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Atrazine-Resistant AMAPA

Confirmation and Management of Atrazine-Resistant Palmer Amaranth (*Amaranthus palmeri*) in North Carolina

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Abstract

North Carolina farmers have extensively relied on atrazine for effective weed control in corn, particularly for Palmer amaranth. In 2016, a grower from Washington County, NC, reported a control failure of Palmer amaranth in a corn field treated with atrazine. Greenhouse studies were conducted with the objectives to confirm if the Washington County Palmer amaranth population was resistant to atrazine and to determine other effective postemergence herbicides. Two atrazine-susceptible Palmer amaranth populations collected from Edgecombe and Johnston County were used for comparison. Greenhouse response assays confirmed the Washington County population is resistant, with a lethal dose causing 50% mortality (LD_{50}) of 1,253 g ai ha^{-1}. This population was 7- and 9-fold less susceptible to atrazine compared with the Edgecombe (LD_{50}: 208 g ai ha^{-1}) and Johnston County (LD_{50}: 155 g ai ha^{-1}) populations, respectively. Additionally, the LD_{80} (lethal dose causing 80% mortality) of the Washington County population (12,107 ga ai ha^{-1}) was more than five times greater than the maximum labeled rate for atrazine. A subsequent herbicide screen that utilized field labeled rates suggested that 2,4-D, dicamba, fomesafen, glufosinate, and mesotrione provide at least 79% or greater control of the Washington County population. The Washington County population was not effectively controlled by thifensulfuron or glyphosate, both of which have widespread resistance documented throughout North Carolina. The results of these experiments provide evidence that the Washington County population is resistant to atrazine, glyphosate, and thifensulfuron, and effective herbicides to manage this population were confirmed.

Nomenclature: 2,4-D; atrazine; dicamba; glufosinate; glyphosate; mesotrione; thifensulfuron; Palmer amaranth, *Amaranthus palmeri* S. Watson.

Keywords: dose-response**,** herbicide resistance, weed management

Introduction

Palmer amaranth is consistently ranked as one of the most common and troublesome weeds for U.S. growers (Van Wychen 2025). Native to the southwest United States (Sauer 1972), the species has proliferated throughout the Central and Southeast United States (Ward et al. 2013; Webster and Grey 2015). Palmer amaranth has been documented to produce over 600,000 seeds per plant (Keeley et al. 1987) and is capable of rapid germination and accelerated growth (Burke et al. 2007; Horak and Loughin 2000; Steckel et al. 2004). Palmer amaranth has been considered by far the most successful invader of disturbed habitats (Ward et al. 2013). Palmer amaranth can cause extensive yield losses in row crops such as soybean [*Glycine max* (L.) Merr.], corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), sweetpotato (*Ipomoea batata* L.), and grain sorghum (*Sorghum bicolor* L.) due to competitive interference (Klingaman and Oliver 1994; Massinga et al. 2001; Meyers et al. 2010; Moore et al. 2004; Morgan et al. 2001).

Palmer amaranth, due to high fecundity and being a dioecious species, has a high potential for a rapid development of herbicide resistance (Borgato et al. 2025; Roberts and Florentine 2021).

This species has evolved resistance to nine herbicide modes of action: acetolactate synthase (ALS [(WSSA Group 2)]-inhibitors, microtubule (WSSA Group 3)-inhibitors, synthetic auxins (WSSA Group 4), photosystem II [PSII (WSSA Group 5)]-inhibitors, 5-enolpyruvyl-shikimate-3-phosphate synthase [EPSPS (WSSA Group 9)]-inhibitors, glutamine synthetase (WSSA Group 10)- inhibitors, protoporphyrinogen oxidase [PPO (WSSA Group 14)]-inhibitors, very-long-chain-fatty-acid inhibitors [VLCFA (WSSA Group 15)]-inhibitors, and 4-hydroxyphenylpyruvate dioxygenase [HPPD (WSSA Group 27)] inhibitors (Heap 2025). In addition, populations with resistance to multiple modes of action and/or cross-resistance to multiple herbicide families within the same mode of action are widespread across the United States (Heap 2025).

Atrazine affects photosynthesis by preventing electron transfer at the reducing site of photosynthesis system II in the chloroplasts and subsequently causes an overproduction of free radicals that disrupt cell membrane integrity (Cooper et al. 2007; Shimabukuro and Swanson 1969). Atrazine can be used for preemergence and postemergence weed control and is effective on many annual broadleaf and certain grass weed species (Knuesli 1970; Solomon et al. 1996), being primarily used in sorghum (*Sorghum bicolor* (L.) Moench), sugarcane (*Saccharum* L.) and

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corn (Williams II et al. 2010). Atrazine is extensively used in U.S. corn production, with over 63% of corn hectares treated annually (Armel et al. 2003), where an average of 32 million kilograms is applied across 30 million hectares (EPA 2020). Previous to the introduction of glyphosate-tolerant crops, triazines were the most common herbicides used in corn production between 1990 and 2005, applied to 62 to 89% of corn land (Webster and Nichols 2012).

