on Weed Control in Soybean in North Carolina** ......................................................................................................................................... 115
  References ............................................................................................................................................... 119
LIST OF TABLES

Chapter 1. Evolved Resistance to Herbicides in Palmer Amaranth Accessions Collected in the North Carolina Coastal Plain

Table 1. Herbicide treatments used for Palmer amaranth resistance screening ................................. 7
Table 2. Primers used for competitive allele specific polymerase chain reaction assays on surviving Palmer amaranth plants following an application of fomesafen ............................................... 8
Table 3. The dose of fomesafen needed to reduce Palmer amaranth biomass 50% (GR50) or kill 50% of the population (LD50) for various population from North Carolina ........................................................................................................... 21
Table 4. The dose of mesotrione needed to reduce Palmer amaranth biomass 50% (GR50) or kill 50% of the population (LD50) for various population from North Carolina ...................................................................................................................... 25

Chapter 2. Influence of Soybean Population and Postemergence Herbicide Application Timing on In- and Subsequent-Season Weed Control and Economic Returns

Table 1. Pearson correlation coefficients quantifying the relationships between in-season soybean plant population, Palmer amaranth control, and soybean yield when pooled or sorted by herbicide application timings .............................................. 53
Table 2. Pearson correlation coefficients quantifying the relationships between Palmer amaranth densities in cotton at 3, 7, and 15 WAP and cotton yield following various soybean plant populations and Palmer amaranth control in soybean the previous season ..................................................................................................................... 54

Chapter 3. Palmer Amaranth (Amaranthus palmeri) Growth and Seed Production When in Competition with Row Crops in North Carolina

Table 1. Crop varieties and planting populations at each experimental location ............................................ 79
Table 2. Herbicides utilized in each crop system ................................................................................................. 80
Table 3. Parameter estimates and goodness of fit using the four-parameter logistic function to fit male and female Palmer amaranth (Amaranthus palmeri) height when emerging with and three wks after with corn, cotton, peanut, and soybean crops ........................................................................................................................................... 81
Table 4. Parameter estimates and goodness of fit using the four-parameter logistic function to fit male and female Palmer amaranth (Amaranthus palmeri) width when emerging with corn, cotton, peanut, and soybean crops ................................................. 83
Table 5. Parameter estimates and goodness of fit using the quadratic function to fit male and female Palmer amaranth (Amaranthus palmeri) width when emerging three wks after corn, cotton, peanut, and soybean crops ........................................ 84
Chapter 4. The Influence of Postemergence Herbicide Timing and Frequency on Weed Control and Soybean Yield

Table 1. Herbicides included in the soybean studies at Rocky Mount and Lewiston, NC in 2016, 2017, and 2018 ........................................... 103

Table 2. Application timing and herbicide treatments for the soybean studies at Rocky Mount and Lewiston, NC in 2016, 2017, and 2018 ......................... 104

Chapter 5. The Effect of Nozzle Selection and Carrier Volume on Weed Control in Soybean in North Carolina

Table 1. Palmer amaranth and large crabgrass control following preemergence and postemergence applications at 4, 7, and 15 wk after treatment in Rocky Mount, NC in 2017 and 2018 ............................................................. 120
Chapter 1. Evolved Resistance to Herbicides in Palmer Amaranth Accessions Collected in the North Carolina Coastal Plain

Figures 1a-d. Survival frequencies for the 110 screened Palmer amaranth populations from the North Carolina Coastal Plain ........................................... 13

Figures 2a-c. Graphical representation of the KASP assays completed for the ΔG210, R128G, and R128M mutations .................................................................. 17

Figure 3. Number of mechanisms of action survived by the 110 total Palmer amaranth populations tested from the North Carolina Coastal Plain region ........ 18

Figure 4. Dose-response curves of biomass reduction Palmer amaranth populations from North Carolina following fomesafen application ..................... 20

Figure 5. Dose-response curves of survival for Palmer amaranth populations from North Carolina following fomesafen application .............................. 23

Figure 6. Dose-response curves of biomass reduction Palmer amaranth populations from North Carolina following mesotrione application ..................... 24

Figure 7. Dose-response curves of survival Palmer amaranth populations from North Carolina following mesotrione application .............................. 27

Chapter 2. Influence of Soybean Population and Postemergence Herbicide Application Timing on In- and Subsequent-Season Weed Control and Economic Returns

Figure 1. The influence of soybean plant population and herbicide application timing on late season Palmer amaranth control ........................................... 55

Figures 2a-b. The influence of soybean population or herbicide application timing on soybean yield ................................................................. 56

Figure 3. The influence of herbicide application timing in soybean on Palmer amaranth densities three wk after planting cotton the following season .......... 57

Chapter 3. Palmer Amaranth (Amaranthus palmeri) Growth and Seed Production When in Competition with Row Crops in North Carolina

Figure 1. Male and female Palmer amaranth (Amaranthus palmeri) heights modeled against growing degree days (base 10 C) when competing with corn, cotton, peanut, and soybean crops ............................................. 85

Figure 2. Male and female Palmer amaranth (Amaranthus palmeri) percent of maximum heights modeled against growing degree days (base 10 C) when competing with corn, cotton, peanut, and soybean crops ................. 86

Figure 3. Growing degree days required for Palmer amaranth (Amaranthus palmeri) to reach 10, 50, and 90% of maximum height when competing with corn, cotton, peanut, and soybean crops .................................. 87
Figure 4. Male and female Palmer amaranth (*Amaranthus palmeri*) growth rates by growing degree day (GDD; base 10 C) when competing with corn, cotton, peanut, and soybean crops................................................................. 88

Figure 5. Male and female Palmer amaranth (*Amaranthus palmeri*) widths modeled against growing degree days (base 10 C) when competing with corn, cotton, peanut, and soybean crops................................................................. 89

Figure 6. Fresh biomass of male and female Palmer amaranth (*Amaranthus palmeri*) from Cohort 1 at harvest (14 wks after crop planting) . ......................... 90

Figure 7. Fresh biomass of Palmer amaranth (*Amaranthus palmeri*) from Cohort 2 at harvest (17 wks after crop planting) .................................................... 91

Figure 8. Correlations between final height, width, or fresh biomass and total seed production of Palmer amaranth (*Amaranthus palmeri*) from Cohort 1 ........ 92

Figure 9. Seed production of female Palmer amaranth (*Amaranthus palmeri*) from Cohort 1 when competing with corn, cotton, peanut, and soybean crops .... 93

Figure 10. Correlations between final height, width, or fresh biomass and total seed production of Palmer amaranth (*Amaranthus palmeri*) from Cohort 2 .... 94

Figure 11. Seed production of female Palmer amaranth (*Amaranthus palmeri*) from Cohort 2 when competing with corn, cotton, peanut, and soybean crops .... 95

Chapter 4: The Influence of Postemergence Herbicide Timing and Frequency on Weed Control and Soybean Yield

Figures 1a-d. Broadleaf weed and annual grass control in twin- and narrow-row soybean as influenced by herbicide timing and frequency ....................... 113

Figures 2a-d. Soybean yield and net economic return in twin- and narrow-row soybean as influenced by herbicide timing and frequency ....................... 114
Evolved Resistance to Herbicides in Palmer Amaranth Accessions Collected in the North Carolina Coastal Plain

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**Declarations of interest:** none.

**Abstract.** Palmer amaranth (*Amaranthus palmeri* S. Wats.) populations resistant to acetolactate synthase-inhibiting herbicides and glyphosate have become commonplace throughout the state of North Carolina (NC) in the USA. This has caused farm managers to rely more heavily on herbicides with other mechanisms of action (MOA) for Palmer amaranth control, especially protoporphyrinogen oxidase- and glutamine synthetase inhibitors. In the fall of 2016, seeds from Palmer amaranth populations were collected from the NC Coastal Plain, the state’s most productive agricultural region. In separate experiments, plants with 2 to 4 leaves from the 110 populations were treated with field use rates of glyphosate (840 g ae ha\textsuperscript{-1}), glufosinate-
ammonium (451 g ai ha\(^{-1}\)), fomesafen (280 g ai ha\(^{-1}\)), mesotrione (105 g ai ha\(^{-1}\)), or thifensulfuron-methyl (17.5 g ai ha\(^{-1}\)). Percent visible control and survival were evaluated three weeks after treatment. Survival frequencies were highest and most common following glyphosate (99\%) or thifensulfuron-methyl (96\%) treatment. Forty-two populations had survivors after mesotrione application with 17\% survival in one population. Four populations survived fomesafen treatment, while none survived glufosinate. Dose-response studies showed a 1.9- to 2.6-fold increase in fomesafen needed to kill 50\% of two populations (LD\(_{50}\)); however, these rates were far below the field use rate (less than 5 g ai ha\(^{-1}\)). In two populations following mesotrione dose-response studies, a 2.4- to 3.3-fold increase was noted with LD\(_{90}\) values approaching the field use rate (72.8 and 89.8 g ai ha\(^{-1}\)). Screening of the progeny of individuals surviving mesotrione confirmed the presence of resistance alleles, which was reflected in a higher number of survivors at the 1X rate compared to the parent population, confirming resistance to mesotrione. These data suggest Palmer amaranth resistant to chemistries other than glyphosate and acetolactate synthase inhibitors are present in NC, which highlights the importance of integrated weed management approaches to mitigate the evolution and spread of herbicide-resistant populations.

**Key words:** Herbicide resistance; multiple resistance.
1. Introduction

Palmer amaranth (*Amaranthus palmeri* S. Wats.) is the most problematic weed in the United States (US) (Van Wychen et al., 2016). It is highly competitive with immense fecundity and has the ability to replenish the soil seedbank in one generation (Mahoney et al., 2019; Webster and Grey, 2015). Palmer amaranth is an obligate cross-pollinator, which has high genetic variation (Chandi et al., 2013). Its pollen can move long distances (Sosnoskie et al., 2012). Along with intense herbicide selection pressure, these characteristics have favored the evolution of Palmer amaranth populations with resistance to several mechanisms of action (MOA) with some populations expressing multiple resistance (Heap, 2019; Ward et al., 2013). The first case of herbicide-resistant Palmer amaranth was reported in South Carolina in 1989 to the microtubule assembly inhibitor trifluralin (Heap, 2019). To date, 28 states have reported herbicide-resistant Palmer amaranth, with 16 reporting resistance to two or more herbicide MOAs. Rapid occurrence and spread of herbicide-resistance in this weed continue to be of great concern for the agricultural community. Recently, Palmer amaranth populations in the mid-southern and midwestern US resistant to POST applications of protoporphyrinogen oxidase (PPO) inhibitors have been documented with resistance confirmed through genetic and metabolic pathways (Giacomini et al., 2017; Salas et al., 2017; Varanasi et al., 2018a). In North Carolina (NC), Palmer amaranth resistant to the acetyl-CoA carboxylase (ACC) inhibitor thifensulfuron was first reported in 1995 followed by reports of resistance to glyphosate in 2005 (Heap, 2019). In the most recent screening of 134 Palmer amaranth populations collected in 2010 predominantly from the NC Coastal Plain, Poirier et al. (2014) reported more than 95% populations were resistant to the ALS inhibitor thifensulfuron-methyl or glyphosate with 95% of those populations
being resistant to both herbicides. Additionally, these populations were screened with fomesafen (PPO-inhibitor) and glufosinate (glutamine synthetase inhibitor) and no resistance was detected.

While the research by Poirier et al. (2014) was informative, a more current resistance screen is needed due to the recent reported resistance to PPO inhibitors in the mid-southern and midwestern US and the potential for rapid evolution of herbicide-resistant Palmer amaranth populations. Additionally, a baseline survey is needed for 4-hydroxphenylpyruvate dioxygenase (HPPD) inhibitors for future resistance monitoring as the release of HPPD-tolerant cotton and soybean varieties approaches (Wechsler, 2018). Early detection and characterization of herbicide-resistant populations is critical in order to mitigate the spread of resistance and maintain the efficacy of current herbicide technologies long-term through adequate stewardship programs (Burgos et al., 2013). Therefore, the objectives of this research were to 1) quantify the frequency of ALS- and glyphosate-resistant Palmer amaranth populations from the NC Coastal Plain and 2) determine the presence of and characterize glufosinate-, HPPD-, and PPO-resistant populations.

2. Materials and Methods

2.1 Plant materials

In the fall of 2016, Palmer amaranth populations were collected from fields predominantly in the NC Coastal Plain, the state’s primary row crop producing region (USDA, 2018a). Field samplings were not predetermined and was done at random when enough females were present in a field for collection. The sampling region was selected to ensure it overlapped with the area sampled in previous resistance screenings completed in NC by Whitaker (2009) and Poirier et al. (2014) to allow for inferences about changes in herbicide resistance frequencies over time. Generally, 10 to 15 female plants were selected from a random 10 by 10 m area within
a field. Seeds were collected from each female plant and pooled to represent the variability of the population in each field. In all, 125 populations were collected from the following cropping systems: 12 in cotton (*Gossypium hirsutum* L.), 34 in peanut (*Arachis hypogaea* L.), 58 in soybean (*Glycine max* (L.) Merr.), and 21 in sweetpotato (*Ipomoea batatas* (L.) Lam.).

Herbicides applied during the season were not determined for any field. Plant material was transported to the Method Road Greenhouse Complex (Raleigh, NC), where it was air-dried, threshed, and cleaned. Cleaned seed was stored at 4 C until testing.

**2.2 Herbicide resistance bioassay**

Due to poor germination of some populations, only 110 of the 125 were tested with all herbicides. In the greenhouse, approximately 30 seeds cell\(^{-1}\) were sown into 50-cell trays (Greenhouse Megastore, Danville, IL, USA) containing potting soil mix (Sungro Fafard 4P Mix, Agawam, MA, USA) and watered daily. The greenhouse was maintained at 35 ± 5 C with metal halide lighting (400 µmol m\(^{-2}\) s\(^{-1}\); Hubbell Lighting Inc., Greenville, SC, USA) supplementing natural light for 14 h daily. After emergence, plants were thinned to one plant cell\(^{-1}\) with 10 continuous cells equaling one replication. Each experiment consisted of five replications and was repeated in time.

In separate experiments, Palmer amaranth at the 2- to 4-leaf stage was treated with fomesafen, glyphosate, glufosinate-ammonium, mesotrione, or thifensulfuron-methyl at the rates shown in Table 1. Nontreated plants from each population were included in all experiments. Herbicides (excluding mesotrione) and rates were similar to those previously used by Poirier et al. (2014) when screening NC Palmer amaranth populations for herbicide resistance. Per label recommendations, a nonionic surfactant (0.25% v v\(^{-1}\); Induce adjuvant, Helena Chemical Co., Collierville, TN, USA) was included with fomesafen and thifensulfuron-methyl. Also, a liquid
solution of urea ammonium nitrate (32% N) at 4.7 L ha\(^{-1}\) was included with thifensulfuron-methyl. Mesotrione treatments included 1% (v v\(^{-1}\)) crop oil concentrate (Agri-Dex, Helena Chemical Co., Collierville, TN, USA) and 4.7 L ha\(^{-1}\) urea ammonium nitrate. Herbicides were applied with a CO\(_2\)-pressurized backpack sprayer (8002EVS nozzle) calibrated to deliver 187 L ha\(^{-1}\) at 207 kPa.

Three weeks after treatment, injury was visually estimated on a scale 0 to 100% (0 = no visible injury and 100 = plant death) and survivors were counted. Visual estimates of percent control included foliar chlorosis and necrosis and plant stunting. Survival was determined by counting the number of survivors (i.e. plants with functional green leaves and apical meristems) and dividing by the number of plants treated per replication. Data were subjected to ANOVA using PROC GLIMMIX in SAS (SAS 9.4, SAS Institute Inc., Cary, NC, USA). Palmer amaranth population and experimental run were considered fixed variables with replication considered as random. Populations completely controlled with the tested rates in both experimental runs were considered as the control group (i.e., no resistant individuals). Dunnett’s Procedure (\(\alpha = 0.05\)) was utilized to determine significant difference for survival frequencies. For the PPO-inhibitor screen, a known resistant population from Arkansas was included (Salas et al., 2017).

2.3 KASP assays for Palmer amaranth surviving PPO-inhibitors

Plants surviving following the fomesafen application were transplanted into 15-cm diameter pots filled with the same growth media. After allowing regrowth for approximately two weeks, 100 mg (± 5 mg) of young leaf tissue were clipped from the plant and placed in a 96-well plate. Plates were then placed in an air-tight container with silica gel mesh desiccant (Silica gel 6-12 mesh desiccant Grade H Type II; VWR International, LLC., Radnor, PA, USA) for three days until fully desiccated. A stainless-steel bead was added to each tube, capped, and placed in
a -80 C for one to two days. Tissues were then ground (Geno/Grinder 2000, PEX SamplePrep., Metuchen, NJ, USA) at 1,600 strokes min\(^{-1}\) for one to two minutes and then placed back in the -80 C overnight. The following morning DNA was then extracted following the sbeadex plant kit (NAP41615; LGC Biosearch Technologies, Middlesex, UK) protocol using an oKtopure\textsuperscript{TM} robotic platform (LGC Biosearch Technologies, Middlesex, UK). Concentration of DNA was then determined, and concentrations were adjusted to approximately 5 to 10 ng µL\(^{-1}\). Using an Apricot liquid handler (Personal Pipettor; Apricot Designs, Inc., Covina, CA, USA), 3 µl of molecular biology reagent grade water (Sigma-Aldrich, Inc., St. Louis, MO, USA) was pipetted into 384-well polymerase chain reaction (PCR) plates and spun to ensure the water was at the well bottom. The handler was then used to transfer 2 µL of extracted DNA solution to the 384-well plates and spun. Plates were then dried at 65 C for 1 hour. At least 2 blank wells per 96-well plate were included.

Table 1. Herbicide treatments used for Palmer amaranth resistance screening.

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Trade name</th>
<th>Rate (g ai ae ha(^{-1}))(^{a})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glufosinate</td>
<td>Liberty 280 SL</td>
<td>451</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>Roundup PowerMax II</td>
<td>840</td>
</tr>
<tr>
<td>Fomesafen</td>
<td>Reflex</td>
<td>280</td>
</tr>
<tr>
<td>Mesotrione</td>
<td>Callisto</td>
<td>105</td>
</tr>
<tr>
<td>Thifensulfuron-methyl</td>
<td>Harmony 50 SG</td>
<td>17.5</td>
</tr>
</tbody>
</table>

\(^{a}\)Based on the product labels, all herbicides were applied at a full labeled rate.

Thifensulfuron-methyl rate was based on sulfonylurea-tolerant soybean.
Table 2. Primers used for competitive allele specific polymerase chain reaction assays (KASP) to detect reported mutations conferring resistance to protoporphyrinogen oxidase inhibitors in surviving Palmer amaranth plants following an application of fomesafen at 280 g ai ha\(^{-1}\).

