ABSTRACT

ARGUETA MENENDEZ, RONEL JOSUE. Investigating a Multiple Herbicide-resistant Palmer amaranth (*Amaranthus palmeri*) population from North Carolina. (Under the direction of Dr. Charles W. Cahoon)

Amaranthus palmeri S. Watson (Palmer amaranth) is a problematic annual C4 weed native to the southwestern United States and Mexico. Over time, this weed has successfully expanded into the southeastern United States, where it causes significant economic losses in major row crops such as corn, soybeans, cotton, and sorghum. In 2023, a farmer in Duplin County, North Carolina, reported difficulties in controlling A. palmeri in a soybean field treated with glufosinate and dicamba. Therefore, the objectives of this study were: to evaluate the response of a putative resistant population from Duplin County (R) to various herbicides through dose-response experiments, and second, to determine effective herbicide options for managing this population. For comparison, two herbicide-susceptible populations were used as controls: one from Bertie County (Sus 1) and another from Columbus County (Sus 2). These populations were confirmed to be 2,4-D, dicamba, fomesafen, glufosinate, and mesotrione. The experimental design followed a completely randomized design with seven replications and two runs. Treatments were applied to plants of 7-10 cm in height. Subsequently, plant survival was evaluated 28 days after treatment, and biomass was collected and dried at 65 °C. To analyze the data, LD50 and BR50 values for each population were calculated from a three-parameter log-logistic model. A resistance ratio (R/S) was calculated by comparing the R population to the susceptible population. Overall, the results suggest that the Duplin County population has evolved five-way multiple resistance to 2,4-D, fomesafen, glyphosate, mesotrione, and thifensulfuron. Importantly, this research documents the first confirmed case of 2,4-D *A. palmeri* resistant in North Carolina.

Investigating a Multiple Herbicide-resistant Palmer amaranth (*Amaranthus palmeri*)
Population from North Carolina

by
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DEDICATION

To my father, Jorge Ronel Argueta Ramirez; my mother, Sandra Lorena Menendez Polanco; my grandparents, Juana de la Cruz Polanco Juarez, Jorge Menendez; my uncle, Jorge Luis Menendez Polanco; and myself.

BIOGRAPHY

Ronel Josué Argueta was born in the town of Coatepeque, Guatemala. He attended a Catholic school, Liceo Coatepeque, for elementary and middle school. He then pursued his secondary studies at EFA San Marcos Agricultural Training School, where he specialized in agriculture. With the support of to a half-scholarship and the unconditional encouragement of his parents, he continued his education at the Zamorano Pan-American Agricultural School in Honduras, earning a degree in Agricultural Science and Production.

His interest in agriculture was sparked at an early age when he accompanied his father to his rubber plantation. As a graduation project, he researched the diversity and economic importance of the *Tabanidae* family in Honduras. His professional experience began with an internship in 2020 at the University of Florida, where he contributed to strawberry disease research. In 2021, he gained additional experience working in lemon, rubber, and swine production (2021). Later that year, he joined the weed science laboratory for small grains and soybeans at North Carolina State University. This experience allowed him to perfect his English and build valuable relationships that continue to shape his journey.

Beyond academics, Ronel describes himself as a tireless dreamer, driven by self-improvement and resilience, inspired by his mother and grandmother. A Star Wars and Harry Potter fan. He enjoys appreciating the beauty of nature, spending time with friends, and discovering new places to visit. For him, every emotion, be it joy or sadness, is part of the journey, where he always remembers the words of Rainer Maria Rilke: "Let everything happen to you / Beauty and terror / Just keep going / No feeling is final."

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To my father, Jorge Ronel Argueta Ramirez, for teaching me that dreams have no limits, to never give up, and to always think big. To my mother, Sandra Lorena Menendez Polanco, whose love and example of resilience have been my role model. To my grandparents, Juana de la Cruz Polanco Juarez and Jorge Menendez, for being the source of my joy and all my love. And to my uncle, Jorge Luis Menendez Polanco, whose support has been key to becoming the person I am.

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Finally, and not as an act of self-centeredness, I thank myself for continuing to fight and seek new goals.

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CHAPTER I

Literature Review

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Palmer Amaranth (*Amaranthus palmeri* Wats.)

Palmer Amaranth (*Amaranthus palmeri* S. Watson) is a summer annual weed that belongs to the *Amaranthaceae* family. Native to the desert southwestern United States and northeastern Mexico, it is highly adapted to hot, dry environments through its use of C4 photosynthesis (Ehleringer 1983). Over time, *A. palmeri* has undergone significant geographical expansion, spreading north and east across the United States (Webster and Grey 2015). Its invasive success is largely attributed to its rapid growth rate, which surpasses that of other *Amaranthaceae* species (Horak and Loughin 2000). Its remarkable ecological plasticity, including the ability to compete effectively under low-light conditions (Jha et al. 2008) and its vigorous growth, enables dense populations to drastically reduce crop yields (Burke et al. 2007). These traits have established *A. palmeri* as a major economic threat to agronomic crops such as soybean (*Glycine max*), corn (*Zea mays*), cotton (*Gossypium hirsutum*), and sorghum (*Sorghum bicolor*), making it one of the most problematic weeds in the United States (Chaudhari et al. 2020; Van Wychen 2023).

Morphology, biology, and reproduction

Morphologically, *A. palmeri* is characterized by its robust growth habit. The plant can reach up to 2 m in height and bear rhomboid-lanceolate leaves with notably long petioles (Sauer 1955). Each plant is capable of producing as many as 600,000 small (1-1.5 mm) seeds, which can disperse long distances (Keeley et al. 1987; Sauer 1955; Sosnoskie et al. 2012). *A. palmeri* is also dioecious, producing separate male and female flowers (Steckel 2007a), a trait observed in only about 6% of all plant species (Renner 2014). However, recent studies have reported that male *A. palmeri* flowers exhibit transient hermaphroditism. This means that male flowers initially develop both stamens (male organs) and carpels (female organs), but the carpels are aborted during development, leaving only functional stamens. In contrast, female flowers exclusively develop carpels, with no stamens present (Wu et al. 2023). Another trait that favors the competitiveness of *A. palmeri* is its rapid germination, which occurs more efficiently compared to other Amaranthus species under comparable conditions. (Steckel et al. 2004).

Herbicide Resistance Mechanisms

Herbicide resistance is defined as a plant's ability to survive and reproduce following exposure to a herbicide dose that would normally be lethal (WSSA 2025). This trait arises through natural selection acting on genetic variation within a weed population (Vencill et al. 2012). Two primary mechanisms drive herbicide resistance: target site resistance (TSR) and non-target site resistance (NTSR). TSR occurs when genetic mutations alter the herbicide's binding site, often through a single amino acid substitution, thereby reducing herbicide efficacy (Délye 2013). For example, a mutation at the glycine-210 position confers *A. palmeri* resistance to PPO inhibitors (Salas et al. 2016). Another form of TSR is gene amplification, in which multiple copies of a target gene lead to overproduction of the corresponding target enzyme, therefore reducing the herbicides activity (Heap 2014a). In 2010, it was demonstrated that *A. palmeri* resistance to glyphosate was conferred by amplification of the EPSPS gene, the first documented case of herbicide resistance linked to this specific mechanism (Gaines et al. 2010; Tranel 2017). More recently, amplification of the chloroplast glutamine synthetase gene has also been associated with glufosinate resistance in *A. palmeri* (Carvalho-Moore et al. 2022). NTSR encompasses other mechanisms that do not involve modifications at the herbicide's site of action, such as reduced absorption, limited translocation, sequestration/compartmentalization, and enhanced metabolism (Powles and Yu 2010).

Reduced herbicide absorption can result from changes in the plant cuticle (Délye 2013), while reduced translocation inhibits the movement of herbicides molecules within the plant, preventing the herbicide from reaching its site of action (Powles and Yu 2010). Enhanced metabolism involves overactivation of detoxifying enzymes, such as cytochrome P450 and glutathione-S-transferase, allowing plants to survive herbicides (Moss 2017). Sequestration or compartmentalization isolates the herbicide in cellular structures, such as vacuoles, or binds it to cell walls, preventing the herbicide from reaching its target site (Heap 2014a). Most alarming, NTSR often enable weeds to evolve resistance to multiple herbicides.