Through extensive usage, the first case of atrazine resistance in Palmer amaranth was reported in Texas and subsequently was confirmed in Georgia, Kansas, Iowa, Nebraska, New York, and Wisconsin (Heap 2025). Resistance to atrazine in *Amaranthus* species is commonly associated with a target-site mutation in the *psbA* gene encoding the D1 protein of photosystem II, particularly a Ser264Gly substation that reduces atrazine binding at the Q_B-binding site (Devine and Preston 2000; Hirschberg and McIntosh 1983). Despite reports of atrazine resistance in Palmer amaranth since 1993, it is likely that the reproductive fitness penalties associated with triazine (PSII-inhibitors) target-site resistance have halted the occurrence and spread of this mutation in A*maranthus* species (Kohrt et al. 2017; Sibony and Rubin 2003; Soltani et al. 2008). However, nontarget-site resistance mechanisms have also been reported, most commonly involving enhanced metabolic detoxification mediated by the increased glutathione *S*-transferase (GST) activity (Anderson and Gronwald 1991). Because nontarget-site resistance mechanisms do not appear to impose measurable fitness penalties in *Amaranthus* species (Gray et al. 1995), they may persist and spread more readily within populations and have been documented among *Amaranthus* species (Chahal et al. 2019; Chaudhari et al. 2020; Nakka et al. 2017; Wu et al. 2018). Thus, nontarget-site resistance associated with enhanced metabolic activity may facilitate sustained persistence and dissemination of atrazine resistance in Amaranthus populations. Despite this potential, atrazine generally remains effective in North Carolina, as a survey of 120 Palmer amaranth accessions demonstrated that atrazine continued to control Palmer amaranth (Moore et al. 2021).

However, in 2016, a farmer from Washington County, NC reported a control failure of Palmer amaranth in a corn field treated with atrazine postemergence. Plants from the Washington County Palmer amaranth population were collected for further analysis to determine if atrazine resistance was present. The objectives of this research were to determine if the Washington County Palmer amaranth population had developed resistance to atrazine and effective postemergence herbicides to control this population.

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Materials and Methods

Plant material.

Mature seedheads from the Washington County (WC) Palmer amaranth plants surviving atrazine were collected in 2016 from the atrazine-treated field. The seeds were threshed, cleaned, and combined into a single sample and stored at 4 C. Two known atrazine-susceptible Palmer amaranth populations from North Carolina, collected from the Upper Coastal Plains Research Station (35° 53′ 33.2″ N, 77° 40′ 49.8″ W) located in Edgecombe County (EC) and the Central Crops Research Station (35° 40′ 5.36″ N, 78° 30′ 6.63″ W) located in Johnston County (JC) were selected for comparison. Mature seedheads from both susceptible populations were collected during the fall of 2020 and cleaned and stored as described above.

Postemergence dose-response experiment.

Seeds from the WC, EC and JC populations were sown in March 2023 into separate 52x25x6 cm plastic trays containing a 4:1 mixture of potting media (Sunshine® Mix #1 [Sun Gro Horticulture, Agawam, MA, USA]) and sand. The trays received 5 grams of Osmocote Flower Food Granules (14-14-14) as fertilizer. Plants were watered as needed through overhead irrigation. Once germinated, two seedlings (1 to 3 cm tall) from each population were transplanted into 8x8 cm square plastic pots containing the previously described growing medium. The greenhouse conditions were set for diurnal fluctuations of 30/20 C and 12:12 h day/night photoperiods, where supplemental light was provided (600 – 1000 µmol $m^{-2}s^{-1}$).

The experimental design consisted of a randomized complete block with four replications and two experimental runs replicated in space, utilizing two adjacent greenhouses set for the same environmental conditions as described above. Atrazine (Aatrex® 4L, Syngenta®, Greensboro, NC, USA) was applied postemergence to plants 7- to 9-cm in height (4- to 6-leaf) with a CO_2-powered track-mounted sprayer equipped with a Teejet 8002EVS nozzle (TeeJet Technologies, Wheaton, IL, USA) 46 cm above the target height at an output of 140 L ha^{-1}. Atrazine doses consisted of 0, 56, 177, 560, 1770, 5600 and 17,700 g ai ha^{-1} along with crop oil concentrate (Crop Oil Concentrate; Helena Chemical Co., Collierville, TN) at 10 mL L^{-1} as recommended by the label for postemergence applications.

Plant survival was evaluated at 21 days after treatment (DAT) by visually assessing each plant as either alive (i.e., new, green vegetative tissue present) or dead (i.e., no green vegetative tissue present). Survival was recorded at the experimental unit level (two plants per pot), where the number of surviving plants per unit ranged from 0 to 2. Subsequently, for graphical representation purposes, percent survival for each population and herbicide rate was calculated as the mean number of surviving plants per experimental unit divided by the total number of treated plants (two) and expressed as a percentage. Following the survival assessment at 21 DAT, aboveground dry biomass was collected by clipping individual shoots at the soil surface, placing them into paper bags, drying 65 C for 72 hours, and recording dry weight. Relative dry biomass was calculated for each experimental unit as a percentage of the non-treated control within each population.

Effective postemergence herbicide screen.