<table>
<thead>
<tr>
<th>Name(^a)</th>
<th>Sequence (5’ – 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔG210 mutant</td>
<td>GAAGGTGACCAAGTTCTAGTCTAGCAGGTGAGATCTCCACA</td>
</tr>
<tr>
<td>ΔG210 wild type</td>
<td>GAAGGTGAGGAAGAATGCTGATTGAGATGCTCCACC</td>
</tr>
<tr>
<td>ΔG210 common reverse(^b)</td>
<td>GCAGTTTGTTATAGTGTTATGACCCTTT</td>
</tr>
<tr>
<td>R128G mutant</td>
<td>GAAGGTGACCAAGTCTAGTCTAGCAGGTGAGATCTCCACA</td>
</tr>
<tr>
<td>R128G wild type</td>
<td>GAAGGTGACCAAGTCTAGTCTAGCAGGTGAGATCTCCACA</td>
</tr>
<tr>
<td>R128M mutant</td>
<td>GAAGGTGACCAAGTCTAGTCTAGCAGGTGAGATCTCCACA</td>
</tr>
<tr>
<td>R128M wild type</td>
<td>GAAGGTGACCAAGTCTAGTCTAGCAGGTGAGATCTCCACA</td>
</tr>
<tr>
<td>R128G/M common reverse(^b)</td>
<td>CTTATAAATTGCTTTTCTGTCACAGCCAA</td>
</tr>
</tbody>
</table>

\(^a\)Abbreviations: Glycine 210 deletion, ΔG210; arginine to glycine substitution, R128G; arginine to methionine substitution, R128M.

\(^b\)Common reverse primers amplify the region of interest for both mutant and wild type sequences.
Genomic DNA sequences surrounding the known mutations conferring resistance to PPO-inhibitors in Palmer amaranth were developed based on Giacomini et al. (2017) and Salas et al. (2017) for the G210 and R198 mutations, respectively (Table 2). Kompetitive allele specific PCR (KASP) assays were completed according to the LGC group (LGC, 2019). Resistant, wild type, and common primer final concentrations were 0.16, 0.16, and 0.32 µM, respectively. Following the 34 base thermocycling procedure, fluorescence was read using a (PHERAstar Plus, BMG LABTECH Inc., Cary, NC, USA) and data analyzed using the KlusterCaller software (LGC Biosearch Technologies, Middlesex, UK). Additional cycles (3 per run) were completed as needed to ensure clustering.

2.4 Herbicide dose response bioassay

A dose response bioassay was performed on populations with the highest survival following the fomesafen and mesotrione resistance screening. Populations EDG16 and HAL16 (2 and 10% survival, respectively) were included in the fomesafen bioassay while BLA16 and EDG16 (17 and 8% survival, respectively) were included in the mesotrione assay. Adequate plant numbers allowed for progeny to be collected from BLA16 survivors following the mesotrione resistance bioassay and included in the dose response study. Two populations that were 100% controlled in the initial herbicide resistance bioassay (DUP16 and NAS16) were used as field-collected “susceptible populations.” A documented PPO- and HPPD-susceptible Palmer amaranth population (SS) was also included in the dose-response bioassay (Chaudhari et al., 2017). Dose response bioassays for glyphosate and thifensulfuron-methyl were not conducted as these have been well defined in previous work (Chaudhari et al., 2017; Poirier et al., 2014; Whitaker, 2009). Palmer amaranth seeds were planted in 10-cm square pots (Greenhouse Megastore, Danville, IL, USA) containing potting soil mix (Sungro Fafard 4P Mix, Agawam,
MA, USA) and watered daily. Seedlings were thinned to three plants pot\(^{-1}\). When plants reached the 4- to 6-leaf stage, fomesafen and mesotrione were applied as previously described with 280 and 105 g ai\(^{-1}\), respectively, considered as 1X rates. Herbicide rates (nine total) for fomesafen ranged from 0.1 to 631 g ai ha\(^{-1}\) (1/2,919 to 2.25X rate) while mesotrione rates ranged from 0.8 to 210 g ai ha\(^{-1}\) (1/128 to 2X rate). A nontreated control was included in each replication. Unfortunately, the BLA16 progeny population had poor germination and all rates were not able to be tested. As such, mesotrione was screened for this populations at 0.25, 0.5, and 1X rates to compare mortality against the original field population to determine resistance heritability. Eight total plants were screened at 0.25 and 0.5X rates while twelve total plants across the two runs (four or six replications of one plant pot\(^{-1}\) ) were treated at the 1X rate.

The experiment was a randomized complete block design with four replications and was repeated two and three times for fomesafen and mesotrione, respectively. At two (fomesafen bioassay) and three weeks (mesotrione bioassay) after treatment, survivors were counted, and the aboveground tissue was harvested. Aboveground tissue samples were placed in a brown paper bag and dried for three to four days at 65 C. Percent dry biomass reduction compared to the nontreated control was calculated from these samples. Survival and biomass reduction data were fit in SigmaPlot v.14 (Systat Software, Inc., San Jose, CA, USA) to a four-parameter logistic model

\[
y = c + (d - c) \left/ \left(1 + \left(\frac{x}{m}\right)^{-b}\right)\right.\]

[1]

where \(y\) is the percent biomass reduction relative to the nontreated control or survival, \(c\) is the minimum value, \(d\) is the maximum value, \(m\) is the point in the curve halfway between the minimum and maximum values, and \(b\) is the hillslope. The dose needed to kill 50% of the
population (LD\textsubscript{50}) or reduce the biomass by 50% (GR\textsubscript{50}) was calculated using the above equation.

3. Results and Discussion

3.1 Herbicide resistance bioassay and genetic assays

All Palmer amaranth populations were controlled 100% by glufosinate (data not shown). Poirier et al. (2014) observed similar results with 134 Palmer amaranth populations collected predominantly from the NC Coastal Plain during 2010. In 2018, glufosinate-tolerant cotton and soybean varieties accounted for approximately 65 and 20% of all US plantings, respectively (Unglesbee, 2018; USDA, 2018b). When considering the large genetic diversity in Palmer amaranth and extensive gene flow between populations, one can deduce that great stewardship is required in order to reduce selection pressure, contain the dispersal of emerging resistant genotypes, and maintain those MOAs that are still effective long-term (Chandi et al., 2013; Franssen et al., 2001; Powles and Yu, 2010). However, the data suggest that with proper herbicide rate and application timing, glufosinate can effectively manage resistant biotypes within these populations that escape other herbicide MOAs discussed below. Additionally, while no survivors were observed following glufosinate applications, the data can be utilized as a benchmark for future screenings of Palmer amaranth from the region.

Palmer amaranth survival frequency from glyphosate treatment differed across populations (P < 0.0001; F = 15.6) with only one population being completely controlled by glyphosate (Figure 1a). Of the 110 populations tested, nine had a relatively low survival (1 – 30%) with 20 consisting of moderate survival (31 – 60%). However, six of the nine low-survival populations (2 – 14% range) were not significantly different from the control (P > 0.05) suggesting lack of genetic homogeneity, thus causing high error variance. Most populations (72)
had high survival (61 – 90%), with the remaining eight populations surviving at > 90%. Overall, 80 of the 110 populations, or roughly 73%, had survival greater than 60%. The authors acknowledge that sampling plants in the fall may bias survival in those plants from cotton and soybean fields, as glyphosate was most likely used. The survival frequencies from glyphosate treatment differed significantly among cropping systems (P < 0.0001; F = 39.3) with being highest in soybean (83%) followed by cotton and sweetpotato (70 and 69%, respectfully) then peanut (62%). Thus, a high number of survivors were found in populations from every cropping system. This is expected because glyphosate-resistant cotton, corn, and soybean cultivars are widely grown in rotation by farmers across this region of NC (USDA, 2018a). Crop rotation maybe practiced, but in the majority of cases, the base chemical weed control across crops contains glyphosate. Therefore, there is generally no relief from glyphosate selection pressure. Palmer amaranth allowed to produce seed at the end of the season, like those selected for this research, may occur in many systems thus adding seed to the soil seedbank which may remain viable for years after burial (Sosnoskie et al. 2011).

Resistance to ALS inhibitors was high, as indicated by high survival frequencies following thifensulfuron-methyl across populations with only four populations being completely controlled. Most survival fell into the low to moderate categories (Figure 1b); although 37 populations (2 – 16% range) were not significantly different from the control (P > 0.05), again suggesting genetic heterogeneity within these populations. Excluding those, 32 populations had low survival with 31 categorized as moderately resistant, all of which had significantly more survivors than the designated control. While survival was relatively low compared to those with glyphosate, the rate applied was four times the manufacturer’s recommended rate for non-
sulfonylurea-tolerant soybean, suggesting that these survivors are resistant (Anonymous, 2012).

The remaining six populations had high survival of 61 – 80%.

Figure 1a-d. Survival frequencies for the 110 screened Palmer amaranth populations from the North Carolina Coastal Plain following an application of a) glyphosate (840 g ae ha\(^{-1}\)), b) thifensulfuron-methyl (17.5 g ai ha\(^{-1}\)), c) fomesafen (280 g ai ha\(^{-1}\)), and d) mesotrione (105 g ai ha\(^{-1}\)). All herbicides were applied at a full labeled rate.

Thifensulfuron-methyl rate was based on sulfonylurea-tolerant soybean. Plants were designated survivors if the plants had functional green leaves and apical meristems.

The survival and resistance observed in the tested populations were like those reported by Poirier et al. (2014), who sampled in the same general region. Those researchers designated resistance in a population if any survivors were present following glyphosate and thifensulfuron-
methyl at 840 g ae ha\(^{-1}\) and 17.5 g ai ha\(^{-1}\), respectively, which were the same rates evaluated in our study. Poirier et al. (2014) determined that 98 and 97\% of the 134 tested Palmer amaranth populations contained individuals resistant to glyphosate and thifensulfuron-methyl, respectively. Comparing our populations under the same metrics, 99 and 96\% were resistant to these herbicides, respectively. These data suggested that resistance to glyphosate and thifensulfuron has remained relatively similar from 2010 to 2016. Whitaker (2009) screened 290 Palmer amaranth populations collected from NC in 2005 for resistance to glyphosate and thifensulfuron-methyl and reported that 49 (17\%) and 52 (18\%) populations were resistant to these herbicides, respectively. These data illustrate how in a five to ten-year period there was a rapid and widespread incidence of glyphosate- and ALS-resistant Palmer amaranth occurring in NC.

The resistance to ALS inhibitors and glyphosate has created limited PRE and POST herbicide control options for Palmer amaranth in crops such as cotton, peanut, and soybean (NCSU, 2019). Growers have increased reliance on PPO-inhibitor herbicides, such as fomesafen, in these crops in order to manage the resistant biotypes. While 106 of the 110 populations were completely controlled with fomesafen, four populations had 1 – 10\% survivors (Figure 1c). Three of the four populations had very low survival of 1 – 2\%, yet one population (EDG16), was in the 10\% range. The populations with 2 (HAL16) and 10\% survival were statistically different from the susceptible control (P = 0.0222 and < 0.0001, respectively). Survivor for the four populations (1 – 10\%) were less than the Arkansas resistant standard (36\% survival; P < 0.0001). While seemingly very low survival, when one considers the immense fecundity of Palmer amaranth, these frequencies can quickly increase if selection pressure remains high. Previous research has shown that Palmer amaranth in competition with cotton may produce up to 312,000 seeds plant\(^{-1}\) (Webster and Grey, 2015). More recently in NC, the average seed production from
Palmer amaranth in competition with corn, cotton, peanut, and soybean was approximately 51,000, 534,000, 443,000, and 273,000 seeds plant$^{-1}$, respectively (Dissertation chapter 2).

Other states in the mid-southern and midwestern US have documented that genetic mutations (Giacomini et al., 2017; Salas et al., 2017) and enzymatic herbicide metabolism (Varanasi et al., 2018a) are the mechanisms conferring resistance to PPO-inhibitors in their Palmer amaranth populations. In North Carolina, ragweed was determined to have a single nucleotide polymorphism at the R98 position (synonymous with R128 in Palmer amaranth) conferring resistance to PPO-inhibitors (Heap, 2019). Figures 2a-c illustrate KASP analysis for the ΔG210, R128G, and R128M mutations which are known to confer resistance to PPO-inhibiting herbicides (Giacomini et al., 2017; Salas et al., 2017). Survivors from the Arkansas populations all contained the ΔG210 mutation (Figure 2a) which has been previously reported in this population (Salas et al., 2017). Survivors from the NC populations did not express the ΔG210 mutation (Figure 2a), with no population expressing the R128G (Figure 2b) or R128M mutations (Figure 2c). While no resistance cluster occurred for the R128 mutations to visualize this, the primers designed for these mutations were in a manner which FAM fluorescence would be expressed for the wild-type or non-mutant DNA. Fluorescence readings suggested amplification occurred and was confined to the FAM fluorescence region suggesting the wild-type DNA was amplified. Further research to characterize these populations which will be discussed later.

Sixty-eight populations were completely controlled by mesotrione (Figure 1d). Of the remaining 42 surviving populations, 41 had survival in the 1 – 10% range with 37 of these having less than 5% survival. Unlike the populations surviving fomesafen, these populations were located in many counties across the NC Coastal Plain. Populations having 2% survival or
less (30 total) were not significantly different from the susceptible control (P > 0.05) likely due to lack of genetic homogeneity, thus causing large error variance. One population from Bladen County, NC consistently had higher survival, at 17% on average. These Palmer amaranth populations surviving mesotrione are the first documented incidence of resistance to a HPPD inhibitor in NC. Currently, HPPD-resistant Palmer amaranth has been reported in Kansas, Nebraska, and Wisconsin (Heap, 2019). Nakka et al. (2017) reported that rapid detoxification of mesotrione within a Palmer amaranth populations from Kansas compared to a susceptible (2.45-fold faster) was one mechanism conferring resistance. Additionally, this population had a 12-fold increase in HPPD gene expression compared to the susceptible, which also contributed to the observed resistance. Corn production area in NC averaged approximately 359,000 total ha over the past 10 years (USDA, 2018a). Mesotrione is labeled for weed control in corn (Anonymous, 2018). With the oncoming release of HPPD-tolerant cotton and soybean, the area treated with mesotrione could potentially increase nearly 3.4-fold or reach 1,215,000 ha in NC alone (Unglesbee, 2018; USDA, 2018a). Proper stewardship for this technology is a must; otherwise, evolution of HPPD-resistant Palmer amaranth populations will occur quickly and become widespread.
**Figures 2a-c.** Graphical representation of the KASP assays completed for the ΔG210 (a), R128G (b), and R128M (c) mutations. Populations are color coded according to the legend in Figure 2a. Four populations from North Carolina are represented (EDG16, HAL16, PNL16, and UPC16) along with the herbicide-resistant standard from Arkansas (AR).
Figure 3. Number of mechanisms of action survived by the 110 total Palmer amaranth populations tested from the North Carolina Coastal Plain region.

Of the 110 Palmer amaranth populations screened, none were completely controlled by all five tested MOAs and only three were controlled by all but one MOA (Figure 3). Most of these populations (65) were controlled by all but two MOAs, which were largely glyphosate and thifensulfuron-methyl. Greater cause for concern lies with Palmer amaranth populations with individuals surviving three (40) or four (2) MOAs. Based on our survey, growers can utilize glufosinate as one tool to manage these populations and mitigate further spread; however, great care should be taken to ensure the optimal application timing and rate are used to mitigate any survivors and reduce selection pressure. These populations illustrate the importance of proper herbicide stewardship in order to protect our current chemical weed management tools as well as to ensure the longevity of coming technologies. Farm managers must ensure that an integrated
weed management approach is implemented and that escapes are minimized to mitigate additions of potentially resistant weed seeds to the soil seedbank.

3.2 Herbicide dose response assays

Further characterization of populations surviving HPPD- and PPO-inhibitors was done. Dose-response assays were conducted to determine the level of resistance compared to a known susceptible population. Increasing the dose of fomesafen reduced the dry weight and survival of all populations tested. The fomesafen dose required to cause a 50% reduction of Palmer amaranth biomass (GR$_{50}$) was 6.3 to 10.7 times greater for all putative resistant populations compared to the susceptible standard (Figure 4 and Table 3); however, these doses were still approximately 80 times lower than the field use rate of fomesafen. Even as the GR$_{90}$ for EDG16 (9.3 g ai ha$^{-1}$) and HAL16 (9.8 g ai ha$^{-1}$) were 2.1 and 2.2 times greater than the SS (4.4 g ai ha$^{-1}$), respectively, these dosages were well below the field use rate. The fomesafen dose required to kill 50% of the population (LD$_{50}$) was 1.4 to 2.6 times greater in the four tested Palmer amaranth populations compared to the susceptible (Figure 5 and Table 3). However, these values are also far below the field use rate of fomesafen. As with the GR$_{90}$, the LD$_{90}$ of EDG16 and HAL16 were well below the field use rate. While this may suggest a decrease in susceptibility of the population, the data suggest field use rates will still adequately control these populations.

Salas et al. (2016) observed greater increases in the R/S ratios of fomesafen-resistant Palmer amaranth compared to a susceptible population and reported the ΔG210 mutation within the PPX2 gene conferred this resistance. Additional research from Arkansas has noted the presence of R128G and R128M resistance-conferring mutations, which have proven to confer resistance, are also present in the state (Varanasi et al., 2018a). In addition to the target site mutations, metabolic resistance has also been indirectly implicated in one Palmer amaranth
population from Arkansas (Varanasi et al. 2018b). The authors suggested that resistance was due to an increase in cytochrome P450 monooxygenases (P450s) and glutathione S-transferases (GSTs) activity.

**Figure 4.** Dose-response curves based on biomass reduction of Palmer amaranth populations from North Carolina following fomesafen application. Biomass reduction (% of nontreated) was recorded 14 days after fomesafen application. Treatments means (n = 12) plus one standard error are plotted with the regression curves. A four-parameter logistic model best described the data, $y = c + \{(d - c)/(1 + (x/m)\exp(-b))\}$ where $y$ is the biomass reduction, $c$ is the minimum value, $d$ is the maximum value, $m$ is the point in the curve halfway between the minimum and maximum values, and $b$ is the hillslope. The dotted vertical line denotes the field use rate.
The known resistance-conferring *PPO* gene mutations were not found in the NC Palmer amaranth populations (Figures 2a-c) which had survivors at the field use rate of fomesafen, suggesting survival may either be metabolism-based, conferred by a novel (but weak) target site mutation, or translocation-based. While overall survival, GR$_{50}$, and LD$_{50}$ values were relatively low in these populations, they are still concerning due to the immense fecundity of Palmer amaranth (Mahoney et al., 2019). Previous research by Norsworthy et al. (2014) has illustrated how quickly resistance can spread throughout a field. Within three years of introduction, Palmer amaranth infested 95 to 100% of the field. This demonstrates the critical need for a zero-tolerance policy for survivors as one escape can become an increasing problem in a short amount of time.