Cases of resistance in *Amaranthus palmeri*

Cross-resistance occurs when a weed survives two or more herbicides sharing the same site of action, whereas multiple resistance refers to a weed biotype resistant to herbicides from different sites of action (Heap 2014b). One of the most concerning aspects of *A. palmeri* is its ability to rapidly evolve herbicide resistance and disseminate it efficiently over long distances, largely through pollen-mediated gene flow and genetic exchange between populations (Ward et al. 2013). To date, this weed has evolved resistance to nine different sites of action. (Heap 2025). The first documented case of resistance in *A. palmeri* was in the 1990s in South Carolina, where populations were no longer controlled by trifluralin, a microtubule-inhibitor (Gossett et al. 1992). Shortly thereafter, in 1995, resistance to thifensulfuron, an ALS-inhibitor, was reported (Horak and Peterson 1995). Subsequent studies have identified that resistance to ALS-inhibitors can occur through both TSR and NTSR mechanisms (Nakka et al. 2017b).

In 2005, the first documented case of *A. palmeri* surviving glyphosate was reported in Georgia, followed by North Carolina later that same year (Heap 2025; Sosnoskie and Culpepper 2014). The primary mechanism of resistance was determined to be amplification of the EPSPS gene (Chandi et al. 2012; Whitaker et al. 2013). Populations with multiple resistance to both glyphosate and ALS-inhibitors are now commonly found in North Carolina (Mahoney et al. 2020; Poirier et al. 2014). In subsequent years, resistance to photosystem II-inhibitors, such as atrazine, has been reported in Michigan, Nebraska, Iowa, and North Carolina (Chahal et al. 2019; Heap 2025; Kohrt et al. 2017). Additionally, resistance to PPO-inhibitors has been reported in Arkansas (Salas et al. 2016; Varanasi et al. 2018), primarily caused by a mutation in the glycine-210 gene (Salas et al. 2016). This has been further discovered in Georgia more recently (Randell-Singleton et al. 2024). Resistance to HPPD inhibitors, such as mesotrione, has also developed in Kansas and Iowa, arising through both TSR and NTSR mechanisms (Hamberg et al. 2024; Kumar et al. 2019; Nakka et al. 2017a).

The threat posed by *A. palmeri* has intensified, particularly with the recent documentation of resistance to synthetic auxins, such as dicamba, in Tennessee and Arkansas (Foster and Steckel 2022; Heap 2025). Resistance to glufosinate, a glutamine synthetase inhibitor, has also been documented in North Carolina and Arkansas (Jones et al. 2024b; Priess et al. 2022). This resistance is associated with extra copies of the chloroplast glutamine synthetase gene (Carvalho-Moore et al. 2022). Other studies indicate that glufosinate resistance may be oligogenic, potentially involving a small number of genes, including maternally inherited chloroplast genes (Jones et al. 2024b).

Resistance detection and experimental methods

Determining weed resistance to herbicides requires an accurate diagnosis as a fundamental step (Beckie et al. 2000). To achieve this, it is essential to use standardized detection tests that are effective and accessible to researchers (Burgos 2015; Burgos et al. 2013). A dose-response is an experiment used in various scientific disciplines (Ritz et al. 2019) to evaluate an organism's ability to survive different doses of a chemical (Burgos et al. 2013). These bioassays can be performed under field conditions, in controlled areas such as greenhouses, or in laboratories using petri dishes. Bioassays performed in controlled areas are the most reliable for identifying cases of herbicide resistance (Heap 1994). Several authors recommend that experiments begin by collecting seeds from plants that survived herbicide applications in the field. These seeds should be stored under cold conditions until germination, using species-specific protocols. Once seedlings reach adequate size and uniformity, they are treated with different herbicide rates, including 0, 0.5X, 1X, and 2X the recommended field rate to assess phytotoxicity (Panozzo et al. 2015). Resistance is confirmed statistically by comparing complete dose-response curves of the presumably resistant population against a susceptible population (Beckie et al. 2000; Heap 1994). Nonlinear regression is recommended for accurate analysis, as it allows estimation of key parameters, such as the herbicide dose required to reduce plant dry weight by 50% (Brain and Cousens 1989; Heap 1994).

Management strategies

Effective management of *A. palmeri* requires strategies that integrate diverse approaches, including cultural, mechanical, and chemical methods of control (Everman and Contreras 2025; Woyessa 2022). Cultural strategies include using cover crops like cereal rye (*Secale cereale* L.), which can initially suppress *A. palmeri* but lose effectiveness over time (Wiggins et al. 2016), along with competitive crops, reduced row spacing, and higher planting density. Cultural tactics often lead to rapid canopy closure, reduced weed density, and give crops like cotton, soybean, wheat, and corn an advantage when competing for resources (Jha et al. 2017). In particular, the use of narrow row spacing can minimize weed emergence and competitiveness, decrease seed production, and help conserve soil moisture (Molin et al. 2004).

Mechanical control includes physical techniques to eliminate weeds. Tillage has been the primary practice throughout time, consisting of tilling the soil at different intervals to eliminate weeds (Seely 1952; Timmons 2005). Other techniques include mowing or cutting, which involve the mechanical removal of the aerial parts of weeds (Hatcher and Melander 2003). Electrocution is another method of mechanical control; its success depends on factors such as the intensity and duration of the electric shock, the specific weed species, its morphological characteristics, and growth stage (Bajwa et al. 2015). An experiment conducted in Missouri has confirmed that this method can eliminate resistant weeds at the end of the season in soybean fields, drastically reducing the weed seed bank (Schreier et al. 2022). Another mechanical strategy is harvest weed seed control (HWSC), which involves collecting and destroying weed seeds during harvest, often using impact mills that are integrated into the combine (Winans et al. 2023). In Missouri, seed destroyers such as the Seed Terminator® damaged 94% of tall waterhemp (*Amaranthus tuberculatus*) seeds, rendering them non-viable and reducing the soil seed bank by 97% (Winans et al. 2023).

For chemical control, herbicides remain the primary tool utilized by growers. These chemical compounds are specifically designed to control or eliminate unwanted vegetation (US EPA 2015). Effective chemical management often combines preemergence and postemergence herbicides (Hager et al. 2002). However, the rapid growth of *A. palmeri* significantly reduces the window for postemergence herbicide applications, and poorly timed applications can result in weed escapes (Smeda et al. 2009). While herbicides and herbicide-resistant crops are useful, the efficacy of these herbicides can vary by region (Cahoon et al. 2015). Preventing high weed population densities is critical for limiting their spread and ability to compete with crops (Korres and Norsworthy 2017).

A. palmeri has become a major concern in agriculture because it presents on of the most significant control challenges in various crops throughout the US. Its morphological and reproductive traits confer remarkable adaptability and genetic plasticity. With the increasing number of documented cases of resistance to multiple sites of action, continued research supported by interdisciplinary collaboration, is required to effectively manage *A. palmeri* long-term. Furthermore, integrated weed management programs that combined all of the aforementioned tactics are necessary in continued battle against multiple resistant *A. palmeri* (Norsworthy et al. 2012; Yu et al. 2021).

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CHAPTER II

Characterization of a multiple-resistant Palmer amaranth (*Amaranthus palmeri*) population from North Carolina

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Introduction

Palmer amaranth (*Amaranthus palmeri* Wats.) is a summer annual weed known for its C4 photosynthetic pathway (Ehleringer 1983). Native to northeastern Mexico and the southwestern United States (Sauer 1957), this species has demonstrated remarkable adaptability, spreading into new agricultural regions across the United States (Webster and Grey 2015). As a dioecious species (Steckel 2007), female flowers are capable of producing around 600,000 seeds per plant (Keeley et al. 1987). Its combinations of rapid growth, high fecundity, and accelerated germination provide *A. palmeri* with a competitive advantage over most crops (Steckel et al. 2004). If left unmanaged, *A. palmeri* can cause considerable yield loss in soybeans (*Glycine max*), corn (*Zea mays*), cotton (*Gossypium hirsutum*), and sorghum (*Sorghum bicolor*) (Chaudhari et al. 2020) establishing it as one of the most problematic weeds in the United States (Van Wychen 2023).