Simultaneous experiments were conducted in March 2023 to evaluate effective alternative postemergence herbicides for the WC population. Herbicides were selected based on their common use as postemergence herbicide options in North Carolina corn and soybean production systems (Everman, personal reference). Glyphosate and thifensulfuron resistance in Palmer amaranth are widespread in North Carolina (Mahoney et al. 2020), however they were added to evaluate susceptibility of the WC, EC, and JC populations. The rest of the herbicides were selected due to their effectiveness for controlling glyphosate-resistant Palmer amaranth (Meyer et al. 2015). Alternative PSII-inhibiting herbicides were not included, as the objective of this experiment was to identify effective postemergence control options outside of the PSII mode of action. Seeds from the WC, EC and JC populations were sown and maintained under greenhouse conditions as described above. Single seedlings (1 to 3 cm tall) were then individually transplanted into 4x20 cm cone-tainers (Greenhouse Megastore, Danville, IL) containing the soil medium as described above. The experiment was designed as a randomized complete block with seven replications and two experimental runs replicated in space, using two adjacent greenhouses set up with the same environmental settings as described above. Seven herbicides with different modes of action were applied to plants 7- to 9-cm in height (4- to 6-leaf) using the CO_2-powered track-mounted sprayer with the same settings used in the dose-response experiment. Herbicide doses were applied at 1X labeled field rates with adjuvants per label recommendations (Table 1). A visual assessment of plant survival was performed 21 DAT as described in the dose-response section.

Statistical Analysis

Postemergence dose-response experiment.

Plant survival and biomass reduction data were analyzed using nonlinear regression to estimate population-level dose–response relationships. To model the binomial response of plant survival ("0" = dead, "1" = alive), data were modeled using a two-parameter log-logistic function (Equation 1) in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) with a binomial distribution and logit link, with the upper asymptote fixed at "1" (100% survival):

$$Y = \frac{1}{(1+\exp(b*(\log(x)-\log(e))))} \text{ [1]}$$

Where Y is the probability of plant survival at herbicide dose x, e is the herbicide dose required to reduce survival by 50%, and b is the slope parameter describing the rate of change in survival at e. Population-specific LD_{50} and slope parameters were estimated within a single nonlinear model, with experimental run and replication treated as random effects. Percent survival for graphical representation was calculated as the mean survival per experimental unit divided by the total number of plants per unit (2 plants) and expressed as a percentage. Dose-response curves for each population were plotted in Sigmaplot 14.0 for graphical representation of the observed survival data (Figure 1).

Dry biomass data were analyzed using PROC GLIMMIX in SAS 9.4 where population and rate were considered main effects while experimental run and replication were treated as random effects. Dry biomass data were modeled using a three-parameter log-logistic equation (Equation 2) in Sigmaplot 14.0 (Systat Software, Palo Alto, CA, USA):

$$y = \frac{a}{[1 + (x \,/\, x0)b} \text{ [2]}$$

Where a is the upper asymptote, x is the herbicide rate, $x0$ equals the BR_{50} (herbicide dose required to reduce dry biomass by 50%), and b is the slope at $x0$. In addition BR_{50} estimates, BR_{80} values (herbicide dose required to cause 80% mortality and/or dry biomass reduction) were calculated from the fitted models to allow further comparison among populations.

Resistance factors (RF) were calculated to determine atrazine susceptibility shifts between the WC (putative resistant) and the EC and JC (known susceptible) populations, where the LD_{50}/BR_{50} value of WC population were divided by the LD_{50}/BR_{50} values of the EC and JC populations. An RF value statistically greater than 2.0 suggests the population is herbicide-resistant (Burgos et al. 2013).

Effective postemergence herbicide screen.

Plant survival and biomass reduction data were subjected to ANOVA using PROC GLIMMIX in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) ($\alpha = 0.05$) where population and herbicide were considered main effects while experimental run and replication were considered random effects. The main effect of population was not significant ($P = 0.36$), however the herbicide used had a significant main effect on plant survival ($P < 0.001$), and a significant population by herbicide interaction ($P < 0.0001$) revealed that herbicide response varied by population. Means for the effective herbicide screen were separated using Fisher's Least Significant Difference ($P \leq 0.05$) and control data from nontreated plants and treatments that incurred complete control (e.g., 100% control) were excluded from statistical analysis to not violate the constant variance assumption of analysis of variance. Ninety-five percent confidence intervals were calculated to determine whether any treatment was like the treatments excluded from the analysis. Data from the effective herbicide screen were further evaluated using the mixed-model ANOVA framework previously described. Population, herbicide, and their interaction were examined to assess whether herbicide performance differed between the putative resistant and susceptible populations.

Results and Discussion

Postemergence dose-response experiment

The WC population exhibited the lowest susceptibility to atrazine as shown by plant mortality (Figure 1). The estimated LD_{50} for the WC population treated with atrazine was 1,402 g ai ha^{-1}, in contrast to 218 and 161 g ai ha^{-1} for the EC and JC populations, respectively (Table 2). The RF values for the WC population were 6.4 and 8.7 when compared to the EC and JC populations, respectively. Decreased susceptibility was further demonstrated by the WC population as the LD_{80} was 12,107 g ai ha^{-1}, whereas the EC and JC populations exhibited LD_{80} values of 453 and 365 g ai ha^{-1}, respectively (Figure 1; Table 2).