**Table 3.** The dose of fomesafen needed to reduce Palmer amaranth biomass 50% (GR$_{50}$) or kill 50% of the population (LD$_{50}$) for populations from North Carolina, USA.$^a$

<table>
<thead>
<tr>
<th>Population</th>
<th>GR$_{50}$ (g ai ha$^{-1}$)</th>
<th>R/S$^b$</th>
<th>LD$_{50}$ (g ai ha$^{-1}$)</th>
<th>R/S</th>
</tr>
</thead>
<tbody>
<tr>
<td>DUP16</td>
<td>3.5 ± 0.3$^c$</td>
<td>10.7</td>
<td>3.5 ± 0.3</td>
<td>1.4</td>
</tr>
<tr>
<td>EDG16</td>
<td>3.4 ± 0.5</td>
<td>10.5</td>
<td>6.4 ± 0.6</td>
<td>2.6</td>
</tr>
<tr>
<td>HAL16</td>
<td>2.6 ± 0.4</td>
<td>7.9</td>
<td>4.7 ± 0.5</td>
<td>1.9</td>
</tr>
<tr>
<td>NAS16</td>
<td>2.1 ± 0.2</td>
<td>2.1</td>
<td>3.8 ± 0.4</td>
<td>1.5</td>
</tr>
<tr>
<td>SS$^d$</td>
<td>0.3 ± 0.2</td>
<td>---</td>
<td>2.5 ± 0.2</td>
<td>---</td>
</tr>
</tbody>
</table>

$^a$The 1X rate for fomesafen is 280 g ai ha$^{-1}$.

$^b$Resistance levels (R/S) calculated using the GR$_{50}$ or LD$_{50}$ values of the field collected populations relative to the susceptible standard.

$^c$Value ± standard error.

$^d$Susceptible standard.
As with fomesafen, increasing the dose of mesotrione reduced the dry biomass and survival of all populations tested. The dose required to cause a 50% reduction in Palmer amaranth biomass (GR$_{50}$) was 1.2- to 2.8-fold greater in all populations compared to the susceptible standard (Figure 6 and Table 4). Populations BLA16 and EDG16 also had 1.6- to 2.3-fold increase in GR$_{50}$ compared to the other designated “susceptible” populations (DUP16 and NAS16). The GR$_{50}$ for populations BLA16 (13.4 g ai ha$^{-1}$) and EDG16 (11.4 g ai ha$^{-1}$) were about 12.5% of the POST field use rate. The GR$_{90}$ for these populations were 65.8 and 43.5 g ai ha$^{-1}$, respectively, which was 1.9 to 2.9 time greater than the SS (23.1). The dose required to kill 50% of the Palmer amaranth populations (LD$_{50}$) was 1.2 to 3.3 times greater in all populations compared to the susceptible standard (Figure 7; Table 4) with BLA16 and EDG16 again having increased LD$_{50}$ values compared to DUP16 or NAS16 (1.6- to 2.7-fold). The LD$_{90}$ rates for HAL16 (89.8 g ai ha$^{-1}$) and EDG16 (72.8 g ai ha$^{-1}$) were increased compared to the susceptible standard (33.4 g ai ha$^{-1}$) and represent 86 and 69% of the full field use rate, respectively. Of note, three and two plants out of 36 sprayed from BLA16 and EDG16, respectively, survived the field use rate while two survived the 2X rate from the BLA16 population.

While these GR$_{90}$ and LD$_{90}$ rates approximately half- and three-quarters of the field use rate, respectively, it is generally accepted that plants are more susceptible to herbicides when grown in the greenhouse compared to the field. Nonetheless, these rates may occur in the field which allow these plants to produce seed. Reduced rates can be realized if the weed is too large at application, weather conditions prohibit adequate control, or weeds are present in difficult-to-spray areas (i.e. turnarounds, field edges, etc.) where seed can then be distributed at harvest or by tillage the following season (Norsworthy et al., 2012). Repeated exposure to (unintentional)
reduced rates can lead to resistance evolution over several generations (Busi and Powels, 2009; Busi et al., 2013).

Figure 5. Dose-response curves of survival for Palmer amaranth populations from North Carolina following fomesafen application. Survival (%) was recorded 14 days after fomesafen application. Treatments means (n = 12) plus one standard error are plotted with the regression curves. A four-parameter logistic model best described the data, $y = c + \left(\frac{d - c}{1 + (x/m)\exp(-b)}\right)$ where $y$ is the percent survival, $c$ is the minimum value, $d$ is the maximum value, $m$ is the point in the curve halfway between the minimum and maximum values, and $b$ is the hillslope. The dotted vertical line denotes the field use rate.
Figure 6. Dose-response curves of Palmer amaranth populations from North Carolina, USA following mesotrione application, using biomass reduction data. Biomass reduction (% of nontreated) was recorded 21 days after mesotrione application. Treatments means (n = 16) plus one standard error are plotted with the regression curves. A four-parameter logistic model best described the data, \( y = c + \frac{(d - c)}{[1 + (x/m)\exp(-b)]} \) where \( y \) is the biomass reduction, \( c \) is the minimum value, \( d \) is the maximum value, \( m \) is the point in the curve halfway between the minimum and maximum values, and \( b \) is the hillslope. The dotted vertical line denotes the field use rate.

When comparing the BLA16 progeny population at the 0.25, 0.5, and 1X rate to the original field population, survival after mesotrione treatment was increased in the progeny population. Both the field population (19 of 36 plants) and progeny (8 of 8 plants) had significant survival at the 0.25X rate. Survival of the progeny remained high at the 0.5X rate (7 of 8 plants)
but was less for the field population (9 of 36 plants). At the 1X rate, only 3 of 36 plants survived from the field population while 6 of 12 plants survived among the progeny population. These data demonstrate that the ability to survive mesotrione treatment was inherited by the progeny of the BLA16 population. The frequency of survivors also increased in the progeny. Thus, we documented the first HPPD-resistant Palmer amaranth population in the southeastern US.

Table 4. The dose of mesotrione needed to reduce Palmer amaranth biomass 50% (GR$_{50}$) or kill 50% of the population (LD$_{50}$) for various population from North Carolina, USA.

<table>
<thead>
<tr>
<th>Population</th>
<th>GR$_{50}$ (g ai ha$^{-1}$)</th>
<th>R/S$^a$</th>
<th>LD$_{50}$ (g ai ha$^{-1}$)</th>
<th>R/S $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLA16</td>
<td>13.6 ± 3.5</td>
<td>2.8</td>
<td>38.0 ± 2.7</td>
<td>3.3</td>
</tr>
<tr>
<td>DUP16</td>
<td>6.0 ± 1.5</td>
<td>1.2</td>
<td>14.1 ± 1.1</td>
<td>1.2</td>
</tr>
<tr>
<td>EDG16</td>
<td>11.4 ± 2.4</td>
<td>2.3</td>
<td>27.5 ± 2.1</td>
<td>2.4</td>
</tr>
<tr>
<td>NAS16</td>
<td>7.2 ± 2.0</td>
<td>1.5</td>
<td>17.1 ± 1.2</td>
<td>1.5</td>
</tr>
<tr>
<td>SS$^c$</td>
<td>4.9 ± 1.6</td>
<td>---</td>
<td>11.5 ± 0.9</td>
<td>---</td>
</tr>
</tbody>
</table>

$^a$Resistance levels (R/S) calculated using the GR50 or LD50 values of the field collected populations relative to the susceptible standard.

$^b$Value ± standard error.

$^c$Susceptible standard.

Resistance to HPPD-inhibiting herbicides has previously been documented in *Amaranthus* species. Kaundun et al. (2017) reported a mesotrione-resistant waterhemp (*Amaranthus tuberculatus* (Moq.) Sauer) population from Nebraska, USA having a 45.5-fold higher GR$_{50}$ compared to a susceptible population. The authors hypothesized that resistance could be due to increased metabolism or reduced translocation of mesotrione in the plant.
Olivera et al. (2017) reported a 13.2-fold increase in mesotrione required to achieve 50% control of another waterhemp population from Nebraska compared to a susceptible population. The authors also reported a 17.8-fold increase in GR$_{50}$ using dry biomass data value of the resistant vs. the susceptible population. Reported R/S ratios for HPPD-resistant Palmer amaranth populations are generally lower than what has been observed in waterhemp. Jhala et al. (2014) determined a 4.6- to 4.9-fold increase in GR$_{50}$ values for mesotrione when comparing a resistant Palmer amaranth population to two susceptible populations. To achieve 50% control of the resistant population, 3.5- to 4.7-fold more mesotrione was needed. The mechanism for resistance was not determined, but cross resistance to other HPPD-inhibiting herbicides was noted. Küpper et al. (2018) reported a 3.3-fold increase in LD$_{50}$ of a Palmer amaranth population from Nebraska with tembotrione. The authors hypothesized that metabolism, likely by cytochrome P450s, conferred the resistance. A higher level (10.1- to 17.8-fold) of Palmer amaranth resistance to mesotrione was reported in Kansas (Nakka et al. 2017). In this case, the resistant plants metabolized mesotrione faster and also produced more copies of the $HPPD$ gene, were determined to confer resistance in this population. Until today, HPPD-resistant Palmer amaranth has not been documented in the southeast (Heap, 2019). Further research and characterization on the EDG16 and HAL16 populations is warranted.

Resistance to glyphosate and ALS-inhibitor herbicides within Palmer amaranth populations continue to be commonplace throughout the NC Coastal Plain region. Farm managers often use other herbicide MOAs to combat resistant populations. The data suggest little to no fitness penalties associated with these resistance traits (Ward et al., 2013), which allowed the rapid spread of resistance alleles in the NC Coastal Plain with time. Widespread resistance to glyphosate and ALS inhibitors has imposed heavy reliance on other herbicides such as
glufosinate and PPO-inhibitors. While the production of glufosinate-tolerant crops has continued to increase (USDA, 2018b), no survivors following glufosinate application were observed in any population at the tested rate. Of great concern are the survivors following fomesafen and mesotrione applications.

**Figure 7.** Dose-response curves of Palmer amaranth populations from North Carolina, USA following mesotrione application, using survival data. Survival (%) was recorded 21 days after mesotrione application. Treatments means (n = 16) plus one standard error are plotted with the regression curves. A four-parameter logistic model best described the data, $y = c + \{(d - c)/(1 + (x/m)\exp(-b))\}$ where $y$ is the percent survival, $c$ is the minimum value, $d$ is the maximum value, $m$ is the point in the curve halfway between the minimum and maximum values, and $b$ is the hillslope. The dotted vertical line denotes the field use rate.
More populations were observed surviving mesotrione application than surviving fomesafen; however, because of the immense fecundity of Palmer amaranth, even the relative low incidence of survival following fomesafen is still worrisome. Two populations (BLA16 and EDG16) had 1.6- to 3.3-fold increase in LD$_{50}$ values compared to three susceptible populations. Furthermore, BLA16 was confirmed resistant to mesotrione based on increased survivors and GR$_{50}$ value in the progeny population compared to the parent population. This is the first detected HPPD-resistant population in the southeastern US and is a concern, considering the oncoming release of HPPD-tolerant cotton and soybean. A coordinated effort among growers is necessary to monitor the populations in their current location and prevent their spread to others. Future work will seek to characterize these surviving populations and determine multiple-resistance frequencies and the mechanisms conferring the potential resistance.

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The Influence of Soybean Population and Postemergence Herbicide Application Timing on In- and Subsequent-Season Weed Control and Economic Returns

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Abstract

Overreliance on herbicides for weed control has led to the evolution of herbicide-resistant Palmer amaranth populations. Farm managers should consider the long-term consequences of their short-term management decisions, especially when it pertains to the soil weed seedbank. The objectives of this research were to determine how soybean population and POST herbicide application timing affects in-season weed control and soybean yield and how those variables influence weed control and cotton yields the following season. Soybean was drilled (19-cm row spacing) at a low, medium, and high population (268,000, 546,000, and 778,000 plants ha⁻¹, respectively). Herbicides were applied at the VE, V1, or V2 to V3 soybean growth stage. Nontreated plots were also included to assess the effect of population alone. The following season, cotton was planted directly behind the soybean treatments to understand the management influence on Palmer amaranth densities from soybean in the subsequent crop. Using an herbicide increased Palmer amaranth control compared to no treatment. When applied at the V1 or V2 to V3 soybean stage, weed control in the high population increased 17 to 23% compared to the low population. Economic return was not influenced by soybean population and was increased 72 to 94% with herbicide application compared to no treatment. In the subsequent cotton, Palmer amaranth densities were 24 to 39% lower at 3 wk after planting when following soybean sprayed with herbicides compared to soybean without herbicides. Additionally, Palmer amaranth densities in cotton were 19% lower when soybean was treated at the VE stage compared to later stages. Thus, increasing soybean population can improve Palmer amaranth control without adversely effecting economic returns and can reduce future weed densities. Reducing the weed seedbank and selection pressure from herbicides are critical in mitigating resistance evolution. To that end, integrating some simple cultural adjustments provide significant benefits.

**Key words:** weed interference, resistance management, cultural practices.

**Introduction**

Controlling weeds is critical in agricultural systems as they have the potential to reduce yields, crop quality, and harvesting efficiencies (Jones et al. 1997; Smith et al. 2000; Ward et al. 2013). Herbicides are an essential tool for weed control as they increase farming efficiencies and profits and have also allowed for soil conservation practices (Kudsk and Streibig 2003; Price et al. 2011). However, overreliance on herbicides for weed control has led to the evolution of herbicide-resistant weed populations and has threatened some of these beneficial practices (CAST 2012; Kudsk and Steibi 2003; Price et al. 2011). Integrated approaches recommended to reduce selection pressure from herbicides for weed control include, but are not limited to, proper herbicide application timing, optimum crop row spacing, cover cropping, crop rotation, and optimum seeding rates (Bell et al. 2015; Harker 2013; Johnson and Hoverstad 2002; Mischler et al. 2010; Vann et al. 2016).

Increasing plant population has been proven to increase weed control but brings forth concerns regarding increased seed cost due to technology fees of herbicide-tolerant crops (Bell et al. 2015; Harder et al. 2007; Hoffner et al. 2012; Nice et al. 2001; Schultz et al. 2015). In soybean, prior to resistant weed problems, a second application of glyphosate increased the profit margin over increasing seeding rates (Norsworthy and Oliver 2001). In other words, investing in more glyphosate application was more profitable than increasing the soybean population to
control weeds. Other researchers reported increased economic returns with glyphosate- and glufosinate-tolerant soybean planted at high (342,000 to 560,000 plants ha\(^{-1}\)) vs low (122,000 to 221,000 plants ha\(^{-1}\)) densities (Hoffner et al. 2012). Harder et al. (2007) reported a similar trend when glyphosate-tolerant populations ranged from 124,000 to 445,000 plants ha\(^{-1}\). Yet, the previous studies may not be representative with the onset and widespread incidence of glyphosate-resistant Palmer amaranth which continues to be one of the most troublesome and damaging weeds in soybean not only in North Carolina (NC; Poirier et al. 2014), but throughout the United States (Heap 2019).

While preemergence (PRE) herbicides are generally recommended for weed management programs, resource constraints or climatic conditions may prevent farmers from applying PRE herbicides, or compromise the efficacy of such herbicides (Everman et al. 2018). Herbicide application timing is critical when relying on a POST herbicide programs to ensure adequate control is achieved. Gower et al. (2002) applied glyphosate to glyphosate-resistant corn (\textit{Zea mays} L.) when giant foxtail (\textit{Setaria faberi} Herm.) was 5-, 10-, or 15-, 23-, or 30-cm in height. When late single applications were made (23- or 30-cm height), weed control was generally better compared to a single early (5-cm height) application. Less control with the early treatment was attributed to weed re-infestation; however, greater weed interference occurred with late applications and resulted in reduced corn yields. In another study, a mixed, natural weed population in corn was treated with a single application of glyphosate when average weed heights were 5-, 10-, 15-, 30- or 60-cm (Nelson 2007). Weed density (plants m\(^{-2}\)) and biomass (g m\(^{-2}\)) were greater when glyphosate was applied to 5-cm tall weeds in one of two years. The same result was obtained in in soybean across two years of study. Therefore, applying glyphosate too early is not advisable as it would allow for higher weed re-infestation. Weed re-infestation after
an early herbicide application, from a late-season flush of weeds, may adversely affect the soil weed seedbank as the late flush may still produce seed. Palmer amaranth can continually throughout the growing season and has the ability to produce seed even when emerging many weeks after the crop (Jha and Norsworthy 2009; Webster and Grey 2015). Additionally, control may be adversely affected if contact herbicides are applied late, when the weeds are large, allowing for regrowth and eventual seed production (Bellinder et al. 2003; Corbett et al. 2004; Tharp et al. 1999).

Much of the previous research has focused on glyphosate-based programs. Currently in NC, the most troublesome broadleaf weed in soybean is Palmer amaranth, which commonly possesses multiple resistance to glyphosate and acetolactate synthase (ALS) inhibitor herbicides (Poirier et al. 2014; Van Wychen 2016). Coupling chemical and cultural weed control is needed to effectively manage Palmer amaranth presently and when considering future management challenges. Data are needed on how soybean population and POST herbicide application timing influence in-season Palmer amaranth control and soybean yield. Additionally, a missing aspect in previous research is how management practices in one season affect weed populations in a subsequent crop. Thus, the objectives of this study were to 1) determine how soybean population and POST herbicide application timing affect in-season weed control and soybean yield and 2) how those variables influence weed control and cotton yields the following season.

**Materials and Methods**

**In-season soybean.** Five field experiments were conducted at the Upper Coastal Plain Research Station (35.897 N, -77.675 W) near Rocky Mount, NC, in 2016, 2017, and 2018. Soils were an Aycock very fine sandy loam (fine-silty, siliceous, subactive, thermic Typic Paleudult) and a
Goldsboro fine sandy loam (fine-loamy, siliceous, subactive, thermic Aquic Paleudult). Soybean (AG 69X6; Monsanto Co., St. Louis, MO) was planted in mid- to late-June on 19-cm rows using a grain drill following disk ing and field cultivation. Seeding rate was altered in order to provide low, medium, and high soybean populations. Stowe et al. (2018) recommends approximately 415,000 plants ha\(^{-1}\) for June-planted soybean on 19-cm rows in NC. Soybean stand counts recorded 2 wk after planting (WAP) showed that populations averaged approximately 268,000, 546,000, and 778,000 plants ha\(^{-1}\) for the low, medium and high populations, respectively. Plot sizes were 3.7 m by 10.7 to 13.7 m.

The trials were arranged in a randomized complete block design with four replications. In addition to the effect of plant population, herbicide application timing was also evaluated. For this treatment, fomesafen (Reflex, Syngenta Crop Protection, LLC., Greensboro, NC) and clethodim (Select 2EC, Valent USA Co., Walnut Creek, CA) were applied at 280 and 210 g ai ha\(^{-1}\), respectively, at 7 to 8 days after planting (DAP; EPOST), 14 to 16 DAP (MPOST), or 21 to 25 DAP (LPOST). Soybean was at the VE, V1, or V2 to V3 growth stages at these timings, respectively (Fehr et al. 1971). Nontreated plots were included to evaluate the effect of soybean population alone.