Over the years, *A. palmeri* has evolved resistance to nine different herbicide sites of action, including cross-resistance within the same herbicide site of action and multiple resistance to herbicides from different sites of action within a single population (Heap 2014b, 2025). This widespread resistance is driven in part by intensive herbicide selection pressure and the species' obligate outcrossing, which generates high genetic diversity among offspring (Moore 2021; Ward et al. 2013). The first cases were reported in the early-to-mid 1990s, with resistance to a microtubule inhibitor (trifluralin) in South Carolina (Gossett et al. 1992) and to ALS inhibitors, such as thifensulfuron, in Kansas (Horak and Peterson 1995). In 2005, the first cases of *A. palmeri* resistance to glyphosate, an EPSP synthase inhibitor, were documented in Georgia and shortly thereafter in North Carolina (Heap 2025; Sosnoskie and Culpepper 2014). By 2010, populations with multiple resistance to both glyphosate and thifensulfuron were commonplace in NC (Mahoney et al. 2020; Poirier et al. 2014). In subsequent years, resistance expanded to other herbicide groups, including photosystem II inhibitors (atrazine) in states like Michigan, Nebraska, and North Carolina (Chahal et al. 2019; Heap 2025; Kohrt et al. 2017). Resistance to PPO inhibitors, such as fomesafen, was reported in populations from Georgia and Arkansas (Randell-Singleton et al. 2024; Salas et al. 2016; Varanasi et al. 2018), while resistance to HPPD inhibitors (mesotrione) emerged in Kansas and Iowa (Hamberg et al. 2024; Kumar et al. 2019; Nakka et al. 2017a). More recently, in 2022, resistance to dicamba, a synthetic auxin, was confirmed in Tennessee (Foster and Steckel 2022). Resistance to glutamine synthase inhibitors (glufosinate) has been documented nationally, with the first case in North Carolina reported in 2024 (Jones et al. 2024b). With the rate of herbicide discovery at a near standstill, growers must increasingly rely on integrated weed management strategies that combine cultural, mechanical, and chemical methods to effectively control *A. palmeri* and other troublesome weed species (Beckie and Harker 2017; Woyessa 2022).

In 2023, a farmer in Duplin County, North Carolina, reported control failure of *A. palmeri* with dicamba and glufosinate. This research evaluates the response of this putatively resistant population through dose-response experiments and seeks to identify effective herbicide options for its management.

Materials and Methods

Plant Material

In the summer of 2023, a farmer reported that dicamba and glufosinate failed to control *A. palmeri* in Duplin County, North Carolina. Seeds were collected from female plants that survived glufosinate and dicamba in a preliminary field experiment. Seeds were cleaned and stored at 5°C at North Carolina State University. Seeds of the putative resistant Duplin County population (R) were sown in 21 x 28 cm trays containing commercial growing medium (Sunshine Mix #1, Sungro Horticulture, Agawam, MA, USA). Two *A. palmeri* populations collected in 2022 from Bertie County (Sus 1) and Columbus County (Sus 2), North Carolina, were used as the susceptible populations, as they were effectively controlled by 2,4-D, dicamba, glufosinate, fomesafen, and mesotrione in a previous study (Alsdorf 2024). Once the plants were 1–2 cm in height, they were transplanted into 7 cm pots using the same commercial substrate, and approximately 1 g of Osmocote® Flower Food Granules (14-14-14; Scotts Company, Marysville, OH, USA).

Dose-Response Experiment

Dose-response experiments were conducted under controlled conditions with a 12-hour photoperiod, day/night temperatures of 31/24°C with diurnal fluctuations and overhead irrigation, to evaluate resistance levels to the herbicides thifensulfuron, 2,4-D, dicamba, glyphosate, glufosinate, fomesafen, and mesotrione. Dose-response experiments for each herbicide were conducted independently. The experimental design was a randomized complete block (replicates as blocks) with seven replications and two experimental runs. Each herbicide was applied when plants reached 7–10 cm in height. Applications were made using a CO_2-pressurized track sprayer (Devries Gen4 Research Track Sprayer) equipped with TeeJet® TTI

11002 VS nozzles (TeeJet nozzles; Spraying Systems Co., 77 Wheaton, IL) for 2,4-D and dicamba and TeeJet® XR 11002 VS nozzles (TeeJet nozzles; Spraying Systems Co., Wheaton, IL) for all other herbicides. The herbicides, rates, adjuvants, and product manufacturers are listed in Table 2.1. Due to thifensulfuron and glyphosate-resistant *A. palmeri* being dominant in North Carolina, only two rates, plus a nontreated check of each, were evaluated (Table 2.1) (Mahoney et al. 2020).

Data Collection

Visual estimates of injury were collected 7, 14, 21, and 28 days after treatment (DAT) using a scale from 0% (no symptoms) to 100% (complete plant death) (Frans et al. 1985). At 28 DAT, survival was recorded using a binary system where 0 = dead plant and 1 = alive plant. At the end of the experiment (28 DAT), aboveground biomass was collected and stored in individual paper bags and dried at 65°C for 72 hours to obtain dry aboveground biomass. Biomass reduction was calculated using the following formula:

$$\mathrm{BR} = 100 \mathrm{~X} \left(1 - \frac{T}{C}\right)$$

BR is biomass reduction relative to the non-treated plants, T is the treated plant weight, and C is the weight of the non-treated plants.

Statistical analysis

Data was subjected to analysis of variance (ANOVA) using PROC GLIMMIX in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). Plant survival and biomass reduction were modeled using a three-parameter log-logistic equation:

$$\mathrm{Y{=}a}/\left[1 + \left(\frac{x}{x0}\right) b\right]$$

Where a is the upper asymptote, x is the herbicide rate, x0 equals the LD_{50} (lethal dose to control 50% of the population) or BR_{50} (dose to reduce biomass by 50%) rate, and b is the slope at x0 (Jones et al. 2024b). The data obtained for the LD_{50} and BR_{50} for each *A. palmeri* population were obtained from the regression equations. The resistance ratio (Burgos 2015) was calculated by dividing the LD_{50} of the R population by the LD_{50} of the two susceptible populations (Sus 1 and Sus 2).

Results and Discussion

Dose-response results are discussed separately by ascending herbicide group as defined by the Weed Science Society of America (WSSA). For each herbicide, survival and biomass data are shown by population and rate. Although the two-way interaction of rate by population was not significant for thifensulfuron, glyphosate, dicamba, and glufosinate, results are still presented at the population level to provide clarity on population responses.

Thifensulfuron (WSSA Group 2)

There were no significant differences with the control treatment when thifensulfuron was applied at 90 g ai ha^{-1} (2x labeled rate), resulting in 88%, 50%, and 75% survival in the R, Sus 1, and Sus 2 populations, respectively (Figure 2.1). Likewise, no differences were observed when thifensulfuron was applied at 180 g ai ha^{-1} (4x labeled rate), with survival ranging from 25% to 75% across populations (Figure 2.1). These results indicate that each of these populations can survive excessively high rates of thifensulfuron, suggesting that they are resistant. Due to decades of selection pressure on ALS-inhibitors, susceptible *A. palmeri* is rare, and is further supported by past resistance screenings in North Carolina (Mahoney et al. 2020; Poirier et al. 2014).

2,4-D (WSSA Group 4)

The LD_{50} for the R population was 915 g ae ha^{-1}, compared with 365 and 283 g ae ha^{-1} for Sus 1 and Sus 2, respectively (Figure 2.2). This corresponded to an R/S ratio of 2.5 for Sus 1 and 3.2 for Sus 2 (Table 2.2). These findings were supported by BR_{50} values, which indicated that the R population required 296 g ae ha^{-1} to reduce biomass by 50%, approximately 3.74- and 6.16-fold higher than Sus 1 and Sus 2, respectively (Figure 2.3, Table 2.3). No LD_{90} value could be calculated for the R population as the evaluated rates never reached that level of control. Differential survival between the susceptible populations and the R population across the tested rates suggests that the R population is 2,4-D-resistant. This finding is significant as it represents the first documented case of 2,4-D-resistant *A. palmeri* in North Carolina. Resistance to 2,4-D has been reported in Kansas (Kumar et al. 2019).