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Dry biomass reduction data results follow a similar trend as plant mortality, as the WC population displayed the lowest biomass reduction following treatment with atrazine compared with the other two populations (Figure 2). The BR_{50} was 1,448 g ai ha^{-1} for the WC population, compared with 212 and 59 g ai ha^{-1} for the EC and JC populations, respectively (Figure 2; Table 3). Corresponding RF values for the WC population were 6.8 and 24.5 relative to the EC and JC populations, respectively (Table 3). Furthermore, the BR_{80} for the WC population was 12,835 g ai ha^{-1}, whereas the BR_{80} was 531 and 214 g ai ha^{-1} for the EC and JC populations, respectively.

The WC population survived atrazine rates that were lethal to two known susceptible populations. The lethal rates of atrazine in this research were comparable with other research confirming atrazine resistance (Jhala et al. 2014; Kohrt et al. 2017; Nakka et al. 2017; Besançon et al. 2026). Additionally, the WC population exhibited survival at atrazine rates exceeding the labeled maximum, as indicated by the LD_{80} value of 12,107 g ai ha^{-1}. This rate is more than five times the maximum labeled application rate of 2,240 g ai ha^{-1}. These results suggest that the WC population is resistant to atrazine (Burgos 2013; Burgos et al. 2015).

In Kansas, a confirmed atrazine-resistant Palmer amaranth population exhibited BR_{50} values of 11,386 g ai ha^{-1} (Nakka et al. 2017). In Michigan, Kohrt et al. (2017) reported a BR_{50} of 1,200 g ai ha^{-1}, while a Palmer amaranth population from Nebraska reported that the maximum tested rate of atrazine (13,450 g ai ha^{-1}) resulted in only 20% control and a 25% biomass reduction (Jhala et al. 2014). Most recently, two Palmer amaranth populations from New York (Besançon et al. 2026) reported 50% survival at approximately 21,355 g ai ha^{-1} and 50% biomass reduction at 278 g ai ha^{-1}.

While this study did not investigate the physiological or molecular basis of atrazine resistance in the WC population, previous research in *Amaranthus* species has shown that resistance to PSII-inhibiting herbicides can arise through both target-site and nontarget-site mechanisms. The most widely reported target-site mechanism involves the Ser264Gly substitution in the *psbA* gene, which reduces atrazine binding at the D1 protein and can confer cross-resistance to other triazine herbicides (Devine and Preston 2000; Beckie and Tardiff 2012). Nontarget-site resistance mechanisms, including enhanced metabolic detoxification mediated by glutathione S-transferases, have also been documented and may contribute to more variable responses among PSII-inhibiting herbicides (Anderson and Gronwald 1991; Nakka et al. 2017). Although the specific mechanism in the WC population remains unknown, these previously

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reported resistance pathways help contextualize the observed reduction in atrazine sensitivity and suggest that additional work would be useful to clarify whether cross-resistance to other Group 5 herbicides may also be present.

It is also important to note that atrazine activity and observed injury responses may vary depending on application method and exposure pathway. Previous research has demonstrated that atrazine can be absorbed through both foliar and root tissues, and that whole-plant response may reflect contributions from both pathways following postemergence application (Thompson and Slife 1969; Thompson and Slife 1970). Foliar uptake of atrazine is known to be variable and influenced by environmental and physiological conditions at the time of application, while root uptake following soil deposition can also contribute to overall efficacy (Dexter et al. 1966; Smith and Nalewaja 1972; Thompson and Slife 1970). Because this study evaluated only postemergence foliar applications, direct comparisons to soil-applied atrazine responses cannot be made, and interpretation is limited to foliar exposure conditions.

Effective postemergence herbicide screen.

Plant survival in the WC population varied widely among the herbicides evaluated, ranging from complete control to no effect (Table 4), therefore results are presented separately by herbicide and ordered by ascending WSSA herbicide group number.

Thifensulfuron.

Thifensulfuron was ineffective against the WC population, with 100% plant survival observed, consistent with the EC and JC populations, in which all plants also survived treatment (Table 4). This response aligns with previous reports demonstrating widespread ALS-inhibitor resistance in Palmer amaranth across North Carolina, where statewide screenings have shown minimal control with thifensulfuron (Alsdorf 2024; Moore et al. 2021; Poirier et al. 2014). Given the lack of response in the WC population and the well-documented statewide prevalence of ALS resistance, the WC population was concluded to be resistant to thifensulfuron.

Dicamba.

Treatment with dicamba reduced survival of the WC and JC populations to 14%, whereas the EC population exhibited 64% survival (Table 4). Further research is warranted to better characterize the EC population's response to dicamba. Previous research in Nebraska reported 91–100% control of glyphosate-resistant Palmer amaranth with dicamba, accompanied by reductions in biomass and seed production (de Sanctis et al. 2021). Similarly, Moore et al. (2021)

observed consistent dicamba efficacy in North Carolina, although variability in control was also noted in herbicide resistance screening conducted by Alsdorf (2024).

2,4-D.