Herbicides were applied with a hand-held, CO\(_2\)-pressurized backpack sprayer calibrated to deliver 140 L ha\(^{-1}\) at 131 kPa fitted with AIXR 11002 nozzles (TeeJet\(^{\circledast}\) flat-fan nozzles, Spraying Systems Co., Wheaton, IL). Other production practices, including fertility, insect and disease management, were conducted in accordance with Cooperative Extension Service recommendations for North Carolina (Stowe et al. 2018). Palmer amaranth control was visually estimated on a scale of 0 to 100 (0 = no control and 100 = complete control) approximately 18 WAP. Foliar chlorosis, necrosis, plant stunting, and reduction in weed population and size were
considered when making the visual ratings. Soybean was harvested using a combine and yield was adjusted to 13% moisture content.

A soybean production budget created by the North Carolina State University Agriculture and Resource Economics Department was used to estimate net economic return (Bullen et al. 2019). The conventional-till soybean production system in the Coastal Plain region of NC was customized for the seeding rates, hauling cost, and herbicide treatments. Seeding rates were approximately 315,000, 642,000, and 915,000 seeds ha\(^{-1}\) for the low, medium, and high populations, respectively. Seed costs for Xtend and Roundup Ready soybean were both used in calculating net returns and were priced at $0.36 and $0.32 1,000-seeds\(^{-1}\), respectively. A custom herbicide application cost of $12.36 ha\(^{-1}\) was added to the $55.18 ha\(^{-1}\) herbicide cost. Average soybean prices from 2010 to 2018 ($0.41 kg\(^{-1}\)) was estimated from the United States Department of Agriculture Nations Agriculture Statistics Service (USDA-NASS 2019).

**Subsequent cotton.** The following season, cotton DP 1646 B2XF (Monsanto Company, St. Louis, MO) was planted behind the soybean crop into conventionally-prepared seed beds (91-cm row spacings) at a seeding rate of approximately 143,500 seeds ha\(^{-1}\). A PRE herbicide was not applied following planting, allowing for Palmer amaranth counts to be determined behind each soybean treatment from the previous year. While the existing seedbank was not quantified, previous research has shown that the average viability of Palmer amaranth seed buried 1- to 10-cm was 20 to 30% compared to seed buried only 6 (66 to 74%) to 9 months (54 to 64%; Sosnoskie et al. 2013). Therefore, the Palmer amaranth densities observed are likely to predominantly be seeds of plants from the previous season. In-row Palmer amaranth densities were quantified at 3 WAP by averaging counts from three 0.5-m\(^2\) samples taken randomly from the two center rows of the four-row plots. After counting, dicamba (Engenia, BASF Co.,
Research Triangle Park, NC) plus glyphosate (Roundup PowerMax II, Monsanto Co., St. Louis, Mo.) was applied at 561 and 947 g ai ha\(^{-1}\), respectively. Application parameters were similar to those used in soybean except TTI 10002 nozzles (TeeJet flat-fan nozzles, Spraying Systems Co., Wheaton, IL) were used in accordance with label recommendations. At 7 WAP (4 wk after POST), the weeds were counted again then the plots were sprayed with glyphosate (947 g ha\(^{-1}\)) one week later. A late-season count was done 19 WAP (15 wk after POST). Cotton was harvested in late October using a small-plot, spindle picker.

**Data analysis.** All data collected in soybean and cotton were analyzed using PROC GLIMMIX in SAS (SAS 9.4, SAS Institute Inc., Cary, NC). Palmer amaranth counts taken in cotton were square root transformed in order to meet normality. Soybean population, herbicide application timing, and their interaction were considered fixed effects, while replication and environment (year by field combination) were considered random. Means were separated according to Fisher’s protected LSD at \(\alpha = 0.05\). Relationships among variables were determined using Pearson Correlation coefficients with the correlation procedure (PROC CORR) in SAS.

**Results and Discussion**

**In-season soybean.** A significant positive relationship between soybean population and Palmer amaranth control was observed when pooled over all environments and herbicide application timings (\(P < 0.0001, r = 0.40\)) suggesting that weed control increases as soybean population increases regardless of herbicide application (Table 1). When examining the relationship of soybean population and Palmer amaranth control by herbicide treatment timing, a significant positive relationship was also present and increased as herbicides were applied later in the season (\(P < 0.0001\) to 0.0012, \(r = 0.37\) to 0.70). These results suggest that application timing is critical
for adequate weed control and becomes more critical as soybean population decreases, placing
more pressure on the herbicide for control. Plant populations and yield were correlated overall (P
= 0.0005, r = 0.22), but only with nontreated soybean (P = 0.0151, r = 0.35) when examining
relationships by herbicide timing.

The interaction of soybean population and herbicide application timing influenced Palmer
amaranth control (P = 0.0017; F = 5.4). When herbicides were not applied, Palmer amaranth
control increased as soybean population increased (17 to 38% increase); however, compared to
any soybean population which received an herbicide application, regardless of timing, control
was 14 to 81% lower (Figure 1). When herbicides were applied EPOST, control was similar (86
to 95%) across soybean populations. When soybean population was low, the EPOST herbicide
application timing was more effective than LPOST application (20% increase). When herbicide
was applied LPOST, Palmer amaranth control was improved with the high soybean population
compared to the low population (23% increase). Additionally, when application was delayed
until LPOST, the medium population provided increased control (18% increase) compared to the
low population. Comparing across populations, when the medium and high soybean populations
were treated EPOST, control was increased compared to the low population treated either
MPOST (22 to 24% increase, respectively) or LPOST (27 to 29% increase, respectively).

Norsworthy and Oliver (2001) reported an average of two glyphosate applications (1.12 kg ai ha−
1 each) was required to maintain 90% weed control or greater when soybean seeding rates ranged
from 185,000 to 741,000 seeds ha−1. This dropped to an average of 1.3 applications at seeding
rates above 800,000 seeds ha−1. Higher soybean seeding rates (676,000 plants ha−1) has been
shown to reduce the density and seed production of the difficult-to-control weed sicklepod
[Senna obtusifolia (L.) H.S. Irwin & Barneby] compared to a lower population of 245,000 plants
ha\(^{-1}\) (Nice et al. 2001). Likewise, in another study, the overall weed biomass was less at high soybean populations of 302,000 to 445,000 plants ha\(^{-1}\) compared to that with 192,000 plants ha\(^{-1}\) in (Harder et al. 2007). Herbicide application timing is critical to achieve acceptable control and herbicide efficacy can be improved tremendously with increased soybean populations. High soybean populations alleviate the pressure on herbicide efficacy, thus mitigating the evolution of herbicide-resistant populations.

Only the main effects of soybean population (P = 0.0017; F = 13.9) and herbicide application timing (P = 0.0038; F = 7.9) both influenced soybean yield, but only herbicide application timing affected net economic returns (P < 0.0081; F = 6.4). When averaged over herbicide applications, the low population yielded less (2,880 kg ha\(^{-1}\)) than the medium (3,250 kg ha\(^{-1}\)) and high populations (3,420 kg ha\(^{-1}\)), which were similar (Figure 2a). When pooled over soybean populations, the EPOST (3,520 kg ha\(^{-1}\)), MPOST (3,290 kg ha\(^{-1}\)), and LPOST (3,420 kg ha\(^{-1}\)) timings resulted in similar yields and were greater than the nontreated yield (2,490 kg ha\(^{-1}\); Figure 2b). Similar results were obtained with respect to net economic return. Differences in net return were not detected between soybean cultivars (Xtend and Roundup Ready soybean) and an average seed cost ($0.34 1,000-seeds\(^{-1}\)) was used in the analysis. When soybean was not treated, net returns were lower ($402.82 ha\(^{-1}\)) than the treated soybeans which produced $693.78 to $783.61 ha\(^{-1}\) net returns.

These results suggest that regardless of herbicide application timing, a higher soybean population will reduce weed interference and provide higher yields. Additionally, regardless of the plant population, herbicides remain critical for providing high yields. Higher soybean populations did not reduce net economic return, yet improved Palmer amaranth control. Long-term, the producer can utilize higher populations in tandem with an herbicide program to
mitigate weed seed returning to the soil seed bank while not adversely affecting their annual income. Removing pressure from herbicides for weed control is critical to mitigate resistance evolution. Increasing plant populations to improve weed control may not always be profitable in the short-term. A study by Norsworthy and Oliver (2001) showed increasing soybean yield with population density from 185,000 to 988,000 seeds ha\(^{-1}\), but with reduced gross profit margins at the higher plant populations. This difference may be due to the primary weed being barnyardgrass \([Echinochloa crus-galli (L.) Beauv]\), which is less competitive with, and easier to control in, soybean than \(Amaranthus\) species (Hock et al. 2006). Harder et al. (2007) observed increased soybean yield when herbicides were used compared to no herbicide, regardless of soybean population. Within treatments, the medium and high populations had greater yields than did the low population. The medium soybean population slightly increased net returns compared to the low population, but high and low soybean populations provided similar returns.

**Subsequent cotton.** Significant negative correlations between Palmer amaranth control in soybean the previous year or soybean plant population with Palmer amaranth density in cotton were noted. At 3 WAP cotton, correlation of Palmer amaranth density in cotton with soybean Palmer amaranth control or soybean plant population were both significant \((r = -0.52, P < 0.0001; \text{Table 2})\). By seven WAP cotton, the correlations were weaker with soybean Palmer amaranth control \((r = -0.31, P < 0.0001)\) and soybean plant population \((r = 0.15, P = 0.0122)\), but still evident. By 15 WAP cotton, the correlations were not significant \((P > 0.16)\). These data suggest that as soybean plant population and Palmer amaranth control in soybean increases, Palmer amaranth densities the following season decreases up to 7 WAP cotton. Reducing future weed populations is an important herbicide-resistance evolution mitigation strategy, allowing existing technologies greater longevity (Bagavathiannan and Davis 2018; Norsworthy et al.)
While most farmers will apply PRE herbicides in cotton, inclement weather can prevent the application or inadequate rainfall after application may reduce the efficacy. Minimizing deposits to the weed seedbank in soybean thorough greater crop competition provides high long-term weed management value.

When considering the density count timings individually, only the main effect of herbicide application timing in soybean influenced Palmer amaranth densities in cotton 3 WAP ($P < 0.0001$, $F = 7.7$). Pooled over soybean planting populations, when no herbicide was applied, densities were greatest at 88 plants m$^{-2}$ (Figure 3). Comparing herbicide application timings, Palmer amaranth densities in cotton were lower following the EPOST timing (54 plants m$^{-2}$) in soybean compared to the LPOST timing (66 plants m$^{-2}$), with the MPOST being intermediate (60 plants m$^{-2}$). By 7 WAP cotton, Palmer amaranth densities were not influenced by soybean planting population ($P = 0.8100$), herbicide application timing ($P = 0.3564$), nor their interaction ($P = 0.1363$). Palmer amaranth infestation ranged from 12 to 29 plants m$^{-2}$ (data not shown). At 15 WAP cotton, Palmer amaranth densities ranged from 36 to 57 plants m$^{-2}$ and was not influenced by any treatment in the previous season ($P > 0.30$).

The reduction of Palmer amaranth densities 3 WAP cotton is an important aspect for weed seedbank and resistance management. In soybean, Palmer amaranth control was numerically greatest in the EPOST treatment in general, followed by MPOST and LPOST (Figure 1), which likely contributed to the reduced Palmer amaranth infestation in cotton the following season (Figure 3). While the chances of spontaneous mutation rates are low (Mortimer et al. 1992), the fact remains that the chances of selecting herbicide-resistant weeds increase as the number of individuals placed under the selection pressure increases. Reducing the number of seeds returned to the soil seedbank and the number of individuals exposed to selection pressure.
with herbicides will, in turn, minimize the rate of resistance evolution (Bagavathiannan and Davis 2018; Neve et al. 2011; Norsworthy et al. 2018).

Cotton lint yield was negatively related to Palmer amaranth densities at 3 and 15 WAP (Table 2). The data show that as Palmer amaranth densities increased, cotton yield decreased. Unlike Palmer amaranth densities in cotton following the soybean crop, relationships of cotton lint yield with the previous soybean plant population (P = 0.2959) nor Palmer amaranth control in soybean (P = 0.7114) were not significant. This is further supported by the ANOVA results which showed that cotton lint yield was not influenced by soybean plant population (P = 0.8934), herbicide application timing in soybean (P = 0.3201), nor their interaction (P = 0.2709) the previous season. Cotton lint yield ranged from 1,070 to 1,240 kg lint ha\(^{-1}\) with no apparent treatment effects (data not shown). This is likely due to the relatively high Palmer amaranth densities in the study. Average Palmer amaranth densities never fell below 10 plants m\(^{-2}\).

Increasing the number of herbicides and/or the diversity of herbicide mechanisms of action in a single season did not decrease economic return in glyphosate-tolerant cropping systems (Edwards et al. 2014). In our work, increasing the soybean population did not cause a decrease in economic return even though there was a greater expense due to higher seed cost. Greater weed control with no reduction in short-term economic gain also results in fewer weed seed entering the soil seedbank. In the same study reported by Edwards et al. (2014), the best management practices that resulted in similar short-term economic returns also resulted in fewer weeds in the soil seedbank (Gibson et al. 2015). Increasing the soybean plant population can be
seen in the same light as increasing herbicide diversity and/or the number of herbicide applications when considering integrated management strategies. Both approaches (increased plant population and additional herbicides) make positive impacts on long-term weed management without negatively impacting short-term economics.

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North Carolina soybean production guide. AG-835


Table 1. Pearson correlation coefficients quantifying the relationships between in-season soybean plant population, Palmer amaranth control, and soybean yield when pooled or sorted by herbicide application timings.\(^{a,b}\)

<table>
<thead>
<tr>
<th></th>
<th>Pooled</th>
<th>EPOST</th>
<th>MPOST</th>
<th>LPOST</th>
<th>Nontreated</th>
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<tbody>
<tr>
<td></td>
<td>P &gt; F</td>
<td>r</td>
<td>P &gt; F</td>
<td>r</td>
<td>P &gt; F</td>
</tr>
<tr>
<td>Soybean population vs</td>
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<td>0.40</td>
<td>0.0012</td>
<td>0.37</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Palmer amaranth control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Soybean population vs</td>
<td>0.0005</td>
<td>0.22</td>
<td>0.1542</td>
<td>0.17</td>
<td>0.2582</td>
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<tr>
<td>soybean yield</td>
<td></td>
<td></td>
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</tbody>
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\(^a\)Abbreviations: EPOST, early postemergence; MPOST, mid-postemergence; LPOST, late postemergence.

\(^b\)Herbicide application timings for the EPOST, MPOST, and LPOST corresponded to the VE, V1, and V2 to V3 soybean growth stages, respectively.
Table 2. Pearson correlation coefficients quantifying the relationships between Palmer amaranth densities (plants m\(^{-2}\)) in cotton at 3, 7, and 15 wk after planting (WAP) and cotton yield following various soybean plant populations and Palmer amaranth control in soybean the previous season.

<table>
<thead>
<tr>
<th></th>
<th>3 WAP</th>
<th>7 WAP</th>
<th>15 WAP</th>
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<tr>
<td></td>
<td>P &gt; F</td>
<td>r</td>
<td>P &gt; F</td>
</tr>
<tr>
<td>Soybean population in previous season vs Palmer amaranth density in cotton</td>
<td>0.0001</td>
<td>-0.52</td>
<td>0.0122</td>
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<tr>
<td>Palmer amaranth control in previous soybean vs Palmer amaranth density in cotton</td>
<td>0.0001</td>
<td>-0.52</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Palmer amaranth density in cotton vs Cotton lint yield</td>
<td>0.0001</td>
<td>-0.26</td>
<td>0.1030</td>
</tr>
</tbody>
</table>
Figure 1. The influence of soybean plant population and herbicide application timing on late season (approximately 18 wk after planting) Palmer amaranth control. Soybean populations averaged 268,000, 546,000, and 778,000 plant ha\(^{-1}\) for the low, medium, and high populations, respectively. Early POST (EPOST), mid POST (MPOST), and late POST (LPOST) timings corresponded to VE, V1, and V2 to V3 soybean growth stages, respectively. Bars with the same letters are not significantly different according to Fisher’s Protected LSD at \(\alpha = 0.05\).
Figure 2A-B. The influence of soybean population (A) or herbicide application timing (B) on soybean yield. Early POST (EPOST), mid POST (MPOST), and late POST (LPOST) herbicide timings corresponded to VE, V1, and V2 to V3 soybean growth stages, respectively. Bars with the same letters are not significantly different according to Fisher’s Protected LSD at $\alpha = 0.05$. 
Figure 3. The influence of herbicide application timing in soybean on Palmer amaranth densities three wk after planting cotton the following season. Early POST (EPOST), mid POST (MPOST), and late POST (LPOST) timings corresponded to VE, V1, and V2 to V3 soybean growth stages, respectively. Bars with the same letters are not significantly different according to Fisher’s Protected LSD at α = 0.05.
Palmer amaranth (Amaranthus palmeri) growth and seed production when in competition with row crops in North Carolina

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Abstract. Palmer amaranth (*Amaranthus palmeri* S. Wats.) is a highly competitive weed that can produce short- and long-term problems for farm managers. Research to date has not quantified the growth and development of *A. palmeri* in a manner that allows direct comparisons across cropping systems. Characterizing competition-related parameters would enable better planning of short- and long-term management strategies. Therefore, the objective of this study was to determine the growth and development of male and female *A. palmeri* when competing with corn, cotton, peanut, and soybean. Additionally, total seed production was measured for each cohort of female *A. palmeri* plants in each crop. Two cohorts of *A. palmeri* were allowed to emerge either with the crop (Cohort 1) or three wks after planting (Cohort 2). Height and width measurements were recorded throughout the growing season with fresh biomass and seed production for female plants quantified at harvest. *Amaranthus palmeri* in Cohort 1 reached 10 cm in height approximately 17 to 18 d when it emerged with corn, cotton, peanut, or soybean. Plants in Cohort 2 reached 10 cm in height approximately 3 wk after emergence in cotton, peanut, or soybean while four wks were needed in corn. *Amaranthus palmeri* seed production was greatest in cotton and peanut (approximately 534,00 and 443,000 seed plant$^{-1}$, respectively), followed by soybean (approximately 173,000 seed plant$^{-1}$), and finally corn (approximately 51,000 seed plant$^{-1}$). Seed production from *A. palmeri* in Cohort 2 was still high. Plants competing with cotton and peanut produced greater than 35,000 seed plant$^{-1}$. Plants produced less seeds when competing with soybean (approximately 3,700 seed plant$^{-1}$) or corn (approximately 200 seed plant$^{-1}$). Thus, cotton and peanut were less competitive with *A. palmeri* than soybean or corn. These data illustrate the importance of applying PRE herbicides and adding residual herbicides to POST application for *A. palmeri* management.
**Key words:** Crop competition, fecundity, weed interference

**Introduction**

Palmer amaranth (*Amaranthus palmeri* S. Wats.) is regarded as one of the most problematic weeds in the United States (US; Van Wychen 2016). It possesses robust growth habits (Keeley et al. 1987; Spaunhorst et al. 2018) and has been shown to effectively compete against crops for nutrients (Knight et al. 2017), light (Meyers et al. 2010), and water (Berger et al. 2015). *Amaranthus palmeri* is an obligate cross-pollinator, which possesses a high amount of genetic variation (Chandi et al. 2013), and its pollen can move long distances (Sosnoskie et al. 2012). Along with immense herbicide selection pressure, these characteristics have led to *A. palmeri* populations having evolved resistance to several mechanisms of action with some populations expressing multiple resistance (Heap 2019; Ward et al. 2013). These competitive characteristics make this weed highly problematic. If not controlled adequately, *A. palmeri* threatens the economic viability of major agronomic and horticultural crops (Burke et al. 2007; Knight et al. 2017; Meyers et al. 2010; Webster and Grey 2015).