Dicamba (WSSA Group 4)

The LD_{50} values were comparable across populations, with each ranging between 128 and 150 g ae ha^{-1} (Table 2.4, Figure 2.4). Similarly, the Br_{50} values were 16, 7, and 23 g ae ha^{-1} for the R, Sus 1, and Sus 2 populations, respectively (Table 2.5, Figure 2.5). This data suggests that the susceptible and putatively resistant populations were effectively controlled with low rates of dicamba. These findings are unexpected given the farmer's initial report that *A. palmeri* survived multiple applications of dicamba. Further studies might be required to determine the reasons why plants did not survive dicamba and glufosinate treatments compared to initial field screenings. This contrasts with the cases of dicamba-resistance found in Arkansas and Tennessee (Foster and Steckel 2022; Heap 2025).

Glyphosate (WSSA Group 9)

There were no differences across all populations when glyphosate was applied at 2692 g ae ha^{-1}, resulting in 88%, 100% and 100% survival in the R, Sus 1, and Sus 2 populations, respectively (Figure 2.6). Similarly, there were no differences across all populations at 5384 g ae ha^{-1}, resulting in 50%, 75%, and 50% survival in the R, Sus 1, and Sus 2 populations, respectively (Figure 2.6). These results indicate that each population demonstrated the ability to survive high glyphosate rates, suggesting they are glyphosate-resistant. Although the susceptible populations are not truly glyphosate-susceptible, this is not unexpected, as previous studies in North Carolina reported that 98% and 97% of evaluated populations were thifensulfuron- and glyphosate-resistant, respectively, with 93% exhibiting multiple resistance to both herbicides (Poirier et al. 2014).

Glufosinate (WSSA Group 10)

The LD_{50} obtained for the R population was 103 g ai ha^{-1} (Figure 2.7), representing only a 1.1-fold increase compared with the susceptible populations (Table 2.6). In contrast, the BR_{50} value for the R population was 18 g ai ha^{-1} (Table 2.7, Figure 2.8), which was lower than the values observed for both Sus 1 (26 g ai ha^{-1}) and Sus 2 (21 g ai ha^{-1}) (Table 2.7). The LD_{50} estimates collected in this study were also lower than those previously recorded for susceptible populations (Jones et al. 2024b). Therefore, the populations evaluated in this experiment exhibited greater sensitivity to glufosinate and the herbicide remains effective for control of *A. palmeri*. However, careful stewardship is warranted given that glufosinate-resistant *A. palmeri* has been documented in North Carolina (Jones et al. 2024b).

Fomesafen (WSSA Group 14)

The estimated LD_{50} for the R population was 892 g ai ha^{-1}, while that of Sus 1 and Sus 2 was 76 and 65 g ai ha^{-1}, respectively (Figure 2.9). This corresponded to an R/S ratio of 11.7 for Sus 1 and 13.7 for Sus 2 (Table 2.8). The LD_{90} could not be determined for the R population, indicating that considerably higher rates than those evaluated were required to achieve 90% control. The BR_{50} for the R population was 261 g ai ha^{-1} (Figure 2.10), which was 5.3 and 26.1-fold higher than that of Sus 1 and Sus 2, respectively. These results are supported by the BR_{90} values noted in Table 2.9, which further indicate that the R population is highly fomesafen-resistant. PPO resistance *A. palmeri* has not been previously documented in North Carolina; however, resistance has been reported in other *Amaranthus* species in the state (Jones et al. 2023, 2024a).

Mesotrione (WSSA Group 27)

The R population had an LD_{50} of 652 g ai ha^{-1}, representing an R/S ratio of 4 and 2.14 compared to the Sus 1 and 2 populations, respectively (Figure 2.11, Table 2.10). Across populations, there were no true differences in BR_{50} values; however, the estimated BR_{90} values demonstrated a defined difference. The BR_{90} for the R was 432 g ai ha^{-1}, which was approximately a 1.8- and 2-fold increase compared to the susceptible populations (Figure 2.12, Table 2.11). Collectively, the LD_{50} and BR_{90} values suggest that the R population is mesotrione-resistant. This finding has important implications for field management, as HPPD herbicides like mesotrione often provide variable control of *A. palmeri* when applied alone, whereas tank mixtures with atrazine have demonstrated synergistic activity that provides more consistent control even in resistant populations (Chahal et al. 2019). In 2016, HPPD resistance was documented in North Carolina (Heap 2025).

Conclusion

The *A. palmeri* population from Duplin County, NC, garnered attention after it was observed surviving multiple dicamba and glufosinate applications. Results from this study confirmed resistance to 2,4-D, fomesafen, glyphosate, mesotrione, and thifensulfuron. Notably, this study provides the first confirmed case of 2,4-D and the second fomesafen resistance case in *A. palmeri* in North Carolina. However, the population was effectively controlled by both dicamba and glufosinate under greenhouse conditions. With dicamba not currently labeled for postemergence over-the-top use in soybean and cotton, glufosinate remains an effective option. Reports of survival following dicamba and glufosinate applications are concerning. Further studies will be required to determine the exact causes of these survival outcomes. Nonetheless, the looming threat of evolving resistance underscores that the preservation of effective herbicide modes of action is critically dependent on the adoption of integrated weed management strategies, which combine careful herbicide stewardship with robust non-chemical tactics.

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Tables

Table 2.1: 2,4-D, dicamba, fomesafen, glufosinate, glyphosate, mesotrione, and thifensulfuron rates used in the dose-response assay to test if *A. palmeri* population from Duplin County had evolved resistance to the tested herbicides.

Herbicide	Trade name	Manufacturer	Adjuvant	Rate g ai ha^{-1} – ae ha^{-1}
2,4-D	Enlist One®	Corteva™ agriscience	N/A	0, 16, 33, 66, 133, 266, 533, 1066, 2132, 4264
dicamba	Xtendimax®	Bayer CropScience	N/A	0, 70, 140, 280, 560, 1120, 2240, 4480
fomesafen	Reflex®	Syngenta®	0.25 v/v NIS	0, 17.5, 35, 70, 140, 210, 280, 560, 1120, 2800
glufosinate	Liberty® 280	BASF Corporation	1682 g/ha AMS	0, 20, 40, 60, 90, 120, 180, 240, 475, 715, 955, 1190
glyphosate	Roundup PowerMAX 3®	Bayer Cropscience Lp	N/A	0, 2692, 5384
mesotrione	Callisto®	Syngenta®	1% COC	0, 26, 52, 105, 210, 420, 840
thifensulfuron	Harmony® GT	FMC Corporation	1% COC + 1682 g/ha AMS	0, 90, 180

[a]

Abbreviations: g ai ha^{-1}, grams of active ingredient per hectare; g ae ha^{-1}, grams of acid equivalent per hectare; COC, crop oil concentration; AMS, ammonium sulfate; NIS, nonionic surfactant.

Table 2.2. Parameter estimates from the three-parameter logistic regression for plant survival of the R Duplin County *Amaranthus palmeri* population treated with 2,4-D compared to the susceptible populations: Sus 1 and Sus 2.

	Regression parameters						
Pop	a	x0	b	r^2	LD_{50}	LD_{90}	R/S
R	104.37	914.67	1.14	0.81	915 g ae ha^{-1}		
Sus 1	100.99	364.18	1.40	0.97	365 g ae ha^{-1}	1755 g ae ha^{-1}	2.50
Sus 2	101.53	282.76	1.69	0.98	283g ae ha^{-1}	1047 g ae ha^{-1}	3.23

[a] a is the upper asymptote, x0 equals the LD_{50}, and b is the slope at x0

[b] Abbreviations: Pop, population; R, putative-resistant population from Duplin County; Sus 1, susceptible populations from Bertie County; Sus 2, susceptible population from Columbus County; LD_{50}, lethal dose to control 50% of the population; LD_{90}, lethal dose to control 90% of the population; R/S, resistant ratio [LD_{50} R population / LD_{50} susceptible population].