Treatment of the WC population with 2,4-D resulted in 21% survival, compared with 64% survival for the EC population and 43% for the JC population (Table 4). Although some WC plants survived the 2,4-D treatment, the herbicide remains a useful tool in integrated management programs where tank mixes and mode of action rotation is advised, particularly due to the availability of 2,4-D traited crops that allow over-the-top applications. The relatively high survival of the EC and JC populations suggests that further studies are warranted to evaluate their susceptibility to 2,4-D. These populations (EC and JC) were collected from research station fields with a history of 2,4-D-resistant crop research, where repeated applications of 2, 4-D may have contributed to reduced susceptibility. Resistance to 2,4-D in Palmer amaranth has been recently documented in North Carolina (Argueta 2025; Heap 2025) has also been reported in Kansas (Heap 2025; Kumar et al. 2019). Regional studies previously indicated that 2,4-D remained effective in North Carolina; however, recent findings confirm the presence of 2,4-D–resistant populations in the state, demonstrating that control can vary substantially among populations and environmental conditions (Alsdorf 2024; Argueta 2025; Moore et al. 2021).

Glufosinate.

Glufosinate treatment resulted in no survival of plants from the WC, EC, and JC populations following treatment (Table 4). These results are consistent with earlier North Carolina screens in which 100% of tested populations were effectively controlled (Alsdorf 2024; Mahoney et al. 2020; Poirier et al. 2014). Despite this consistent efficacy, the recent confirmation of glufosinate-resistant Palmer amaranth in North Carolina (Jones et al. 2024B) underscores the urgency of diversifying herbicide programs to delay further resistance development.

Glyphosate.

Glyphosate treatment was ineffective against the WC population, with 93% of plants surviving, compared with 29% and 71% survival in the EC and JC populations, respectively (Table 4). Similar to thifensulfuron, widespread glyphosate-resistant Palmer amaranth has been documented through statewide screens in North Carolina (Alsdorf 2024; Mahoney et al. 2020; Poirier et al. 2014). Consistent with these patterns and the lack of control observed at labeled

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rates, the WC population was concluded to be resistant to glyphosate. Although glyphosate remains valuable for controlling other weed species, it is not recommended for management of this population.

Fomesafen.

Plant survival following fomesafen treatment was 14% for the WC population, and no plants survived from the EC and JC populations (Table 4), suggesting that fomesafen may be an effective herbicide option for management of the WC population, particularly if rotating to a soybean system. Previous research has reported effective Palmer amaranth control with fomesafen in North Carolina (88–96%; Whitaker et al. 2010) and across multiple states (Bond et al. 2006). However, Alsdorf's (2024) statewide herbicide resistance screen in North Carolina documented numerous Palmer amaranth control failures with fomesafen, and further dose-response experiments confirmed fomesafen resistance in the state (Heap 2025). Additionally, postemergence fomesafen resistance in North Carolina has been reported in related *Amaranthus* species such as waterhemp (*Amaranthus tuberculatus*) and redroot pigweed (*Amaranthus retroflexus*) (Jones et al. 2023A; Jones et al. 2023B).

Mesotrione.

Mesotrione completely controlled the WC population, with no plant survival following treatment (Table 4). It also effectively controlled the EC population, however, 29% of the JC population survived treatment. While resistance to HPPD-inhibiting herbicides has emerged in other regions and multiple-resistance based on atrazine resistance mechanisms are documented (Jhala et al. 2014; Ma et al. 2013), no such resistance was observed in the WC population. Nonetheless, the introduction of HPPD-resistant cotton and increasing HPPD herbicide use may raise concerns about future resistance selection pressure. Variable responses to mesotrione reported in the screen conducted by Alsdorf (2024) as well as the results observed in (Mahoney et al. 2020) reinforce the need for caution and diversity in herbicide programs.

A potential limitation of this study is the exclusion of other PSII-inhibiting herbicides from the effective postemergence herbicide screen. Inclusion of additional Group 5 herbicides could have provided further insight into the extent of cross-resistance within PSII-inhibiting herbicides; however, this was not the primary objective of the present study. Instead, the focus was to identify effective postemergence control options following field-observed failure of postemergence atrazine applications in Palmer amaranth. In addition, many alternative PSII-inhibiting herbicides are primarily used as soil-applied residual treatments or have limited utility as postemergence options in North Carolina row-crop systems. Therefore, the herbicide screen was intentionally restricted to commonly used postemergence herbicides representing alternative modes of action. Future research evaluating additional PSII-inhibiting herbicides could further clarify cross-resistance patterns within Group 5 herbicides in this population.

The results of the effective herbicide screen align with findings from previous statewide evaluations conducted in North Carolina (Alsdorf 2024; Mahoney et al. 2020; Moore et al. 2021; Poirier et al. 2014). Across all studies, variability in herbicide efficacy was observed among Palmer amaranth populations (Table 4). Furthermore, glyphosate and thifensulfuron failed to control the WC population, and thifensulfuron was similarly ineffective against the JC and EC populations. These findings are consistent with widespread resistance to both glyphosate and ALS-inhibiting herbicides previously documented in North Carolina Palmer amaranth populations (Poirier et al. 2014; Mahoney et al. 2020; Alsdorf 2024), however they remain a part of weed control progams due to their effectiveness controlling other weed species. Multiple resistance to glyphosate, atrazine, and ALS-inhibitors has been reported in Palmer amaranth populations in Georgia and Michigan (Heap 2025; Kohrt et al. 2017).