Understanding the biology and ecology of weeds is critical in order to mitigate and manage herbicide-resistant populations with particular attention required for weed seedbank dynamics (Bagavathiannan and Davis 2018). Previous research has explored some of these aspects with respect to *A. palmeri*. In Kansas, Horak and Loughin (2000) reported that the height of mid- to late-June emerging *A. palmeri* increased from 0.18 to 0.21 cm growing-degree-day$^{-1}$ (GDD) and reached maximum heights greater than 2 m. When a second cohort emerged approximately three weeks later, height increases were 0.11 to 0.17 cm GDD$^{-1}$ with a maximum height of 174 cm. In Indiana, Spaunhorst et al. (2018) described a similar growth rate of 0.14 to 0.17 cm GDD$^{-1}$ for regionally distinct *A. palmeri* accessions, not competing with crops, when
emerging in mid-June. When *A. palmeri* is growing without competition, it can reach heights of 160 to 269 cm and produce over 613,000 seeds female\(^{-1}\) (Horak and Loughin 2000; Keeley et al. 1987; Sellers et al. 2003; Spaunhorst et al. 2018). When in competition with cotton in Arkansas, *A. palmeri* emerging 0 and 3 wk after cotton reached approximately 138 and 105 cm in height, respectively (Norsworthy et al. 2016). Seed production was still high in these females and was estimated to be 20,000 to 90,000 seeds plant\(^{-1}\). In Georgia, male and female *A. palmeri* attained final heights of approximately 150 and 130 cm when emerging at 0 and 3 wk after cotton, respectively (Webster and Grey 2015). Female plants produced approximately 300,000 and 100,000 seeds plant\(^{-1}\) at these emergence timings, respectively. Differences in seed production across regions and years are influenced by environmental factors, as well as genotypic and ecotypic differences between the populations studied.

While the aforementioned studies are informative, important knowledge gaps exist. For example, much of the previous research has been completed when *A. palmeri* was not growing in competition with crops (Horak and Loughin 2000; Keeley et al. 1987; Sellers et al. 2003; Spaunhorst et al. 2018). Furthermore, the reference studies were primarily located in the mid-southern to western US (Horak and Loughin 2000; Keeley et al. 1987; Norsworthy et al. 2016; Sellers et al. 2003; Spaunhorst et al. 2018). Competition studies have been conducted in the southeastern US, but only with one crop per study (Burke et al. 2007; Meyers et al. 2010; Webster and Grey 2015) and did not always estimate seed production (Meyers et al. 2010). Research is also limited with respect to growth and seed production of *A. palmeri* when emerging at various times following a competing crop (Norsworthy et al. 2016; Webster and Grey 2015). The need for “zero-tolerance” policies for weed seed production have increased over time, especially when considering *A. palmeri* fecundity (Van Wychen 2015). To date, research
which allows for direct comparison of *A. palmeri* growth and seed production in competition with different row crops has not been done. Therefore, the objectives of this research were to (1) measure the growth of male and female *A. palmeri* plants emerging with, and three weeks after corn, cotton, peanut, and soybean; and (2) determine the effects of emergence time and duration of interference on *A. palmeri* seed production.

**Materials and Methods**

Two field experiments were conducted at the Upper Coastal Plan Research Station (35.897° N, -77.675° W) near Rocky Mount, North Carolina (NC) in 2018 using natural *A. palmeri* populations. Soils were an Aycock very fine sandy loam (fine-silty, siliceous, subactive, thermic Typic Paleudult) and a Goldsboro fine sandy loam (fine-loamy, siliceous, subactive, thermic Aquic Paleudult). Corn, cotton, peanut, and soybean were planted on June 5, 2018 on conventionally-tilled field with raised seedbeds spaced 96-cm apart (Table 1). While planting in mid-May was planned, persistent wet conditions delayed 2018 plantings into early June. Crop populations were counted 2 wk after planting (WAP) to determine plant stands (Table 1). One week after planting, eight *A. palmeri* seedlings (Cohort 1) were selected plot\(^{-1}\). Selected seedlings were on the seedbed edges approximately 20 cm from the crop. Additionally, seedlings were spaced to ensure at least a 3 m\(^2\) weed-free area for each plant to mitigate intraspecific competition. Plot sizes were 12 rows by 6 to 9.1 m for each field, respectively. The experimental design was a randomized complete block design with four replications. Crops were randomized within each replication.

One week after seedling selection (2 WAP), the selected plants were covered with inverted cups and herbicides were applied over-the-top to control non-selected weeds (Table 2). Herbicides with limited to no soil residual activity were chosen to avoid unintended effect on the
selected plants through root absorption, and to allow for a second flush of *A. palmeri* (Cohort 2) to emerge the following week. The second cohort of *A. palmeri* was selected in a similar fashion at 3 WAP to simulate those escaping a PRE herbicide. This was done to understand the competitive advantage of crops against later-emerging *A. palmeri*. All selected weeds were then covered, and herbicides were applied to control non-selected weeds. Plots were kept weed-free beyond this date by handweeding or hoeing. Urea ammonium nitrate (32% N) was applied (approximately 5 to 10 cm from the crop base) to corn (135 kg N ha\(^{-1}\)) and cotton (67 kg N ha\(^{-1}\)) 3 to 4 WAP according to the Cooperative Extension Service recommendations for North Carolina (Edmisten et al. 2019; Heiniger et al. 2019). Fertilizer was applied on the opposite side of the crop row from the selected *A. palmeri* plants to prevent physical damage from application equipment. All other pests were controlled using recommended production practices (Edmisten et al. 2019; Heiniger et al. 2019; Jordan et al. 2019; Stowe et al. 2017).

*Amaranthus palmeri* canopy height and width were measured every two weeks following selection throughout the growing season. Measurements for male or female plants were averaged within each plot where more than one plant was present. All plants from Cohort 1 and 2 were harvested approximately 14 and 17 WAP, respectively. Freshly cut shoots were weighed in the field at harvest. Female plants were placed in bags to minimize seed loss, transported to the Method Road Greenhouse Complex (Raleigh, NC) and dried in the greenhouse at 25 to 30 C. When dried, seeds were stripped manually and homogenized using a Thomas-Wiley Lab Mill (Thomas Scientific, Swedesboro, NJ) fitted with a 2 mm sieve. Chaff was then removed similar to Sosnoskie et al. (2014) and the total cleaned seed was weighed. The total number of seeds produced was then calculated by averaging the weight of 100 seeds from 5 separate, 100-seed
counts and extrapolating the total seed number per plant based on the total seed biomass (Sellers et al. 2003).

Weather data were obtained from a weather station approximately 300 m from the field and used to calculate GDD. Growth parameters were plotted against GDD (base 10 C) using a four-parameter logistic function in SigmaPlot (SigmaPlot v. 14.0, Systat Software, San Jose, CA), similar to the methodology by Spaunhorst et al. (2018). Growth parameters included canopy height and width as well as percent of maximum heights and widths. Root mean square error (RSME; equation 1) and modeling efficiency coefficients (MEF; equation 2) were calculated to test the goodness of fit of the model where \( P_i \) is the predicted value, \( O_i \) is the observed value, \( n \) is the number of observations, and \( \bar{O}_i \) is the mean observed value (Archontoulis and Miguez 2015).

\[
RMSE = \left[ \frac{1}{n} \sum_{i=1}^{n} (P_i - O_i)^2 \right]^{1/2} \tag{1}
\]

\[
MEF = 1 - \left[ \sum_{i=1}^{n} (O_i - P_i)^2 / \sum_{i=1}^{n} (O_i - \bar{O}_i)^2 \right] \tag{2}
\]

A RMSE value of zero suggests that the predicted and observed values are a perfect fit to the model. A MEF value close to one suggests high accuracy of model predictions. Models were fit by gender and crop for each replication. Following fitting, GDD needed to reach 10, 50, and 90% of maximum height were calculated and analyzed using PROC GLIMMIX in SAS (SAS 9.4, SAS Institute Inc., Cary, NC). Crop and gender were considered fixed effects while replication and field were considered random. Final height, width, shoot biomass, and seed production were analyzed using PROC GLIMMIX. Data for seed production were log-transformed prior to analysis in order to meet the assumptions of normality, but the untransformed data are presented. Means were separated according to Fisher’s protected LSD at \( \alpha = 0.05 \).
Results and Discussion

**Plant height.** The logistic models built described the relationship of female (RMSE 8.2 to 11.5, MEF 0.97 to 0.99) and male (RMSE 4.0 to 16.1, MEF 0.96 to 1.00) *A. palmeri* heights in Cohort 1 and GDDs (Table 3). Overall, male and female plants grew similarly in height within their respective crops over the study period (Figure 1A). This is reflected when analyzing final *A. palmeri* heights as only the main effects of crop (P < 0.0001, F = 20.7) and gender (P = 0.0008, F = 11.7) were significant. *Amaranthus palmeri* predicted heights in corn (223 cm) was taller than that in cotton (202 cm height), followed by that in peanut (177 cm) and soybean (177 cm), which were similar. When competing with cotton season-long in Arkansas, the final *A. palmeri* heights averaged approximately 138 cm (Norsworthy et al. 2016). The seemingly significant difference in final heights presented by Norsworthy et al. (2016) compared to our research may be due to factors including, but not limited to: environmental differences between research sites and/or the overspray of herbicides by Norsworthy et al. (2106). In Georgia, Webster and Grey (2015) reported male and female *A. palmeri* final heights (149 to 166 cm) being similar when competing with cotton season-long. *A. palmeri* attained heights of 177 cm when competing season-long in NC (Meyers et al. 2010). This maximum height was similar to what *A. palmeri* attained when in season-long competition with peanut in the current research. Burke et al. (2007) also reported a maximum height of 175 cm of *A. palmeri* competing with peanut season-long in NC.

The logistic models described the relationship of female (RMSE 2.1 to 11.5, MEF 0.95 to 0.99) and male (RMSE 9.9 to 24.8, MEF 0.56 to 0.96) *A. palmeri* heights in Cohort 2 and GDDs (Table 3). Crop canopies had a three-week advantage on Cohort 2 which may explain the reduction in goodness of fit of the model as *A. palmeri* growth was more variable. More uniformity among the later emerging female *A. palmeri* suggests that they may be more
competitive compared to male *A. palmeri* as growth was more consistent among replications. As with Cohort 1, only the crop influenced *A. palmeri* height over time (*P* < 0.0001, *F* = 52.3; Figure 1B). The canopy of *A. palmeri* was tallest in cotton (155 cm) and peanut (151 cm), followed by that in soybean (121 cm) and in corn (30 cm). This indicates that the height of competing crop is not the only determinant of the final height of *A. palmeri*. Other physiological traits of the competing crop and the ability of the crop to compete with the weed below ground also modifies the final stature of *A. palmeri*. Corn and soybean have taller or denser canopies than peanut and cotton, thus differentially affecting the ability of *A. palmeri* to compete for sunlight. Emerging 3 WAP reduced *A. palmeri* final height in corn, cotton, peanut, and soybean 87, 23, 15, and 32%, respectively. Webster and Grey (2015) and Norsworthy et al. (2016) reported 11.5 and 24% decrease in *A. palmeri* height when emerging three weeks after cotton compared to emerging with the crop.

When the data for Cohort 1 and 2 were transformed into percent of maximum height modeled against GDD, the growth lines converge more tightly (Figure 2A and B). The GDDs needed to achieve 10, 50, and 90% in Cohort 1 differed by crop (*P* < 0.0001 to 0.0274, *F* = 3.8 to 31.3), but was not influenced by gender of *A. palmeri* nor the interaction of crop and gender (*P* > 0.1). *Amaranthus palmeri* in cotton (387 GDD) took longer to reach 10% of their maximum height compared to those growing with soybean (359 GDD) and corn (361 GDD; Figure 3A). *Amaranthus palmeri* in soybean (758 GDD) took the longest to reach 50% of maximum height compared to all other crops (633 to 672 GDD). Additionally, *A. palmeri* growing with peanut (672 GDD) grew slower than those with corn (633 GDD). When growing with corn and cotton, *A. palmeri* reached 90% of their maximum height (1,026 and 1,002 GDD, respectively) faster than when growing with peanut and soybean (1,096 and 1,088 GDD, respectively). Similar to
Cohort 1, cropping system influenced the GDDs needed to reach certain growth percentages (P < 0.0001 to 0.0287, F = 3.8 to 27.0) in Cohort 2. *A. palmeri* in corn (256 GDD) reached 10% of maximum height faster than those in the other crops (386 to 429 GDD; Figure 3B). Corn (625 GDD) achieved 50% of maximum height quicker than peanut (719 GDD) and soybean (667GDD) but was similar to cotton. Peanut was slowest to reach 50% of maximum height. However, corn took longer to reach 90% of maximum height than cotton or soybean. This may be due to the corn canopy shedding lower leaves as the season progresses which allowed more light penetration for *A. palmeri* to utilize. These data illustrate the ability of *A. palmeri* to adapt to their competitive environment. For example, when competing with cotton *A. palmeri* increased height quickly to compete for light. However, when competing with peanut, a significantly shorter canopy, *A. palmeri* was able to maintain canopy dominance easier, thus, not requiring rapid upward growth.

With respect to Cohort 1, the average and maximum growth rates for *A. palmeri* in corn (0.14 and 0.34 cm GDD\(^{-1}\), respectively) and cotton (0.13 and 0.33 cm GDD\(^{-1}\)) were greater than those measured in peanut (0.11 and 0.25 cm GDD\(^{-1}\), respectively) and soybean (0.11 and 0.25 cm GDD\(^{-1}\), respectively; Figure 4A). Growth rates for Cohort 2 were less than that of Cohort 1 with average rates of 0.02, 0.10, 0.10, and 0.08 cm GDD\(^{-1}\) in corn, cotton, peanut, and soybean, respectively (Figure 4B). Maximum growth rates were also slower at 0.03, 0.24, 0.23, and 0.20, respectively. In Kansas, Horak and Loughin (2000) observed growth rates of 0.18 and 0.21 cm GDD\(^{-1}\) when mid-June planted *A. palmeri* measured 100 and 87 cm, respectively (approximately 50% of maximum height). Spaunhorst et al. (2018) reported growth rates of 0.14 to 0.17 cm GDD\(^{-1}\) to 50% of maximum height. These are in good agreement with this research with growth
rates of 0.18, 0.16, 0.13, and 0.12 cm GDD$^{-1}$ for *A. palmeri* emerging with the crop (Cohort 1) to reach 50% of maximum height in corn, cotton, peanut, and soybean, respectively (Figure 2A).

In 2018 NC had approximately 368,300-, 174,000-, 41,300-, and 667,700 ha of corn, cotton, peanut, and soybean, respectively (USDA-NASS 2019). In soybean and peanut, contact herbicides such as fomesafen and acifluorfen are commonly used for *A. palmeri* control while glufosinate is used in Liberty Link corn, cotton, and soybean as well. The labels of these herbicides recommend application at a maximum height of 10 cm for good control of *A. palmeri*. *Amaranthus palmeri* in Cohort 1 achieved 10 cm in 17 to 18 d in corn, cotton, peanut, and soybean. This means herbicides need to be applied no later than 18 d from weed emergence. In Cohort 2, *A. palmeri* reached 10 cm 30, 19, 21, and 21 d after the 3 WAP mark in corn, cotton, peanut, and soybean, respectively. Thus, later-emerging *A. palmeri* still need to be sprayed no later than 3 wk after emergence in these crops, except for corn. However, how late one can spray herbicides in corn is dictated by the layby growth stage timing.

**Canopy width of *A. palmeri***. The logistic models described the relationship of Cohort 1 female (RMSE 2.1 to 24.8, MEF 0.83 to 0.96) and male (RMSE 4.2 to 8.6, MEF 0.88 to 0.94) *A. palmeri* widths and GDDs (Table 4). Generally, differences in *A. palmeri* canopy width was evident across cropping systems (Figure 5A). Female plants had wider canopies (more branching and more leafy) than male plants. The main effects of crop ($P < 0.0001, F = 91.6$) and gender ($P < 0.0001, F = 25.6$) on final canopy width of *A. palmeri* were significant, but the interaction of the two was not ($P = 0.1511$). The canopy width of *Amaranthus palmeri* in competition with cotton (105.0 cm) and peanut (95.2 cm) was largest, followed by soybean (54.5 cm), and corn (38.4 cm). Averaged over cropping systems, female plants had a greater canopy width (81 cm) than male plants (67 cm). Webster and Grey (2015) also studied canopy development by gender.
and found that female *A. palmeri* plants have wider canopy (126 cm) than the males (93 cm). Our study supports this.

Canopy width in Cohort 2 was less than in Cohort 1 (Figure 5B). The canopy expansion of Cohort 2 followed a quadratic model with GDD for males at RMSE 2.6 to 5.7 and MEF of 0.53 to 0.90 (Table 5). The RMSE for females ranged from 1.8 to 7.5 with MEF values of 0.71 to 0.78). As the season progressed, *A. palmeri* leaves began to senesce, producing a growth curve that best fit a quadratic model. These data suggest that gender did not influence canopy width throughout the season for Cohort 2, nor the final width (*P* = 0.0564). Cropping system did affect canopy width (*P* < 0.0001, *F* = 23.7) as *A. palmeri* plants competing with peanut had wider canopy (36.5 cm) than those competing with soybean (15.6 cm), cotton (18.5 cm), and corn (3.5 cm). The canopy structure of *A. palmeri* mimicked the canopy structure of the crop, becoming most narrow when growing with corn. This reflected canopy modifications to better compete for light in a narrow inter-row of tall-canopy corn.