Table 2.3. Parameter estimates from the three-parameter logistic regression for biomass reduction of the resistant R County *Amaranthus palmeri* population treated with 2,4-D compared to the susceptible populations: Sus 1 and Sus 2.

	Regression parameters						
Pop	a	x0	b	r^2	BR_{50}	BR_{90}	R/S
R	90.96	295.83	-2.16	0.94	296 g ae ha^{-1}	2399 g ae ha^{-1}	
Sus 1	94.28	78.55	-1.54	0.97	79 g ae ha^{-1}	566 g ae ha^{-1}	3.74
Sus 2	98.89	47.98	-1.08	0.99	48 g ae ha^{-1}	401 g ae ha^{-1}	6.16

[a] a is the upper asymptote, x0 equals the GR_{50}, and b is the slope at x0

[b] Abbreviations: Pop, population; R, putative-resistant population from Duplin County; Sus 1, susceptible populations from Bertie County; Sus 2, susceptible population from Columbus County; GR_{50}, lethal dose to control 50% of the population; GR_{90}, lethal dose to control 90% of the population; R/S, resistant ratio [GR_{50} R population / GR_{50} susceptible population].

Table 2.4. Parameter estimates from the three-parameter logistic regression for plant survival of the R Duplin County *Amaranthus palmeri* population treated with dicamba compared to the susceptible populations: Sus 1 and Sus 2.

	Regression parameters						
Pop	a	x0	b	r^2	LD_{50}	LD_{90}	R/S
R	100.13	132.85	1.42	0.99	133 g ae ha^{-1}	643 g ae ha^{-1}	
Sus 1	98.35	127.65	2.02	0.99	128 g ae ha^{-1}	369 g ae ha^{-1}	1.03
Sus 2	97.03	149.55	2.47	0.99	150 g ae ha^{-1}	382 g ae ha^{-1}	0.88

[a] a is the upper asymptote, x0 equals the LD_{50}, and b is the slope at x0

[b] Abbreviations: Pop, population; R, putative-resistant population from Duplin County; Sus 1, susceptible populations from Bertie County; Sus 2, susceptible population from Columbus County; LD_{50}, lethal dose to control 50% of the population; LD_{90}, lethal dose to control 90% of the population; R/S, resistant ratio [LD_{50} R population / LD_{50} susceptible population].

Table 2.5. Parameter estimates from the three-parameter logistic regression for biomass reduction of the R Duplin County *Amaranthus palmeri* population treated with dicamba compared to the susceptible populations: Sus 1 and Sus 2.

	Regression parameters						
Pop	a	x0	b	r^2	BR_{50}	BR_{90}	R/S
R	99.93	15.70	-0.78	0.99	16 g ae ha^{-1}	262 g ae ha^{-1}	
Sus 1	98.31	6.92	-0.57	0.99	7 g ae ha^{-1}	440 g ae ha^{-1}	2.28
Sus 2	98.87	23.04	-0.97	0.99	23 g ae ha^{-1}	248 g ae ha^{-1}	0.69

[a] a is the upper asymptote, x0 equals the GR_{50}, and b is the slope at x0

[b] Abbreviations: Pop, population; R, putative-resistant population from Duplin County; Sus 1, susceptible populations from Bertie County; Sus 2, susceptible population from Columbus County; GR_{50}, lethal dose to control 50% of the population; GR_{90}, lethal dose to control 90% of the population; R/S, resistant ratio [GR_{50} R population / GR_{50} susceptible population].

Table 2.6. Parameter estimates from the three-parameter logistic regression for plant survival of the R Duplin County *Amaranthus palmeri* population treated with glufosinate compared to the susceptible populations: Sus 1 and Sus 2.

	Regression parameters						
Population	a	x0	b	r^2	LD_{50}	LD_{90}	R/S
R	100.69	102.88	2.75	0.99	103 g ai ha^{-1}	228 g ai ha^{-1}	
Sus 1	100.89	89.64	4.36	0.99	90 g ai ha^{-1}	148 g ai ha^{-1}	1.14
Sus 2	102.35	92.29	2.93	0.99	93 g ai ha^{-1}	195 g ai ha^{-1}	1.10

[a] a is the upper asymptote, x0 equals the LD_{50}, and b is the slope at x0

[b] Abbreviations: R, putative-resistant population from Duplin County; Sus 1, susceptible populations from Bertie County; Sus 2, susceptible population from Columbus County; LD_{50}, lethal dose to control 50% of the population; LD_{90}, lethal dose to control 90% of the population; R/S, resistant ratio [LD_{50} R population / LD_{50} susceptible population].

Table 2.7. Parameter estimates from the three-parameter logistic regression for biomass reduction of the R Duplin County *Amaranthus palmeri* population treated with glufosinate compared to the susceptible populations: Sus 1 and Sus 2.

	Regression parameters						
Population	a	x0	b	r^2	BR_{50}	BR_{90}	R/S
R	103.55	17.67	-0.87	0.98	18 g ai ha^{-1}	154 g ai ha^{-1}	
Sus 1	103.45	26.40	-1.14	0.97	26 g ai ha^{-1}	139 g ai ha^{-1}	0.69
Sus 2	100.63	21.07	-1.21	0.98	21 g ai ha^{-1}	123 g ai ha^{-1}	0.85

[a] a is the upper asymptote, x0 equals the GR_{50}, and b is the slope at x0

[b] Abbreviations: R, putative-resistant population from Duplin County; Sus 1, susceptible populations from Bertie County; Sus 2, susceptible population from Columbus County; GR_{50}, lethal dose to control 50% of the population; GR_{90}, lethal dose to control 90% of the population; R/S, resistant ratio [GR_{50} R population / GR_{50} susceptible population].

Table 2.8. Parameter estimates from the three-parameter logistic regression for plant survival of the R Duplin County *Amaranthus palmeri* population treated with fomesafen compared to the susceptible populations: Sus 1 and Sus 2.

	Regression parameters						
Pop	a	x0	b	r^2	LD_{50}	LD_{90}	R/S
R	96.77	891.94	1.27	0.90	892 g ai ha^{-1}		
Sus 1	98.78	75.89	0.96	0.98	76 g ai ha^{-1}	744 g ai ha^{-1}	11.73
Sus 2	101.06	64.53	0.75	0.81	65 g ai ha^{-1}	1196 g ai ha^{-1}	13.72

[a] a is the upper asymptote, x0 equals the LD_{50}, and b is the slope at x0

[b] Abbreviations: Pop, population; R, putative-resistant population from Duplin County; Sus 1, susceptible populations from Bertie County; Sus 2, susceptible population from Columbus County; LD_{50}, lethal dose to control 50% of the population; LD_{90}, lethal dose to control 90% of the population; R/S, resistant ratio [LD_{50} R population / LD_{50} susceptible population].

Table 2.9. Parameter estimates from the three-parameter logistic regression for biomass reduction of the R Duplin County *Amaranthus palmeri* population treated with fomesafen compared to the susceptible populations: Sus 1 and Sus 2.

	Regression parameters						
Pop	a	x0	b	r^2	BR_{50}	BR_{90}	R/S
R	129.73	260.66	-0.44	0.98	261 g ai ha^{-1}	1664 g ai ha^{-1}	
Sus 1	112.49	48.56	-0.55	0.97	49 g ai ha^{-1}	583 g ai ha^{-1}	5.32
Sus 2	106.68	9.90	-0.45	0.87	10 g ai ha^{-1}	394 g ai ha^{-1}	26.1

[a] a is the upper asymptote, x0 equals the GR_{50}, and b is the slope at x0

[b] Abbreviations: Pop, population; R, putative-resistant population from Duplin County; Sus 1, susceptible populations from Bertie County; Sus 2, susceptible population from Columbus County; GR_{50}, lethal dose to control 50% of the population; GR_{90}, lethal dose to control 90% of the population; R/S, resistant ratio [GR_{50} R population / GR_{50} susceptible population].

Table 2.10. Parameter estimates from the three-parameter logistic regression for plant survival of the R Duplin County *Amaranthus palmeri* population treated with mesotrione compared to the susceptible populations: Sus 1 and Sus 2.