Conclusively, these findings indicate that effective herbicide options for the WC Palmer amaranth population remain available. Although the effective herbicide screen reflects conditions at the time this population was first reported in 2016, the results still highlight which herbicides provided control of this atrazine-resistant population. Notably, the grower eliminated the WC population in the following years with the use of dicamba and glufosinate (Everman, personal communication) demonstrating the importance of utilizing several herbicide modes of action. Widespread resistance to glyphosate and ALS-inhibitors, along with early signs of declining efficacy for fomesafen and mesotrione, and recent resistance confirmation to glufosinate in the state of North Carolina, emphasize the increasing limitations in postemergence control options. The WC population represents the first case of the atrazine resistance in Palmer amaranth in North Carolina, along with potentially the second case of a resistance profile consisting of atrazine, glyphosate and thifensulfuron in the Southeastern United States (Heap 2025). Although this population represents an isolated case of atrazine resistance in the state, preventative actions such as including integrated weed management strategies that delay the onset of atrazine resistance are crucial to maintaining its effectiveness. Future research should characterize the susceptibility of the WC population to preemergence herbicides to further provide information on effective control. Further research should also focus on investigating the mechanism of atrazine resistance of the WC population to better understand implications for long-term management, improve management recommendations to slow the spread and occurrence of resistance within the state, and assess the potential for cross-resistance to other PSII-inhibiting herbicides. Additionally, future herbicide screens should include atrazine to inform North Carolina farmers where the herbicide remains effective.

These results indicate that although several postemergence herbicide options remain effective for control of the WC Palmer amaranth population, the overall herbicide toolbox is becoming increasingly constrained by widespread resistance to multiple modes of action. The continued reliance on a limited number of effective postemergence herbicides, particularly dicamba and glufosinate, raises concern regarding increased selection pressure and the potential for accelerated evolution of additional resistance traits. While no additional cases of atrazine resistance have been confirmed from this population since its initial detection, the presence of resistance to multiple herbicide sites of action highlights the vulnerability of current management systems when diversity in control tactics is limited. Without sustained integration of multiple herbicide modes of action and complementary non-chemical strategies, the likelihood of further resistance stacking in Palmer amaranth populations in North Carolina remains high.

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Practical Implications

The confirmation of atrazine resistance in the WC population represents the first documented case of atrazine and three-way resistance in North Carolina Palmer amaranth. Given the critical role of atrazine in corn production systems and its utility in both pre- and postemergence programs, it remains a vital tool of integrated weed management in the region. However, overreliance on atrazine, especially in the absence of other effective practices, increases selection pressure and risks accelerating the spread of resistance across the North Carolina.

Although several effective postemergence herbicides remain available for Palmer amaranth control, their long-term utility depends on diversified management strategies that reduce selection pressure on a limited number of effective chemistries. Effective management will increasingly rely on systems that integrate strong residual herbicide programs and complementary non-chemical tactics, rather than depending primarily on postemergence control. This includes programs that combine multiple herbicide modes of action, rotate chemistries across seasons, and incorporate both pre- and postemergence residual herbicides to improve control duration and reduce repeated selection on the same sites of action.

Beyond herbicide programs, cultural and mechanical tactics are essential components of sustainable Palmer amaranth management. Crop rotation with soybean expands herbicide options and enables more diverse control programs, while cover crops, deep tillage where appropriate, and harvest weed seed control can collectively reduce weed emergence and seedbank replenishment. Without consistent integration of these practices, the likelihood of further resistance evolution and spread in Palmer amaranth populations will increase, threatening the long-term sustainability of chemical weed control in North Carolina cropping systems.

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Competing Interests

Competing interests: The authors declare none.

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Tables

Table 1. Postemergence herbicides evaluated to determine effective control of the Washington County Palmer amaranth population. Herbicide application rates corresponded to a labeled field-use rate (1×). A non-treated control was included in the experiment.

Herbicide	WSSA Herbicide Group	Trade name	Manufacturer	Herbicide rate[b]
2,4-D	4	Weedar® 64	Nurfarm Americas, Inc	533
Dicamba	4	Clarity®	BASF Ag Products	560
Fomesafen[c]	14	Flexstar®	Syngenta Crop Protection, LLC	264
Glufosinate[d]	10	Liberty® 280 SL	BASF Ag Products	328
Glyphosate[d]	9	Roundup PowerMAX® II	Bayer CropScience	1,346
Mesotrione[cd]	27	Callisto®	Syngenta Crop Protection, LLC	105
Thifensulfuron[c]	2	Harmony® SG	FMC Corporation	45

[a] Abbreviations: WSSA, Weed Science Society of America.

[b]Herbicide rates expressed as g ai or ae ha^{-1}.

[c]Crop oil concentrate was added at 1 ml L^{-1}.

[d]Ammonium sulfate was added at 1,680 g ha^{-1}.

Table 2. Regression parameters from the two-parameter logistic equation describing plant survival of the putative atrazine-resistant Palmer amaranth population from Washington County compared with the Edgecombe and Johnston County populations 21 days after postemergence atrazine treatment.

	Regression parameters[a]			
Population	*b*	*e* (LD_{50}, ±SE)	LD_{80}	RF
Washington Co.	0.70	1402 (491)	9,990	----
Edgecombe Co.	2.53	218 (37)	453	6.4
Johnston Co.	1.82	161 (33)	365	8.7

[a]Two-parameter logistic equation: $y = 1/(1 + exp(b*(log(x) – log(e))))$, "*x*" is the herbicide rate, "*e*" equals the LD_{50} (lethal dose to control 50% of the population [survival]) rate and "*b*" is the slope at "*e*".