*A. palmeri* shoot biomass. For *A. palmeri* plants in Cohort 1, the interaction of cropping system and gender (*P* = 0.0002, *F* = 6.7) influenced plant biomass at harvest. Female plants in cotton (2,014 g plant⁻¹) and peanut (1,879 g plant⁻¹) were larger than plants in any other cropping system (Figure 6). This could be explained by the cotton canopy being open and row width being wide, resulting in less competition for light. Similarly, light competition with peanut would be minimal because peanut is low-growing, allowing *A. palmeri* to grow above this crop quickly. Male *A. palmeri* in cotton (934 g plant⁻¹) and peanut (879 g plant⁻¹) were similar and larger than male or female plants in both corn (77 and 195 g plant⁻¹, respectively) and soybean (258 and 434 g plant⁻¹, respectively). Within each cropping system, female plants had 68 to 153% more biomass than their male counterparts. Thus, female plants are always larger than male plants.
This conclusion is in agreement with the data of Webster and Grey (2015) where in females were 167% larger than males when competing with cotton season-long.

The final biomass for *A. palmeri* emerging 3 WAP (Cohort 2) was affected only by cropping system ($P < 0.0001, F = 11.5$), but not by gender ($P = 0.2020$). The interaction of these factors was also not significant ($P = 0.1629$). Cohort 2 of *A. palmeri* in peanut had the largest fresh biomass (166 g plant$^{-1}$) compared to all other crops (Figure 7). *A. palmeri* in cotton (83 g plant$^{-1}$) and soybean (35 g plant$^{-1}$) were similar with those in cotton being larger than those in corn (2 g plant$^{-1}$). When comparing Cohort 1 and Cohort 2 biomass, the biomass for the latter was 85 to 99% less than the former, across cropping systems. In Arkansas, Norsworthy et al. (2016) reported a 64% reduction in female *A. palmeri* biomass when it emerged three weeks after cotton compared to those emerging with the crop. In Georgia, *A. palmeri* biomass was about 80% less, across genders, when emerging 3 wk after cotton as opposed to with the crop (Webster and Grey 2015). Therefore, applying PRE herbicides is critical for controlling the first flush of *A. palmeri*, giving the crop a high competitive advantage. More importantly, *A. palmeri* biomass is tightly correlated with seed production; therefore, small plants mean less seed production. This is discussed further below.

**Seed production of *A. palmeri* in crop competition.** Seed production for Cohort 1 was not correlated with plant height ($r = 0.03$) but was correlated with canopy width ($r = 0.71$) and plant biomass ($r = 0.63$) suggesting that as width or biomass increases, so does seed production (Figure 8). Spaunhorst et al. (2018) reported similar biomass and seed correlations ($r = 0.63$) from *A. palmeri* populations grown in Arkansas. Shwartz et al. (2016) described good correlations of biomass and seed production in *A. palmeri* ($r = 0.79$) and tall waterhemp [*Amaranthus tuberculatus* (Monq.) Sauer; $r = 0.73$] across multiple states. In Georgia, Webster
and Grey (2015) also determined a strong relationship (r = 0.81) of *A. palmeri* biomass and total seed production. Cropping system influenced total seed production (P < 0.0001, F = 48.9) with average seed production in cotton (534,442 seeds plant\(^{-1}\)) and peanut (443,228 seeds plant\(^{-1}\)) being similar and greater than seed production in soybean (173,093 seeds plant\(^{-1}\); Figure 9). *Amaranthus palmeri* in competition with corn (51,024 seeds plant\(^{-1}\)) averaged the lowest seed production.

In California, Keeley et al. (1987) reported upwards of 613,000 seed plant\(^{-1}\) was achieved when *A. palmeri* was grown without competition. When competing with cotton season long in Georgia, Webster and Grey (2015) and Sosnoskie et al. (2014) reported average seed production of 312,000 and 435,000 seeds plant\(^{-1}\), respectively. In soybean, Bensch et al. (2003) reported *A. palmeri* produced approximately 100,000 to 200,000 seeds m\(^{-2}\) when emerging with the crop and competing season long. This production is approximately one-quarter to one-half of the seed production in our study (Figure 9). However, this may be explained by the biomass of plants in the Bensch et al. (2003) study which was reduced to similar proportions when compared to plants in our study. As discussed, seed production is correlated to plant biomass (Figure 8). In Kansas, Massinga et al. (2001) reported *A. palmeri* produced 140,000 m\(^{-2}\) when competing with corn season long. While greater than what was observed in our study (approximately 102,000 seed m\(^{-2}\)), these data illustrate the vast amount of seed that one plant can contribute to the soil seedbank if left to compete season long (Figure 9).

Seed production from *A. palmeri* in Cohort 2 was correlated with plant height (r = 0.85), width (r = 0.81), and biomass (r = 0.90; Figure 10). Cropping system influenced seed production (P < 0.0001; F = 47.3) with trends being similar to Cohort 1. *Amaranthus palmeri* emerging three WAP cotton (38,531 seed plant\(^{-1}\)) and peanut (36,211 seed plant\(^{-1}\)) produced similar amounts of
seed with both being greater than *A. palmeri* in soybean (3,742 seed plant\(^{-1}\); Figure 11). Corn, again, produced the lowest amount, averaging only 224 seed plant\(^{-1}\). Reductions in *A. palmeri* seed production when it emerges after the crop has previously been observed. Webster and Grey (2015) reported *A. palmeri* emerging three WAP cotton was able to produce approximately 76,000 seed plant\(^{-1}\) (76% reduction) while Norsworthy et al. (2016) observed approximately 51,000 seed plant\(^{-1}\) (30% reduction). When emerging 3 to 4 wks after corn, Massinga et al. (2003) measured approximately 1,800 seeds m\(^{-2}\) were produced, a 99% reduction when compared to *A. palmeri* that emerged with corn and competed season long. The presented data support previous research showing the vast reduction is seed production (greater than 90% reduction) when *A. palmeri* emerges wks after the crop. The data demonstrate the importance of controlling escaped *A. palmeri* even when it emerges wks after the crop as it still has the capacity to produce thousands of seeds and the advantage of more competitive crop canopies.

**Research Implications.** *Amaranthus palmeri* is a highly competitive weed that can cause short- and long-term problems for farm managers. In the short-term, farm managers must be cognizant of their POST application timings to ensure adequate control. *Amaranthus palmeri* was 10 cm in height approximately 17 to 18 d after it emerged with corn, cotton, peanut, or soybean. It is imperative to be timely with applications to ensure adequate control of emerged *A. palmeri* is achieved and it is recommended to include a residual herbicide with POST herbicide applications for extended control. The rate of growth beyond this point exceeded 5 cm d\(^{-1}\) creating enormous light competition with the crop. If a PRE herbicide was applied and adequate control was received, farm managers must still be cognizant of their timeliness. *Amaranthus palmeri* which emerges three wks after cotton, peanut, or soybean reached 10 cm in height approximately three wks after emergence. In corn, this height was achieved in approximately
four wks. Robust growth may still occur with a later cohort as growth rates of greater than four cm d\(^{-1}\) was measured.

Long-term, farm managers must consider the weed seedbank. *Amaranthus palmeri* produced approximately 51,000, 534,000, 434,000, and 173,000 seed plant\(^{-1}\) in corn, cotton, peanut, and soybean, respectively. While it is not likely that farm managers would allow *A. palmeri* to emerge with the crop and compete season long, later emerging cohorts still require much attention. Cotton and peanut were again the least competitive and *A. palmeri* was still able to produce greater than 35,000 seed plant\(^{-1}\). Less seed was produced in soybean (greater than 3,000 seed plant\(^{-1}\)) and corn (approximately 200 seed plant\(^{-1}\)). Good growing conditions in our study allowed for uniform crop stands which may not always occur. *A. palmeri* may produce more biomass and seed if a heterogeneous stand allows for more light interception. Nonetheless, these data illustrate why rotating corn is an important feature into an integrated weed management plan as it provides a competitive advantage and alternative herbicide options. *A. palmeri* has shown the ability to morphologically adapt to differing cropping systems (Bravo et al. 2017), thus, multiple short statured crops followed by one taller in nature may provide the greatest benefit (e.g. peanut to sweetpotato to soybean to corn). Further research is needed to better determine these effects and how they best fit into an integrated weed management plan.

Producers must be timely and attentive when cotton follows peanut, or vice-versa, as these were the two least competitive against *A. palmeri*. Ultimately, a zero tolerance for escapes is required with this weed to minimize additions to the weed seedbank. With respect to herbicide-resistance mitigation, the problem can quickly become field-wide if one plant is allowed to escape and reproduce (Norsworthy et al. 2014). Additionally, the chances of selecting an herbicide-resistant individual increases as the population exposed to herbicides increases,
thus, decreasing additions to the seedbank reduces the amount of plants exposed. Further research is needed to understand if competitive pressures faced by the parent effects the growth and development of the offspring and, if so, how one could utilize crop rotation to increase the crops competitive advantage.

Acknowledgments

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References


### Table 1. Crop varieties and planting populations at each experimental location.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Population (plants ha(^{-1}))</th>
<th>Variety</th>
<th>Source</th>
</tr>
</thead>
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<tr>
<td>Corn</td>
<td>84,060</td>
<td>P1847VYHR</td>
<td>Pioneer Hi-Bred Int., Johnston, IA</td>
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<tr>
<td>Cotton</td>
<td>95,440</td>
<td>Delta Pine Bolgard II</td>
<td>Monsanto Company, St. Louis, MO</td>
</tr>
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<td></td>
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<td>Xtendflex</td>
<td></td>
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<td>Peanut</td>
<td>243,980</td>
<td>Bailey</td>
<td>Isleib et al. 2011</td>
</tr>
<tr>
<td>Soybean</td>
<td>134,910</td>
<td>Credenz 7007LL</td>
<td>BASF, Research Triangle Park, NC</td>
</tr>
<tr>
<td>Crop</td>
<td>Active ingredient</td>
<td>Trade name&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Rate (g ai or ae ha&lt;sup&gt;-1&lt;/sup&gt;)</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-------------------</td>
<td>-------------------------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>Peanut&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Acifluorfen</td>
<td>Ultra Blazer</td>
<td>420</td>
</tr>
<tr>
<td></td>
<td>Bentazon</td>
<td>Basagran 4L</td>
<td>1,122</td>
</tr>
<tr>
<td></td>
<td>Clethodim</td>
<td>Select 2EC</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td>Paraquat</td>
<td>Gramoxone SL2.0</td>
<td>140</td>
</tr>
<tr>
<td>Corn, Cotton, and Soybean</td>
<td>Glufosinate</td>
<td>Liberty 280SL</td>
<td>595</td>
</tr>
<tr>
<td></td>
<td>Glyphosate</td>
<td>Roundup PowerMax</td>
<td>947</td>
</tr>
<tr>
<td></td>
<td>Imazethapyr</td>
<td>Pursuit 70WDG</td>
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</tr>
</tbody>
</table>

<sup>a</sup>The first spray included paraquat and bentazon while acifluorfen and clethodim were utilized in the second spray.
Table 3. Parameter estimates and goodness of fit using the four-parameter logistic function to fit male and female Palmer amaranth (*Amaranthus palmeri*) height when emerging with (Cohort 1) and three wks after (Cohort 2) with corn, cotton, peanut, and soybean crops.\(^{a,b}\)

<table>
<thead>
<tr>
<th>Crop</th>
<th>Gender</th>
<th>(b^{c})</th>
<th>(c^{c})</th>
<th>(d^{c})</th>
<th>GDD(_{50})</th>
<th>RMSE(^d)</th>
<th>MEF(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>cm</td>
<td>cm</td>
<td>cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cohort 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>Female</td>
<td>4.0 ± 0.3</td>
<td>2.3 ± 4.4</td>
<td>241.6 ± 6.5</td>
<td>653.4 ± 16.8</td>
<td>11.4</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>3.9 ± 0.3</td>
<td>2.3 ± 3.1</td>
<td>217.4 ± 4.7</td>
<td>648.7 ± 13.4</td>
<td>8.0</td>
<td>0.99</td>
</tr>
<tr>
<td>Cotton</td>
<td>Female</td>
<td>4.1 ± 0.3</td>
<td>2.3 ± 3.2</td>
<td>211.6 ± 5.0</td>
<td>672.5 ± 14.6</td>
<td>8.5</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>4.7 ± 0.7</td>
<td>3.0 ± 5.8</td>
<td>199.9 ± 7.6</td>
<td>642.9 ± 23.5</td>
<td>16.1</td>
<td>0.96</td>
</tr>
<tr>
<td>Peanut</td>
<td>Female</td>
<td>3.7 ± 0.3</td>
<td>2.4 ± 3.1</td>
<td>193.8 ± 6.0</td>
<td>721.0 ± 19.2</td>
<td>8.2</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>4.1 ± 0.2</td>
<td>2.5 ± 1.5</td>
<td>174.4 ± 4.6</td>
<td>671.8 ± 8.4</td>
<td>4.0</td>
<td>1.00</td>
</tr>
<tr>
<td>Soybean</td>
<td>Female</td>
<td>3.8 ± 0.5</td>
<td>3.2 ± 4.4</td>
<td>189.7 ± 6.0</td>
<td>705.8 ± 26.0</td>
<td>11.5</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>3.8 ± 0.2</td>
<td>2.5 ± 2.0</td>
<td>176.1 ± 3.2</td>
<td>668.3 ± 11.5</td>
<td>5.2</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>Cohort 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>Female</td>
<td>2.7 ± 0.5</td>
<td>0.1 ± 1.3</td>
<td>30.8 ± 2.5</td>
<td>628.8 ± 56.3</td>
<td>2.1</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>2.5 ± 2.2</td>
<td>0.4 ± 6.1</td>
<td>43.4 ± 21.4</td>
<td>798.4 ± 396.3</td>
<td>10.3</td>
<td>0.56</td>
</tr>
<tr>
<td>Cotton</td>
<td>Female</td>
<td>4.4 ± 0.4</td>
<td>2.8 ± 3.6</td>
<td>166.4 ± 4.0</td>
<td>637.4 ± 16.0</td>
<td>6.7</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>4.7 ± 0.8</td>
<td>2.5 ± 5.2</td>
<td>146.2 ± 6.0</td>
<td>674.2 ± 27.0</td>
<td>9.9</td>
<td>0.96</td>
</tr>
<tr>
<td>Peanut</td>
<td>Female</td>
<td>3.9 ± 0.8</td>
<td>2.3 ± 6.3</td>
<td>148.2 ± 9.2</td>
<td>699.8 ± 40.6</td>
<td>11.5</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>4.0 ± 0.9</td>
<td>2.4 ± 6.8</td>
<td>168.0 ± 12.4</td>
<td>765.8 ± 47.5</td>
<td>13.0</td>
<td>0.95</td>
</tr>
<tr>
<td>Soybean</td>
<td>Female</td>
<td>3.9 ± 0.8</td>
<td>0.9 ± 5.7</td>
<td>134.1 ± 7.5</td>
<td>668.1 ± 36.7</td>
<td>10.2</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>5.5 ± 3.2</td>
<td>1.7 ± 12.0</td>
<td>110.7 ± 13.4</td>
<td>690.0 ± 77.4</td>
<td>24.8</td>
<td>0.72</td>
</tr>
</tbody>
</table>

\(^{a,b}\) Statistical analysis and comparison.
Table 3 (continued).

Abbreviations: GDD = growing degree days (base 10 C); RMSE = root mean square error; MEF = modeling efficiency.

\[ Y = c + \frac{(d - c)}{1 + (x/GDD_{50})^b}, \]

where \( Y = A. \ palmeri \) height, \( c \) = the minimum height, \( d \) = the maximum height, \( GDD_{50} \) = accumulated GDD since planting at which 50% of the maximum plant height was achieved, and \( b \) = the slope of the regression line.

Values are mean ± standard error.

RMSE values closer to zero indicate predicted values are closer to the observed values.

MEF closer to one suggest model predictions are more accurate.
Table 4. Parameter estimates and goodness of fit using the four-parameter logistic function to fit male and female Palmer amaranth (*Amaranthus palmeri*) width when emerging with (Cohort 1) corn, cotton, peanut, and soybean crops.\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Crop</th>
<th>Gender</th>
<th>$b^c$ ± SE</th>
<th>$c^c$ ± SE</th>
<th>$d^c$ ± SE</th>
<th>GDD\textsubscript{50} ± SE</th>
<th>RMSE\textsuperscript{d}</th>
<th>MEF\textsuperscript{e}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>Female</td>
<td>3.9 ± 1.7</td>
<td>0.1 ± 3.3</td>
<td>41.1 ± 2.1</td>
<td>335.8 ± 32.1</td>
<td>5.8</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>4.4 ± 1.9</td>
<td>0.1 ± 2.4</td>
<td>37.2 ± 1.4</td>
<td>314.7 ± 26.6</td>
<td>4.2</td>
<td>0.88</td>
</tr>
<tr>
<td>Cotton</td>
<td>Female</td>
<td>3.0 ± 0.4</td>
<td>-0.5 ± 4.6</td>
<td>125.7 ± 6.0</td>
<td>564.9 ± 31.3</td>
<td>8.3</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>3.9 ± 0.7</td>
<td>0.7 ± 4.8</td>
<td>96.4 ± 4.0</td>
<td>498.8 ± 27.9</td>
<td>8.6</td>
<td>0.93</td>
</tr>
<tr>
<td>Peanut</td>
<td>Female</td>
<td>2.6 ± 0.5</td>
<td>-0.1 ± 5.3</td>
<td>110.3 ± 9.2</td>
<td>602.2 ± 56.8</td>
<td>9.4</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>3.3 ± 0.5</td>
<td>0.2 ± 3.9</td>
<td>89.1 ± 4.0</td>
<td>514.3 ± 29.7</td>
<td>7.0</td>
<td>0.94</td>
</tr>
<tr>
<td>Soybean</td>
<td>Female</td>
<td>2.2 ± 0.6</td>
<td>0.1 ± 3.8</td>
<td>67.7 ± 7.0</td>
<td>512.9 ± 67.3</td>
<td>6.8</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>3.6 ± 0.8</td>
<td>0.2 ± 2.4</td>
<td>46.6 ± 1.7</td>
<td>377.9 ± 22.0</td>
<td>4.2</td>
<td>0.92</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Abbreviations: GDD = growing degree days (base 10°C); RMSE = root mean square error; MEF = modeling efficiency.

\textsuperscript{b}Y = c + (d - c)/(1 + (x/GDD\textsubscript{50})\textsuperscript{b}), where Y = *A. palmeri* height, c = the minimum height, d = the maximum height, GDD\textsubscript{50} = accumulated GDD since planting at which 50% of the maximum plant height was achieved, and b = the slope of the regression line.

\textsuperscript{c}Values are mean ± standard error.

\textsuperscript{d}RMSE values closer to zero indicate predicted values are closer to the observed values.