	Regression parameters						
Population	a	x0	b	r^2	LD_{50}	LD_{90}	R/S
R	97.65	651.30	1.41	0.95	652 g ai ha^{-1}		
Sus 1	97.34	163.19	2.44	0.99	163 g ai ha^{-1}	401 g ai ha^{-1}	4
Sus 2	82.52	304.02	4.72	0.90	304 g ai ha^{-1}	484 g ai ha^{-1}	2.14

[a] a is the upper asymptote, x0 equals the LD_{50}, and b is the slope at x0

[b] Abbreviations: R, putative-resistant population from Duplin County; Sus 1, susceptible populations from Bertie County; Sus 2, susceptible population from Columbus County; LD_{50}, lethal dose to control 50% of the population; LD_{90}, lethal dose to control 90% of the population; R/S, resistant ratio [LD_{50} R population / LD_{50} susceptible population].

Table 2.11. Parameter estimates from the three-parameter logistic regression for biomass reduction of the R Duplin County *Amaranthus palmeri* population treated with mesotrione compared to the susceptible populations: Sus 1 and Sus 2.

	Regression parameters						
Pop	a	x0	b	r^2	BR_{50}	BR_{90}	R/S
R	110.91	34.37	-0.57	0.99	34 g ai ha^{-1}	432 g ai ha^{-1}	
Sus 1	107.14	35.09	-0.87	0.99	35 g ai ha^{-1}	220 g ai ha^{-1}	0.97
Sus 2	98.52	17.84	-0.90	0.99	18 g ai ha^{-1}	243 g ai ha^{-1}	1.88

[a] a is the upper asymptote, x0 equals the GR_{50}, and b is the slope at x0

[b] Abbreviations: Pop, population; R, putative-resistant population from Duplin County; Sus 1, susceptible populations from Bertie County; Sus 2, susceptible population from Columbus County; GR_{50}, lethal dose to control 50% of the population; GR_{90}, lethal dose to control 90% of the population; R/S, resistant ratio [GR_{50} R population / GR_{50} susceptible population].

Figures

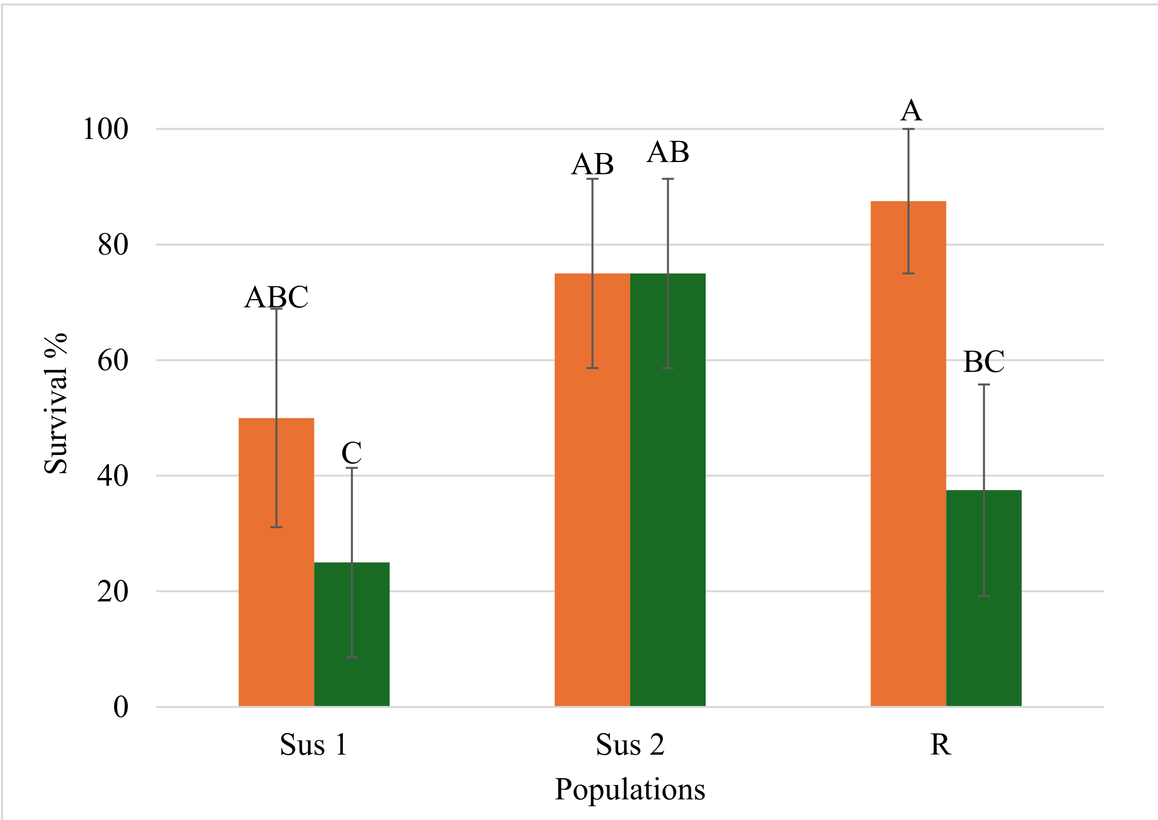

Figure 2.1 Plant survival of susceptible populations (Sus 1, Sus 2), and the R population from Duplin County, *Amaranthus palmeri* treated with thifensulfuron. Legend: orange bar: 90g ai ha^{-1} of thifensulfuron; green bar: 180 g ai ha^{-1} thifensulfuron. Error bars indicate the standard error of the mean.