[b]Abbreviations: LD_{50}, lethal dose (g ai ha^{-1}) to control 50% of the population; LD_{80}, lethal dose (g ai ha^{-1}) to control 80% of the population; RF, resistance factor as determined by dividing the LD_{50} for the Washington County population by the LD_{50} for the Edgecombe and Johnston County populations, SE, standard error.

Table 3. Regression parameters from the three-parameter logistic equation describing plant biomass reduction of the putative atrazine-resistant Palmer amaranth population from Washington County compared with the Edgecombe and Johnston County populations 21 days after postemergence atrazine treatments.

Population	Regression parameters[a]				
	a	*x0* (BR_{50}, ±SE)	*b*	BR_{80}	RF
Washington Co.	96.52	1448 (23)	0.611	12,835	
Edgecombe Co.	94.67	212 (5)	1.42	531	6.8
Johnston Co.	100.02	59 (11)	1.06	214	24.5

[a]Three-parameter logistic equation: $y = a/[1 + (x / x0)b]$, where "*a*" is the upper asymptote, "*x*" is the herbicide rate, "*x0*" equals the BR_{50} (dose to reduce 50% of the dry biomass of the population) rate and "*b*" is the slope at "*x0*".

[b]Abbreviations: BR_{50}, dose (g ai ha^{-1}) to reduce 50% of the dry biomass of the population; BR_{80}, dose (g ai ha^{-1}) to lethal dose to reduce 80% of the dry biomass of the population; RF, resistance factor as determined by dividing the BR_{50} for the Washington County population by the BR_{50} for the Edgecombe and Johnston County Populations, SE, standard error.

Table 4. Plant survival of the Washington County Palmer amaranth population and two atrazine-susceptible populations (Edgecombe and Johnston Counties, NC) 21 days after treatments with postemergence herbicides applied at a labeled field-use rate.

Population	2,4-D		dicamba		fomesafen	glufosinate	glyphosate		mesotrione		thifensulfuron
	% (±SE)										
Washington County	21 (11)	b[a]	14 (10)	b	14 (10)	0 (0)[b]	93 (7)	a	0 (0)	b	100 (0)
Edgecombe County	64 (13)	A	64 (13)	a	0 (0)	0 (0)	29 (13)	b	0 (0)	b	100 (0)
Johnston County	43 (14)	Ab	14 (10)	b	0 (0)	0 (0)	71 (13)	a	29 (13)	a	100 (0)

[a]Means with different letters within the same column indicate statistical differences ($P < 0.05$) using Fischer's LSD.

[b] Treatments that violated the constant variance assumption were not included in the analysis, but 95% confidence intervals were used to determine whether values were similar.

[c]Abbreviations: SE, standard error.