\textsuperscript{e}MEF closer to one suggest model predictions are more accurate.
Table 5. Parameter estimates and goodness of fit using the quadratic function \((Y = y_0 + ax + bx^2)\) to fit male and female Palmer amaranth \((Amaranthus palmeri)\) width when emerging three wks after (Cohort 2) corn, cotton, peanut, and soybean crops.\(^a\)

<table>
<thead>
<tr>
<th>Crop</th>
<th>Gender</th>
<th>(y_0^{b})</th>
<th>(a^{b})</th>
<th>(b^{b})</th>
<th>RMSE(^c)</th>
<th>MEF(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>Female</td>
<td>-0.28 ± 0.990</td>
<td>0.03 ± 0.002</td>
<td>-1.45E-5 ± 1.71E-6</td>
<td>1.8</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>-0.02 ± 1.432</td>
<td>0.02 ± 0.004</td>
<td>-1.25E-5 ± 2.48E-6</td>
<td>2.6</td>
<td>0.53</td>
</tr>
<tr>
<td>Cotton</td>
<td>Female</td>
<td>-1.77 ± 3.171</td>
<td>0.08 ± 0.009</td>
<td>-4.23E-5 ± 5.49E-6</td>
<td>5.8</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>-1.77 ± 1.799</td>
<td>0.07 ± 0.005</td>
<td>-3.70E-5 ± 3.11E-6</td>
<td>3.3</td>
<td>0.86</td>
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<tr>
<td>Peanut</td>
<td>Female</td>
<td>-1.97 ± 4.119</td>
<td>0.07 ± 0.012</td>
<td>-2.70E-5 ± 7.13E-6</td>
<td>7.5</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>-3.40 ± 4.278</td>
<td>0.08 ± 0.012</td>
<td>-3.45E-5 ± 7.41E-6</td>
<td>5.7</td>
<td>0.90</td>
</tr>
<tr>
<td>Soybean</td>
<td>Female</td>
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<td>0.05 ± 0.006</td>
<td>-2.62E-5 ± 3.35E-6</td>
<td>3.5</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>-2.29 ± 2.790</td>
<td>0.05 ± 0.008</td>
<td>-2.76E-5 ± 4.83E-6</td>
<td>5.1</td>
<td>0.61</td>
</tr>
</tbody>
</table>

\(^a\)Abbreviations: GDD = growing degree days (base 10 C); RMSE = root mean square error; MEF = modeling efficiency.

\(^b\)Values are mean ± standard error.

\(^c\)RMSE values closer to zero indicate predicted values are closer to the observed values.

\(^d\)MEF closer to one suggest model predictions are more accurate.
Figure 1. Male (M) and female (F) Palmer amaranth (Amaranthus palmeri) heights modeled against growing degree days (GDD; base 10 C) when competing with corn, cotton, peanut, and soybean crops. *Amaranthus palmeri* was allowed to emerge with the crops (Cohort 1; A) and 3 wk after crop planting (Cohort 2; B). Parameter estimates for Cohort 1 and Cohort 2 can be found in Table 2. Growing degree day 0 represents crop planting (Cohort 1) or 3 wk after crop planting (Cohort 2). Error bars represent 95% confidence intervals. Data are pooled over two field sites.
Figure 2. Male (M) and female (F) Palmer amaranth (*Amaranthus palmeri*) percent of maximum heights modeled against growing degree days (GDD; base 10 C) when competing with corn, cotton, peanut, and soybean crops. *Amaranthus palmeri* was allowed to emerge with the crops (Cohort 1; A) and 3 wk after crop planting (Cohort 2; B). Growing degree day 0 represents crop planting (Cohort 1) or 3 wk after crop planting (Cohort 2). Error bars represent 95% confidence intervals. Data are pooled over two field sites.
Figure 3. Growing degree days (GDD) required for Palmer amaranth (*Amaranthus palmeri*) to reach 10, 50, and 90% of maximum height when competing with corn, cotton, peanut, and soybean crops. Cohort 1 (A) was allowed to emerge with the crop while Cohort 2 (B) emerged 3 wk after planting. Data bars within percent of maximum height categories which have the same letter are not significantly different according to Fisher’s Protected LSD (a= 0.05). Data are pooled over *A. palmeri* gender and two field sites.
Figure 4. Male (M) and female (F) Palmer amaranth (*Amaranthus palmeri*) growth rates by growing degree day (GDD; base 10 C) when competing with corn, cotton, peanut, and soybean crops. Cohort 1 (A) emerged with the crop while Cohort 2 (B) emerged 3 wk after crop planting. Error bars represent 95% confidence intervals. Data are pooled over two field sites.
Figure 5. Male (M) and female (F) Palmer amaranth (*Amaranthus palmeri*) widths modeled against growing degree days (GDD; base 10 C) when competing with corn, cotton, peanut, and soybean crops. *Amaranthus palmeri* was allowed to emerge with the crops (Cohort 1; A) and 3 wk after crop planting (Cohort 2; B). Parameter estimates for Cohort 1 and Cohort 2 can be found in Tables 3 and 4, respectively. Growing degree day 0 represents crop planting (Cohort 1) or 3 wk after crop planting (Cohort 2). Error bars represent 95% confidence intervals. Data are pooled over two field sites.
Figure 6. Fresh biomass of male and female Palmer amaranth (*Amaranthus palmeri*) from Cohort 1 at harvest (14 wks after crop planting). Plants were allowed to emerge and compete season long with corn, cotton, peanut, and soybean. Data bars with the same letter are not significantly different according to Fisher’s Protected LSD ($\alpha = 0.05$). Data are pooled over two field sites.
Figure 7. Fresh biomass of Palmer amaranth (Amaranthus palmeri) from Cohort 2 at harvest (17 wk after crop planting). Plants were allowed to emerge 3 wk after crop planting and compete season long with corn, cotton, peanut, and soybean. Data bars with the same letter are not significantly different according to Fisher’s Protected LSD ($\alpha = 0.05$). Data are pooled over plant gender and two field sites.
Figure 8. Correlations between final height (A), width (B), or fresh biomass (C) and total seed production (n = 80) of Palmer amaranth (*Amaranthus palmeri*) from Cohort 1. Plants were allowed to emerge and compete season long against corn, cotton, peanut, and soybean. Data are pooled over two field sites and four cropping systems.
Figure 9. Seed production of female Palmer amaranth (*Amaranthus palmeri*) from Cohort 1 when competing with corn, cotton, peanut, and soybean crops. Plants were allowed to emerge and compete season long. Data bars with the same letter are not different according to Fisher’s protected LSD at $\alpha = 0.05$. Data are pooled over two field sites.
Figure 10. Correlations between final height (A), width (B), or fresh biomass (C) and total seed production (n = 56) of Palmer amaranth (Amaranthus palmeri) from Cohort 2. Plants were allowed to emerge three wks after the crop and compete season long against corn, cotton, peanut, and soybean. Data are pooled over two field sites and four cropping systems.
Figure 11. Seed production of female Palmer amaranth (*Amaranthus palmeri*) from Cohort 2 when competing with corn, cotton, peanut, and soybean crops. Plants were allowed to emerge three wks after the crop and compete season long. Data bars with the same letter are not different according to Fisher’s protected LSD at $\alpha = 0.05$. Data are pooled over two field sites.
The Influence of Postemergence Herbicide Timing and Frequency on Weed Control and Soybean Yield

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Abstract

Understanding optimal herbicide timing and frequency is critical in order to mitigate weed seed return to the soil seedbank and maximize crop yields. Research was conducted from 2016 to 2018 in North Carolina to determine postemergence (POST) only herbicide application timing and frequency necessary for adequate weed control, soybean yield, and economic return in twin- and narrow-row soybean. Predominant weeds included common ragweed (Ambrosia artemisiifolia), large crabgrass (Digitaria sanguinalis), Palmer amaranth (Amaranthus palmeri), and Texas millet (Urochloa texana). Four POST timings included an early- (EPOST), mid- (MPOST), late- (LPOST) and very late-POST (VLPOST) application in the various combinations. A non-treated control was included for comparison. Regardless of planting
pattern, broadleaf weed control was 9 to 48% higher when herbicides were applied two or more times compared to single EPOST or VLPOST-only applications. Generally, two to three applications were needed to provide 100% annual grass control while single applications only provided 71 to 92% control. Applying herbicides increased yield 21 to 46% when compared with non-treated soybean. In treated soybean, yield following the VLPOST treatment was generally lower compared to other herbicide regimes. Trends were similar with economic return as with soybean yield. The data illustrate the importance of multiple POST applications are needed for adequate weed control and do not adversely affect net returns. While yields were protected with the EPOST only treatment, caution must be taken to mitigate returning weed seed to the soil seedbank, as control for this treatment was less than when herbicides were applied multiple times.

**Nomenclature:** Common ragweed *Ambrosia artemisiifolia* L. AMBEL; Palmer amaranth *Amaranthus palmeri* S. Wats. AMAPA; Texas millet *Urochloa texana* (Buckley) R. Webster PANTE; large crabgrass *Digitaria sanguinalis* (L.) Scop. DIGSA.

**Key words:** Herbicide program, economic return, soybean planting pattern.
**Weed Interference and Control in Soybean**

Weeds compete for essential resources such as water, light, and nutrients and may significantly reduce crop yield (Berger et al., 2015; Coble et al., 1981; Knight et al., 2017). Timing and adequacy of weed control are critical in order to maximize crop yield. In soybean, the critical time of weed control (CTWC) occurs later in narrow-row (7 to 7.5-inch spacing) soybean compared to wide-row (30-inch spacing) soybean (Knezevic et al., 2003; Mulugeta and Boerboom, 2000). Although narrow-row spacing is considered more competitive with weeds than wide-row spacing, these studies suggest the CTWC is around the V3-V4 growth stage, which generally occurs three to four wk after soybean emergence (Jha et al., 2008; Knezevic et al., 2003; Mulugeta and Boerboom, 2000). Research on CTWC in twin-row soybean has not been determined, but research using two crop rows spaced 7.5-inch apart on 36-inch row centers suggests it may be competitively similar to narrow-row soybean and more competitive than wide-row soybean (Chandler et al., 2001; Nelson, 2007). While a PRE herbicide application is generally recommended, resource constraints or climatic conditions may prevent one from being applied (Everman et al., 2018). Understanding POST herbicide application timing and frequency, which effectively manages weeds and protects yield is critical for when PRE herbicides are not applied.

In corn (*Zea mays* L.), Gower et al. (2002) applied glyphosate to giant foxtail (*Setaria faberi* Herm.) at various times with a single or single followed by a sequential application 2 to 3 wk later. Treatments including a sequential application of glyphosate increased weed control compared with single applications regardless of giant foxtail height at the time of application. Less weed control with a single early application compared to a single late application was attributed to weed re-infestation. However, greater weed interference occurred with late
applications and were deleterious to corn yields. Nelson (2007) determined the effect of twin- and single-row soybean spacing and glyphosate timing for weed control. A single application of glyphosate was made when weeds were 2-, 4-, 6-, 12- or 24-inches in height. Weed population and biomass were not affected by row spacing. Among treatment timings, biomass was lowest when treated at 6- to 24-inch height compared to other timings. Jha et al. (2008) utilized glyphosate application timing to determine the influence on Palmer amaranth (*Amaranthus palmeri* S. Wats.) and pusley (*Richardia* spp.) control when applied at the following soybean growth stages: V3, V6, V3/V6, V3/V6/R2. Pusley control was greatest when sequential applications were utilized with the single application timings providing similar control to each other. With respect to Palmer amaranth, all timings performed similarly, and increased control compared to the non-treated. Soybean yields followed the same trend as the pusley control.

While the aforementioned studies were informative, further research is required to address certain knowledge gaps. While twin-row planting has not been widely adopted in North Carolina (NC), interest is growing as increased equipment precision would allow producers to employ twin-row planters across multiple cropping systems (Smith et al., 2018). Furthermore, research to date has not been conducted in NC to determine POST herbicide application timing and frequency needed for weed control in twin- or narrow-row soybean. Additionally, previous research has focused on programs including glyphosate, and currently in NC, the most troublesome broadleaf weed in soybean is Palmer amaranth, which commonly possesses multiple resistance to glyphosate and acetolactate synthase inhibitor herbicides (Poirier et al., 2014; Van Wychen, 2016). Therefore, the objective of this research was to determine the influence of POST herbicide active ingredients, application timing, and frequency in twin- and narrow-row soybean on weed control, crop yield, and net economic return.
Experimental Location and Planting

Field experiments were conducted at the Peanut Belt Research Station near Lewiston-Woodville, NC (2016 and 2017) and Upper Coastal Plain Research Station in Rocky Mount, NC, (2017 and 2018). Soil type was a Goldsboro sandy loam (fine-loamy, siliceous, thermic Aquic Paleudult) at Lewiston-Woodville and an Aycock very fine sandy loam (fine-silty, siliceous, subactive, thermic Typic Paleudult) and a Goldsboro fine sandy loam (fine-loamy, siliceous, subactive, thermic Aquic Paleudult) at Rocky Mount. Dominant weed species in Lewiston-Woodville were common ragweed (2 to 9 plants ft\(^2\); *Ambrosia artemisiifolia* L.) and Texas millet [3 to 6 plants ft\(^2\); *Urochloa texana* (Buckley) R. Webster], while Palmer amaranth (9 to 22 plants ft\(^2\)) and large crabgrass [6 to 17 plants ft\(^2\); *Digitaria sanguinalis* (L.) Scop.] were predominant in Rocky Mount.

In five studies at Rocky Mount, soybean (AG 69X6; Monsanto Co., St. Louis, MO) was planted in mid-June on 7.5-inch row spacing using a grain drill following disking and field cultivation. Soybean stand counts were recorded two wk after planting (WAP). In 3 of the 5 fields, soybean populations were approximately 122,600 plants acre\(^{-1}\), while poor emergence in the other 2 fields reduced populations to approximately 63,100 plants acre\(^{-1}\). In the six studies at Lewiston-Woodville, soybean (AG 69X6) was planted in conventionally tilled and raised seedbeds in mid-June in a twin-row planting pattern. In this configuration, two rows of soybean are planted 7.5-inches apart centered on 36-inch spacings. Soybean populations were approximately 93,500 plants acre\(^{-1}\). Plot sizes in Rocky Mount were 12 ft by 25 to 30 ft and in Lewiston plots were four rows (36-inch spacing) or 12 ft by 30 ft.
Experimental design

The trials were arranged in a randomized complete block design with four replications. Treatments included no treatment or herbicides applied at four POST application timings including an early- (EPOST), mid- (MPOST), late- (LPOST) and very late-POST (VLPOST) application in the following combinations: EPOST alone, EPOST + MPOST (EM), EPOST + MPOST + LPOST (EML), EPOST + MPOST + LPOST + VLPOST (EMLV), MPOST + LPOST + VLPOST (MLV), LPOST + VLPOST (LV), or VLPOST alone (Tables 1 and 2). Soybean stages for the four application timings were VC, V2, V4, and R1, respectively (Fehr et al., 1971). Glyphosate only applications were not included at Rocky Mount due to the high frequency of glyphosate-resistant Palmer amaranth. Applications were made with a hand-held CO₂-pressurized backpack sprayer calibrated to deliver 15-gal acre⁻¹ at 19 psi with AIXR 11002 or TTI 11002 (TeeJet® flat-fan nozzles, Spraying Systems Co., Wheaton, IL) nozzles for treatments including dicamba. Other production practices, including fertility, insect, and disease management, were conducted in accordance with Cooperative Extension Service recommendations for North Carolina (Stowe et al., 2018). Broadleaf and annual grass control was visually estimated on a scale of 0 to 100 (0 = no control and 100 = complete control) 10 to 11 wk after the VLPOST treatment (18 to 19 WAP). Soybean was machine harvested using a combine and yield was adjusted to 13% moisture concentration.

Economic analysis

A soybean production budget created by the North Carolina State University Agriculture and Resource Economics Department was modified for net economic return estimation (Bullen et al., 2019). The conventional-till soybean production system in the Coastal Plain region was customized for the seeding rates, hauling cost, and herbicide treatments. Seeding rates were
approximately 103,600 and 144,500 seeds acre\(^{-1}\) for twin- and narrow-row soybean, respectively. In addition to the herbicide cost shown in Table 2, a $5.00 acre\(^{-1}\) custom application cost was also applied. Average soybean price from 2008 to 2017 ($11.27 bushel\(^{-1}\)) were retrieved from the United States Department of Agriculture – National Agriculture Statistics Service (USDA-NASS, 2018).

**Data analysis**

Data were analyzed separately by location as planting pattern, weed populations, and herbicides differed between the two. For each location, weed control, soybean yield, and net economic return were analyzed using PROC GLIMMIX in SAS (SAS 9.4, SAS Institute Inc., Cary, NC). Treatment was considered a fixed effect, while replication and environment (year by field combination) were considered random. For the Rocky Mount experiments (narrow-row), herbicide active ingredient was also considered a fixed affect as the LPOST and VLPOST treatments differed among experiments (Table 2). Means were separated according to Fisher’s protected LSD at \(\alpha = 0.10\). Relationships among variables were determined using Pearson Correlation coefficients with the correlation procedure (PROC CORR) in SAS.
Table 1. Herbicides included in the soybean studies at Rocky Mount and Lewiston, NC in 2016, 2017, and 2018.

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Trade name(^a)</th>
<th>Rate ((\text{lb ai or ae acre}^{-1}))</th>
<th>Manufacturer</th>
<th>Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acifluorfen</td>
<td>Ultra Blazer</td>
<td>0.37</td>
<td>United Phosphorous Inc.</td>
<td>King of Prussia, PA <a href="http://www.upi-usa.com">www.upi-usa.com</a></td>
</tr>
<tr>
<td>Bentazon</td>
<td>Basagran 4L</td>
<td>1.00</td>
<td>Winfield Solutions</td>
<td>St. Paul, MN <a href="http://www.windfieldunited.com">www.windfieldunited.com</a></td>
</tr>
<tr>
<td>Clethodim</td>
<td>Cleanse 2EC</td>
<td>0.19</td>
<td>Winfield Solutions</td>
<td>St. Paul, MN <a href="http://www.windfieldunited.com">www.windfieldunited.com</a></td>
</tr>
<tr>
<td>Dicamba</td>
<td>Engenia</td>
<td>0.50</td>
<td>BASF</td>
<td>Research Triangle Park, NC <a href="http://www.agriculture.basf.com">www.agriculture.basf.com</a></td>
</tr>
<tr>
<td>Fomesafen</td>
<td>Reflex</td>
<td>0.25</td>
<td>Syngenta</td>
<td>Greensboro, NC <a href="http://www.syngenta-us.com">www.syngenta-us.com</a></td>
</tr>
<tr>
<td>Glyphosate</td>
<td>Roundup PowerMax</td>
<td>0.84</td>
<td>Monsanto</td>
<td>St. Louis, MO <a href="http://www.monsanto.com">www.monsanto.com</a></td>
</tr>
<tr>
<td>Imazethapyr</td>
<td>Pursuit 70WDG</td>
<td>0.06</td>
<td>BASF</td>
<td>Research Triangle Park, NC <a href="http://www.agriculture.basf.com">www.agriculture.basf.com</a></td>
</tr>
</tbody>
</table>

\(^a\)Abbreviations: L, liquid; EC, emulsifiable concentrate; WDG, water dispersible granule.
**Table 2.** Application timing and herbicide treatments for the soybean studies at Rocky Mount and Lewiston, NC in 2016, 2017, and 2018.