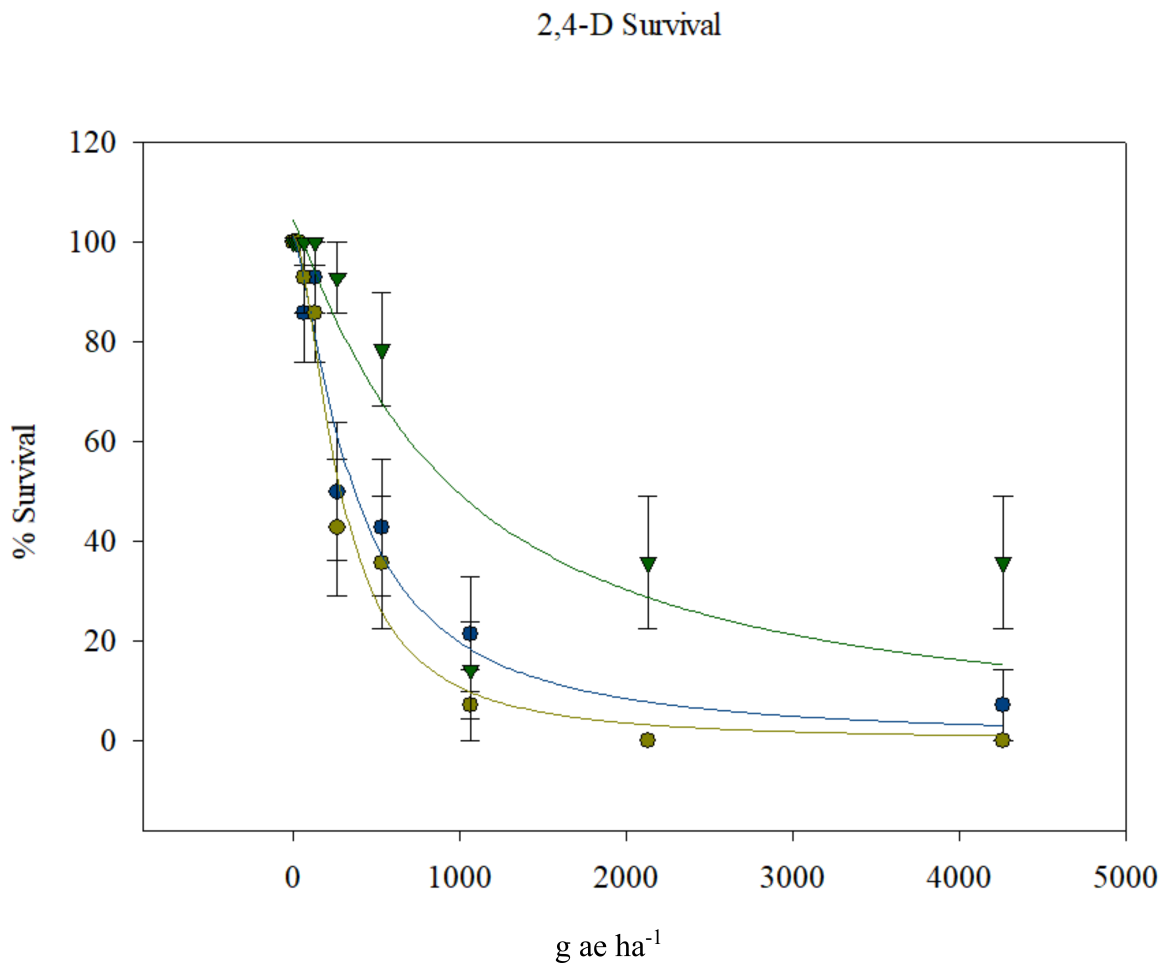

Figure 2.2 Dose-response curve fit to a three-parameter logistic regression model for plant survival of the *Amaranthus palmeri* populations (resistant: R; susceptible: Sus 1 and Sus 2) treated with 2,4-D, pooled across experimental runs. Legend: R: green triangle; Sus 1: blue circle; Sus 2: dark olive circle.

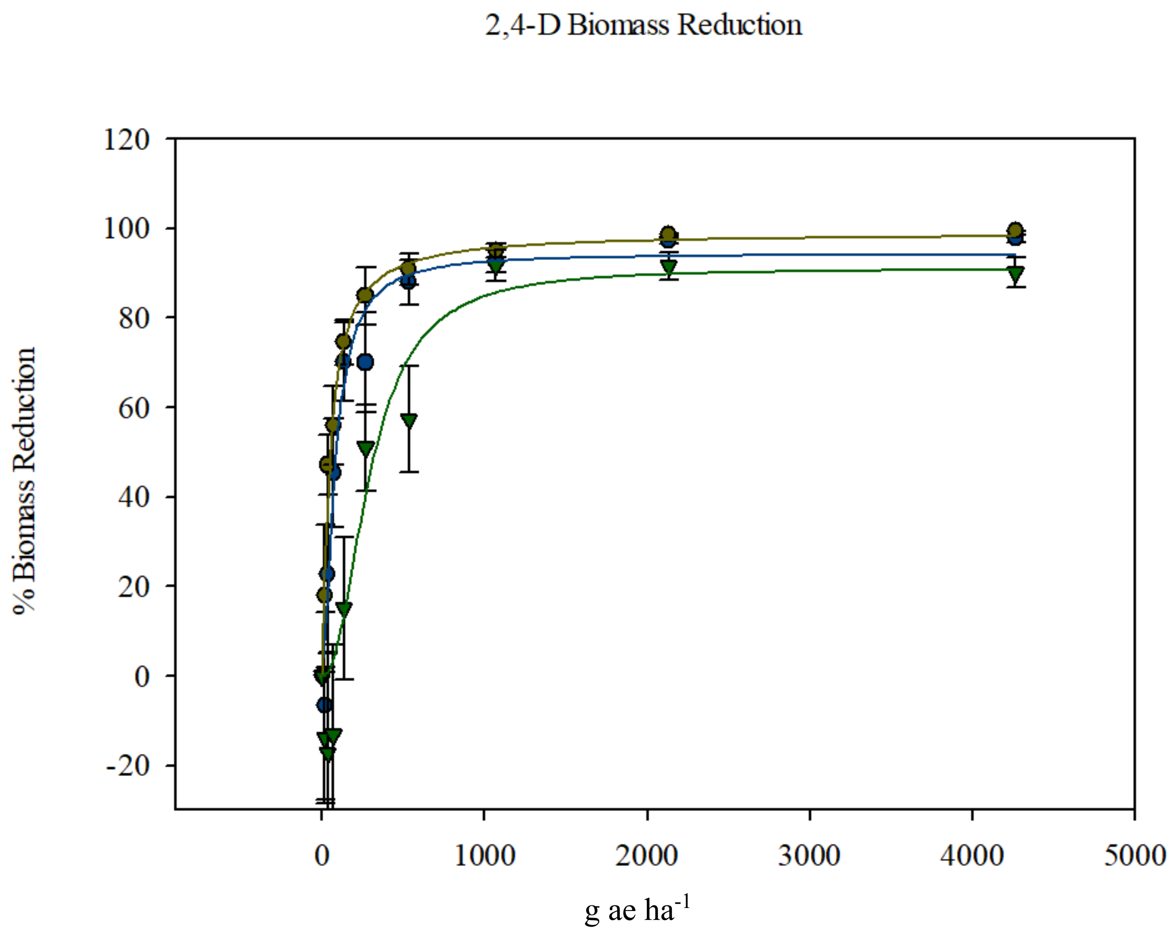

Figure 2.3 Dose-response curve fit to a three-parameter logistic regression model for biomass reduction of the *Amaranthus palmeri* populations (resistant: R; susceptible: Sus 1 and Sus 2) treated with 2,4-D pooled across experimental runs. Legend: R: green triangle; Sus 1: blue circle; Sus 2: dark olive circle.

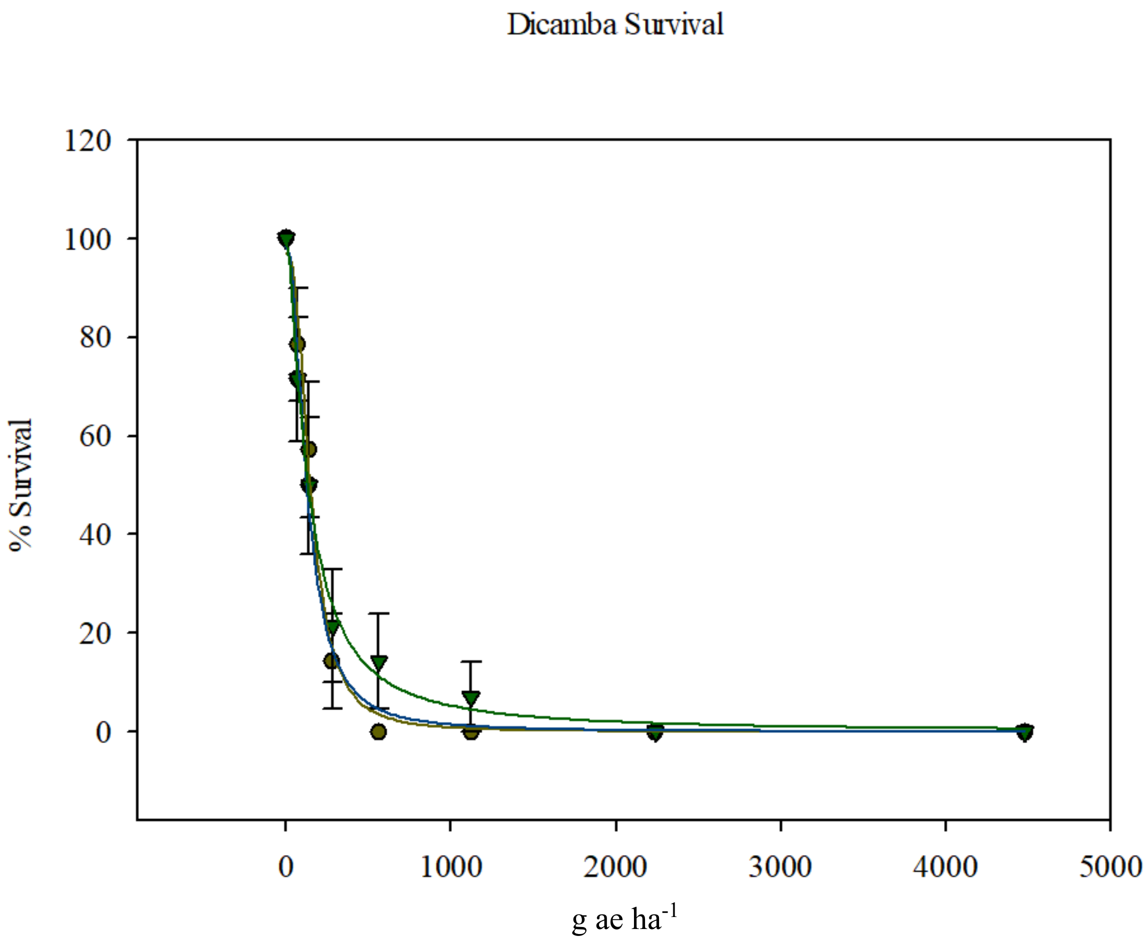

Figure 2.4 Dose-response curve fit to a three-parameter logistic regression model for plant survival of the *Amaranthus palmeri* populations (resistant: R; susceptible: Sus 1 and Sus 2) treated with dicamba, pooled across experimental runs. Legend: R: green triangle; Sus 1: blue circle; Sus 2: dark olive circle.