Figures

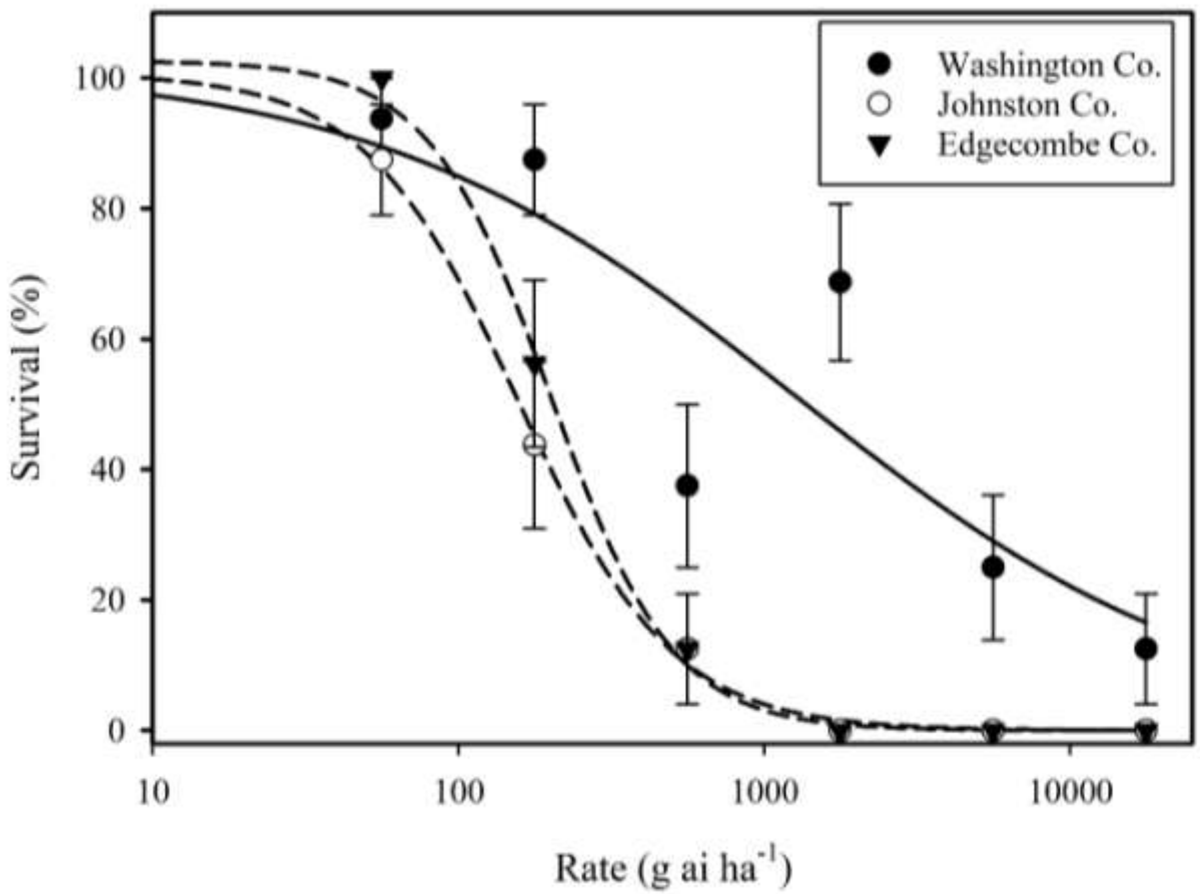

Figure 1. Dose-response curves describing survival of North Carolina Palmer amaranth populations 21 days after postemergence atrazine application. The analysis includes a putative atrazine-resistant population from Washington County and two susceptible populations from Edgecombe and Johnston Counties, NC. Vertical bars represent the standard error (±) of the mean.

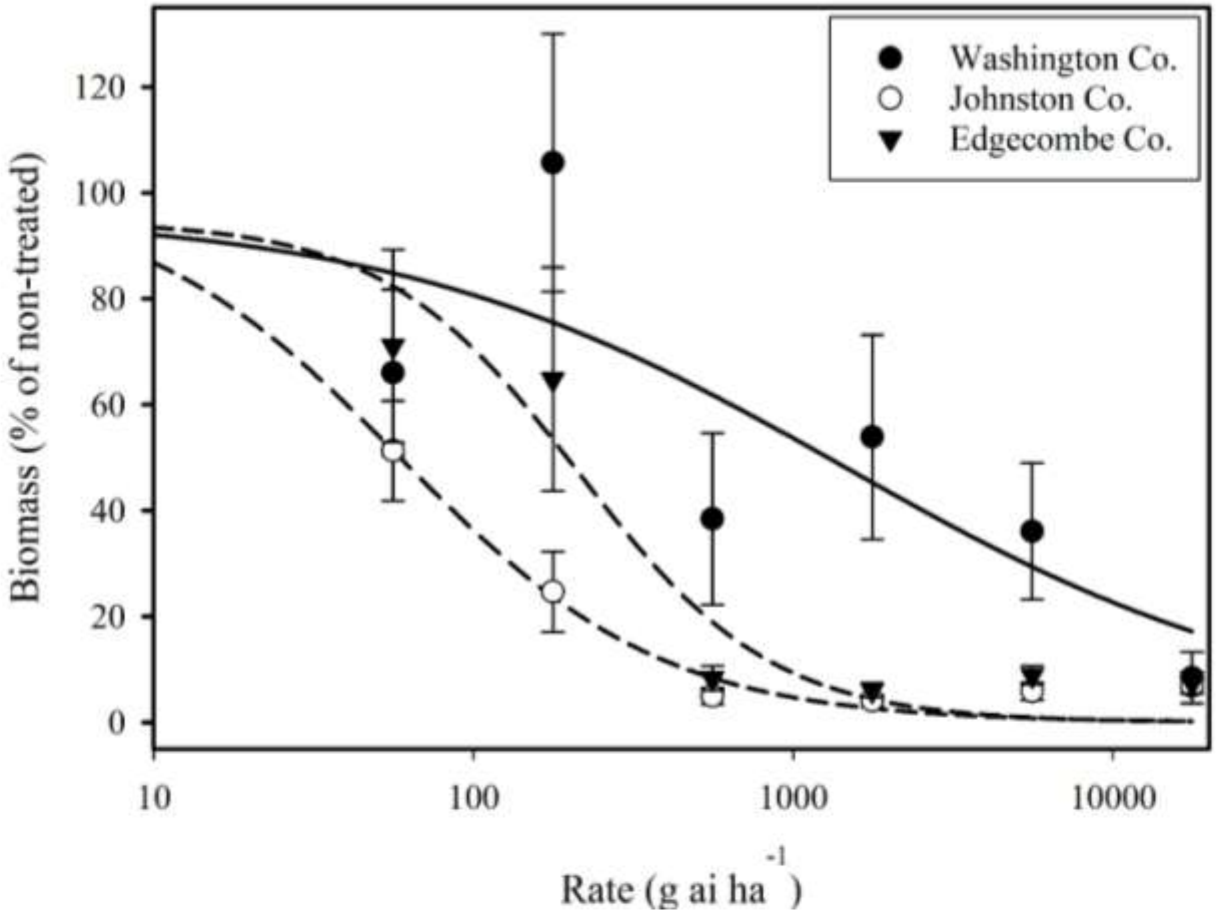

Figure 2. Dose-response curves describing dry biomass reduction of North Carolina Palmer amaranth populations 21 days after postemergence atrazine application. The analysis includes a putative atrazine-resistant population from Washington County and two susceptible populations from Edgecombe and Johnston Counties, NC. Vertical bars represent the standard error (±) of the mean.