<table>
<thead>
<tr>
<th>Location (no. of studies)</th>
<th>Treatmenta</th>
<th>Days after planting</th>
<th>Herbicides</th>
<th>Cost ($/A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rocky Mount (2)</td>
<td>EPOST</td>
<td>11 – 14</td>
<td>Fomesafen + clethodim</td>
<td>$25.97</td>
</tr>
<tr>
<td></td>
<td>MPOSTb</td>
<td>25</td>
<td>Acifluorfen + clethodim</td>
<td>$13.88</td>
</tr>
<tr>
<td></td>
<td>LPOSTc</td>
<td>39 – 40</td>
<td>Imazethapyr + clethodim or fomesafen + clethodim</td>
<td>$10.22 or $25.97</td>
</tr>
<tr>
<td></td>
<td>VLPOST</td>
<td>52 – 55</td>
<td>Bentazon + glyphosate</td>
<td>$26.12</td>
</tr>
<tr>
<td>Rocky Mount (3)</td>
<td>EPOST</td>
<td>10 – 14</td>
<td>Fomesafen + clethodim</td>
<td>$25.97</td>
</tr>
<tr>
<td></td>
<td>MPOST</td>
<td>25</td>
<td>Acifluorfen + clethodim</td>
<td>$13.88</td>
</tr>
<tr>
<td></td>
<td>LPOST</td>
<td>36 – 40</td>
<td>Dicamba + glyphosate</td>
<td>$18.89</td>
</tr>
<tr>
<td></td>
<td>VLPOST</td>
<td>50 – 55</td>
<td>Dicamba + glyphosate</td>
<td>$18.89</td>
</tr>
<tr>
<td>Lewiston-Woodville (6)</td>
<td>EPOST</td>
<td>9 – 14</td>
<td>Fomesafen + clethodim</td>
<td>$25.97</td>
</tr>
<tr>
<td></td>
<td>MPOST</td>
<td>23 – 27</td>
<td>Glyphosate</td>
<td>$7.07</td>
</tr>
<tr>
<td></td>
<td>LPOST</td>
<td>37 – 42</td>
<td>Glyphosate</td>
<td>$7.07</td>
</tr>
<tr>
<td></td>
<td>VLPOST</td>
<td>48 – 56</td>
<td>Glyphosate</td>
<td>$7.07</td>
</tr>
</tbody>
</table>

*Crop oil concentrate (0.13 gal acre\(^{-1}\)) was added with acifluorfen and fomesafen mixtures with clethodim. A nonionic surfactant (0.25\%) was added with the imazethapyr and clethodim mixture.

b*If received the early treatment, only acifluorfen + crop oil concentrate was applied at the mid timing.

c*If plots received the early and mid-treatments prior to the LPOST, imazethapyr + clethodim was applied, otherwise fomesafen + clethodim was applied.
Weed Control, Yield, and Economic Returns

Twin-row Soybean

When pooled over the six environments for twin-row soybean, broadleaf weed control was affected by herbicide application program ($P = 0.0237; F = 2.9$). When herbicides were applied two or more times, broadleaf weed control was similar regardless of specific timing (98 to 100%; Figure 1A). Single applications (EPOST- and VLPOST-only) provided 82 to 89% broadleaf weed control, which was less than the more intensive herbicide regimes. Herbicide program also affected annual grass control ($P = 0.0201; F = 2.9$) in a similar manner to broadleaf weed control. Excluding the MLVPOST application, when herbicides were applied two or more times, annual grass control was 100% and increased compared to the single applications which provided 86 to 92% control (Figure 1C).

With respect to soybean yield, a significant and positive correlation with broadleaf weed control ($P = 0.0041; r = 0.22$) was observed; however, the correlation with annual grass control was not significant ($P = 0.1338$). The data suggest that yield increased as broadleaf weed control increased. Previous research has shown that broadleaf weeds are generally more competitive with soybean than annual grasses (Hock et al., 2006). Soybean yield ($P < 0.0001; F = 7.6$) and economic return ($P < 0.0001; F = 6.5$) were affected by herbicide timing and frequency. For yield, only the non-treated soybean had reduced yields (800 to 1110 lbs acre$^{-1}$ lower) with all other herbicide regimes being similar (Figure 2A). Non-treated soybean also had the lowest economic return compared to any herbicide regime ($112.78$ to $177.72$ acre$^{-1}$ lower; Figure 2C). Within herbicide treatment and timings, economic return for the VLPOST-only treatment ($308.02$ acre$^{-1}$) was less than that for the EML- or EMLV-POST programs ($371.34$ and $372.96$ acre$^{-1}$, respectively).
Narrow-row Soybean

In the narrow-row soybean experiments, broadleaf weed control was only affected by herbicide program (P = 0.0001; F = 9.0) with no differences detected among POST herbicide active ingredients (P = 0.5918). When pooled over the five environments, broadleaf weed control was 18 to 48% lower when an EPOST- or VLPOST-only treatment was applied compared to the other herbicide programs (Figure 1B). Within the sequential herbicide programs, the EML- and EMLV-POST treatments increased broadleaf weed control (18 to 19% increase) compared to the LVPOST treatment with all other programs providing similar control. Annual grass control was also affected by herbicide program alone (P < 0.0001; F = 10.8). Herbicide programs with three or more applications improved control by 29, 16, and 12% compared to the VLPOST, EPOST, and EMPOST, respectively.

For soybean yield, a significant and positive correlation with broadleaf (P = 0.0004; r = 0.30) and annual grass control (P < 0.0001; r = 0.35) was observed. The improved correlation in the narrow-row soybean experiments compared to the twin-row soybean is likely due to the higher predominant weed densities in Rocky Mount compared to Lewiston-Woodville. These data suggest that yield increased as broadleaf and annual grass control increased. Soybean yield (P = 0.0016; F = 3.6) and economic return (P = 0.0658; F = 2.0) were affected by herbicide timing and frequency. For yield, the non-treated soybean had reduced yields (570 to 1,000 lbs acre\(^{-1}\) lower) compared to treated soybean, regardless of specific program (Figure 2B). Within herbicide programs, the EMPOST (3,710 lbs acre\(^{-1}\)) yielded greater than did the VLPOST-only (3,280 lbs acre\(^{-1}\)) with all other programs yielding similarly (3,530 to 3,680 lbs acre\(^{-1}\)). All herbicide programs provided similar economic returns ($313.65 to $382.36 acre\(^{-1}\)) and were greater than that of non-treated soybean ($283.33 acre\(^{-1}\)$).
Generally, broadleaf and annual grass control showed similar trends in both twin- and narrow-row soybeans. Previous research has illustrated weed control in soybean planted on narrow- or twin-row spacing was similar regardless of herbicide treatment (Nelson, 2007). However, more research is needed to directly compare the two planting patterns when competing with similar weed populations. In general, two to three applications were required in order to achieve greater than 90% weed control (Figures 1A-D). In a study by Jha et al. (2008), a single glyphosate application occurring at 5 weeks after soybean emergence (WAE) controlled pusley less than when the application occurred 3 WAE. Both timings were less effective than when glyphosate was applied sequentially at 3 and 5 WAE or 3, 5, and 8 WAE. In cotton, Inman et al. (2018) reported at least three POST applications, occurring two to five WAP, were required to provide more than 95% control for Palmer amaranth with declining control as the number of herbicide applications decreased. Soybean is generally more competitive with weeds than cotton (Zimdahl, 2004), which may explain why fewer applications were needed for adequate weed control in soybean in our studies. Gower et al. (2002) applied glyphosate in corn as single or sequential applications based on weed size to determine the effect on weed control and yield. Excluding 2 of 30 instances, sequential applications of glyphosate increased control of giant foxtail, common lambsquarter (Chenopodium album L.), and velvetleaf (Abutilon theophrasti Medicus) compared to their single application counterpart.

With respect to soybean yield, Jha et al. (2008) observed similar yields when glyphosate was applied POST either two or three times. These timings provided greater yield than a late (5 WAE) single application or no application. However, Jha et al. (2008) reported a single application at three WAE resulted in lower soybean yield compared to multiple herbicide applications. A single early application in our study was able to protect soybean yields (Figures
These differences may be attributed to herbicides utilized for each application. Glyphosate is known to provide little to no residual activity while fomesafen may provide soil residual activity application (Anonymous, 2017 and 2018). In another study, Legleiter et al. (2009) reported soybean yield generally increased with a PRE fb POST program when compared to a PRE or POST only program. Additionally, these more intensive programs generally increased net return over the less intensive counterparts, a similar trend generally observed in our study (Figures 2C and D). In corn, Gower et al. (2002) reported that sequential glyphosate applications did not improve yields compared to its single application counterpart; although on average, yields were 5 to 7% greater when a sequential herbicide application made. While single early applications (2- to 4-inch giant foxtail height) generally yielded similar to the weed-free check, weed re-infestation did occur in these plots. Weed re-infestation was observed in the current presented research as noted by EPOST-only treatments provided less annual grass and broadleaf weed control compared to more intensive or later-timed applications (Figures 1A-D). Gower et al. (2002) noted that caution should be taken to ensure mitigation of weed seed additions to the soil seed bank for future production years.

**Research Implications**

Controlling weeds timely and effectively is critical in order to maximize yields and doing so in an efficient manner is key for producers in order to maximize returns. However, caution and long-term considerations, such as soil weed seedbank dynamics, need to be part of the decision-making process when designing weed management (Gower et al., 2002; Norsworthy et al., 2018). While the EPOST only herbicide treatment provided similar yields to other herbicide combinations (excluding VLPOST-only), both broadleaf and annual grass control ratings were lower for this treatment (Figures 1A-D). Later emerging weeds not controlled by EPOST have
the potential to add significant amounts of seed to the soil weed seedbank and create higher and more problematic weed populations in future crops (Gower et al., 2002; Norsworthy et al., 2018). Our results suggest that at least two POST applications are required in twin- or narrow-row soybean in order to adequately control weeds in a single season while maintaining soybean yield. However, three to four sequential POST applications were needed to ensure 95 to 100% weed control, which are more desirable control levels to manage and reduce weed seedbanks over time. While the production is more costly to the farmer, the data show these costs may not significantly affect the overall net return. However, the improved control each season can provide an overall depletion of weed seed in the seedbank that may pose further management issues long-term. These results illustrate the trade-off growers must face between weed control cost, crop yield, and short vs. medium/long term weed management. Future research should explore additional timing and herbicide (i.e. contact vs systemic) combinations which include residual products. Additionally, a study should include a PRE timing to optimize POST herbicide timing and frequency when a PRE was applied. The use of PRE residual herbicides might reduce the need and cost of POST control applications, while maintaining adequate weed control levels.

Acknowledgements
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References


Figures 1A-D. Broadleaf weed and annual grass control in twin- (A and C, respectively) and narrow-row (B and D, respectively) soybean as influenced by herbicide timing and frequency. Timings include combinations of early (EPOST), mid (MPOST), late (LPOST), and very-late (VLPOST) POST applications at VC, V2, V4, and R1 growth stages, respectively. The first letter of each timing was utilized to abbreviate herbicide timings where multiple applications were made. Bars with the same letters are not significantly different according to Fisher’s protected LSD at $\alpha = 0.1$. 
Figures 2A-D. Soybean yield and net economic return in twin- (A and C, respectively) and narrow-row (B and D, respectively) soybean as influenced by herbicide timing and frequency. Timings include combinations of early (EPOST), mid (MPOST), late (LPOST), and very-late (VLPOST) POST applications at VC, V2, V4, and R1 growth stages, respectively. The first letter of each timing was utilized to abbreviate herbicide timings where multiple applications were made. Bars with the same letters are not significantly different according to Fisher’s protected LSD at $\alpha = 0.1$. 

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**Twin-row soybean**

**Narrow-row soybean**

![Graphs showing soybean yield and net economic return across different herbicide timings and frequencies.](image)

Herbicide timing

Herbicide timing

**Figure 2A.** Soybean yield and net economic return in twin-row soybean as influenced by herbicide timing and frequency.

**Figure 2B.** Soybean yield and net economic return in narrow-row soybean as influenced by herbicide timing and frequency.

**Figure 2C.** Soybean yield and net economic return in twin-row soybean as influenced by herbicide timing and frequency.

**Figure 2D.** Soybean yield and net economic return in narrow-row soybean as influenced by herbicide timing and frequency.
The effect of nozzle selection and carrier volume on weed control in soybean in North Carolina

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Nozzle selection and carrier volume – or gallons per acre (GPA) – can affect weed control and requires consideration in management plans. The interaction of nozzle selection and GPA has been shown to affect POST herbicide efficacy and spray deposition (Creech et al. 2015; Legleiter and Johnson 2016). Research is limited regarding nozzle selection and GPA influence on PRE herbicide efficacy. Borger et al. (2013 and 2015) reported 14 to 25% increased rigid ryegrass (Lolium rigidum Gaudin.) control with trifluralin as GPA increased from 3.2 to 16 gal/acre. Borger et al. (2013) also reported spray coverage increased when using a medium-droplet-size nozzle compared to nozzles delivering coarse droplets; however, weed control was not affected.
Since 2010, the average farm size has been increasing (USDA 2018). Improving efficiency on large farms by increasing acreage covered per application tank increases profit margins. Lessening the GPA applied is more efficient but may affect coverage, thus reducing weed control. Research evaluating the interaction of nozzle selection and GPA on preemergence (PRE) and postemergence (POST) herbicide efficacy is required in order to understand these affects. As such, the objectives of this study were to determine the influence of nozzle selection and GPA on PRE and PRE followed by (fb) POST herbicide efficacy on weeds in a soybean [*Glycine max* (L.) Merr.] crop.

Two field experiments were conducted at the Upper Coastal Research Station (Rocky Mount, NC) on a Goldsboro fine sandy loam (Aquic Paleudult) in 2017 and 2018. In mid-June, following disking and field cultivation, soybean was planted (approximately 120,000 plants/acre) on rows spaced 7.5-inches apart using a grain drill. One day after planting, pyroxasulfone was applied PRE (0.11 lb a.i./acre) fb dicamba plus glyphosate (0.50 + 0.84 lb a.e./acre, respectively) four weeks later. Applications were made with a CO$_2$-pressurized backpack sprayer (approximately 20 inches above the crop canopy) calibrated to deliver 0.13 gal/min at 18 psi fitted with extended range flat fan, air-induction extended range, or Turbo TeeJet Induction 11002 nozzles (TeeJet, Spraying Systems Co., Wheaton, IL). Carrier volumes included 7.5, 15, 30, or 60 gal/acre. Postemergence herbicide applications following PRE treatments were applied similarly. To mitigate the effect on droplet size, speed was altered to achieve the proper rate at each GPA (5.5, 2.8, 1.4, and 0.7 MPH, respectively). Wind speeds ranged from 5 to 8 MPH at each application timing. Treatments were arranged in a randomized complete block design with four replications. Plot size was 6 by 30 ft. Weed control was visually estimated on a scale of 0 to 100% (0 = no injury and 100 = death) 4 wk after treatment (WAT) PRE and 7 and 15 WAT
POST. Natural weed populations consisted predominantly of Palmer amaranth (9 to 22 plants/ft$^2$; *Amaranthus palmeri* S. Wats.) and large crabgrass [6 to 17 plants/ft$^2$; *Digitaria sanguinalis* (L.) Scop.]. Soybean was harvested using a combine and yield was adjusted to 13% moisture content.

Weed control and soybean yield were analyzed using PROC GLIMMIX in SAS (SAS 9.4, SAS Institute Inc., Cary, NC). Nozzle and GPA were considered fixed effects with replication and experimental run considered random. Means were separated according to Fisher’s protected LSD ($\alpha = 0.05$).

While nozzle type, nor its interaction with GPA were significant ($P = 0.0858$ to $0.8185$), the main effect of GPA was significant for weed control ($P < 0.0001$ to $0.0026$; $F = 5.2$ to $11.6$). At 4 WAT PRE, control at 7.5 and 15 gal/acre GPA was similar and less than control at 30 or 60 gal/acre (Table 1); however, the difference was only 8 to 12%. Borger et al. (2013 and 2015) reported a more pronounced effect of GPA with weed control increasing 14 to 25% as GPA increased. The lower range GPAs utilized by Borger et al. (2013 and 2015) may explain why our results are less pronounced. Additionally, whereas Borger et al. (2013 and 2015) reported low rainfall following application, adequate rainfall for herbicide activation occurred within 10 days of our PRE application in 2017 (0.83 inches) and 2018 (2.15 inches). Weed control at 7 and 15 WAT PRE fb POST was similar (Table 1); thus, to eliminate repetition, only 15 WAT POST data are discussed. While control was significantly different ($P = 0.0003$; $F = 7.1$) across GPA, the range was 91 to 96%. Creech et al. (2015) reported decreased weed control with lower GPAs when using glufosinate and lactofen POST. The systemic nature of the POST herbicides used in our experiments likely explain the minimal difference ($\leq 5\%$) in observed control. Soybean yield was not affected by nozzle or GPA and averaged $4,730 \pm 160$ lb/acre (data not shown). The results suggest that adequate weed control may be achieved using lower GPA for PRE.
applications. Additionally, with systemic POST herbicides, GPA may have less impact than when using a contact herbicide and should be researched further. Lower GPAs may help increase efficiency and profitability as more acreage can be treated per application tank. It should be noted, that the selected application nozzle and GPA should be accordance with label recommendations, especially for synthetic auxin herbicides.

Acknowledgements

The authors would like to thank the North Carolina Agricultural Foundation for partial funding and support of this research.
References


Table 1. Palmer amaranth and large crabgrass control following preemergence (PRE) and postemergence (POST) applications at 4, 7, and 15 wk after treatment (WAT) in Rocky Mount, NC in 2017 and 2018.†‡§

<table>
<thead>
<tr>
<th>Carrier volume</th>
<th>Rating date (WAT)</th>
<th>PRE</th>
<th>PRE fb POST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gal/acre</td>
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<td>90 c</td>
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<td>74 a</td>
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<td>95 ab</td>
</tr>
</tbody>
</table>

†Preemergence applications were made one day following planting (June 13, 2017 and June 14, 2018).
‡Postemergence applications were made 4 wk after the PRE application.
§Means within rating date followed by the same letter are not significantly different according to Fisher’s protected LSD ($\alpha = 0.05$). Data are pooled over 2 yr.