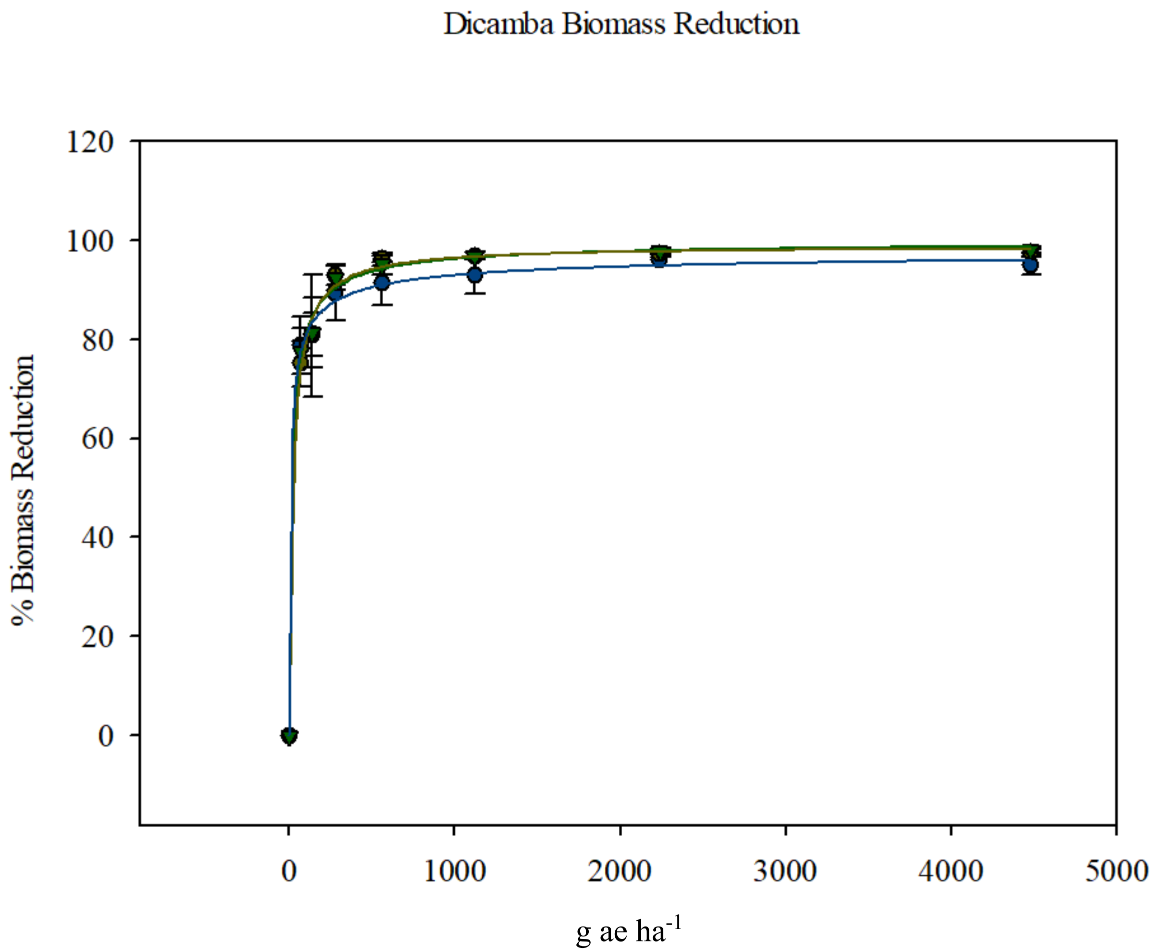

Figure 2.5 Dose-response curve fit to a three-parameter logistic regression model for biomass reduction of the *Amaranthus palmeri* populations (resistant: R; susceptible: Sus 1 and Sus 2) treated with dicamba, pooled across experimental runs. Legend: R: green triangle; Sus 1: blue circle; Sus 2: dark olive circle.

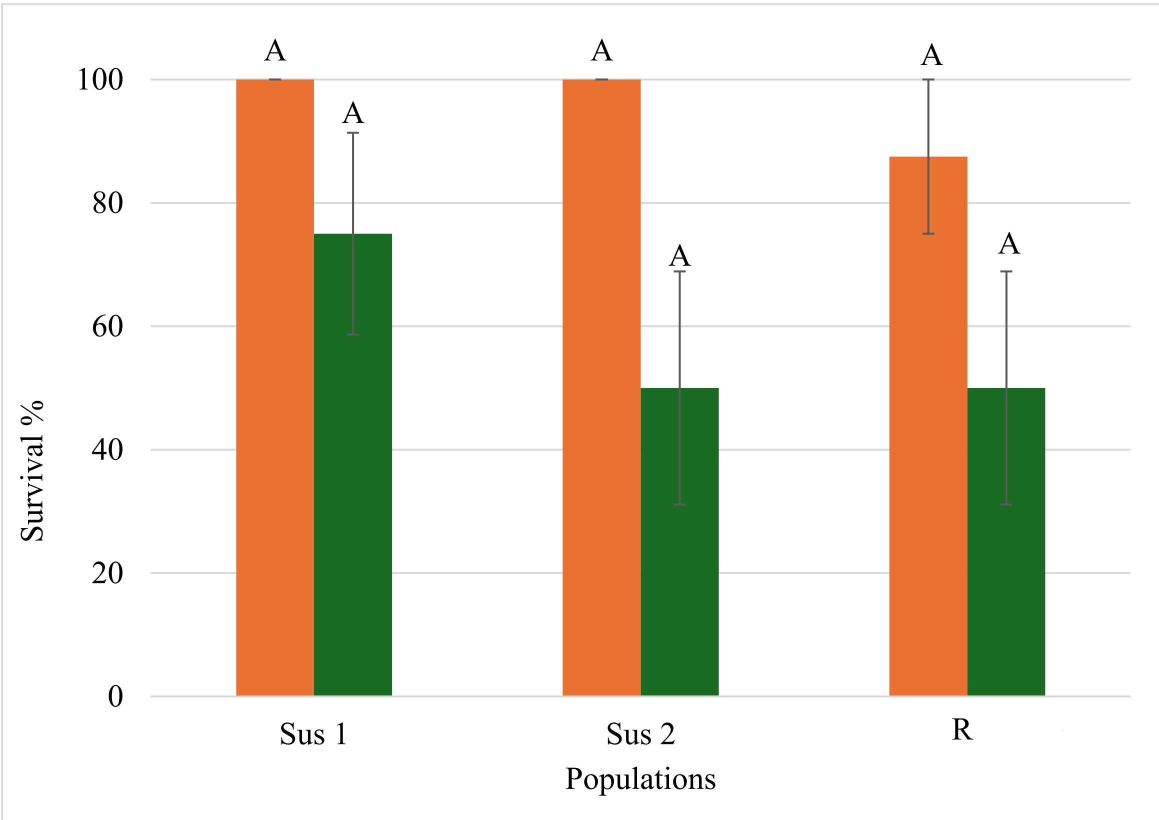

Figure 2.6 Plant survival of Sus 1, Sus 2, and the R population from Duplin County, *Amaranthus palmeri* treated with glyphosate. Legend: orange bar: 2691.64 g ae ha^{-1} of glyphosate; green bar: 5383.83 g ae ha^{-1} of glyphosate. Error bars indicate the standard error of the mean.

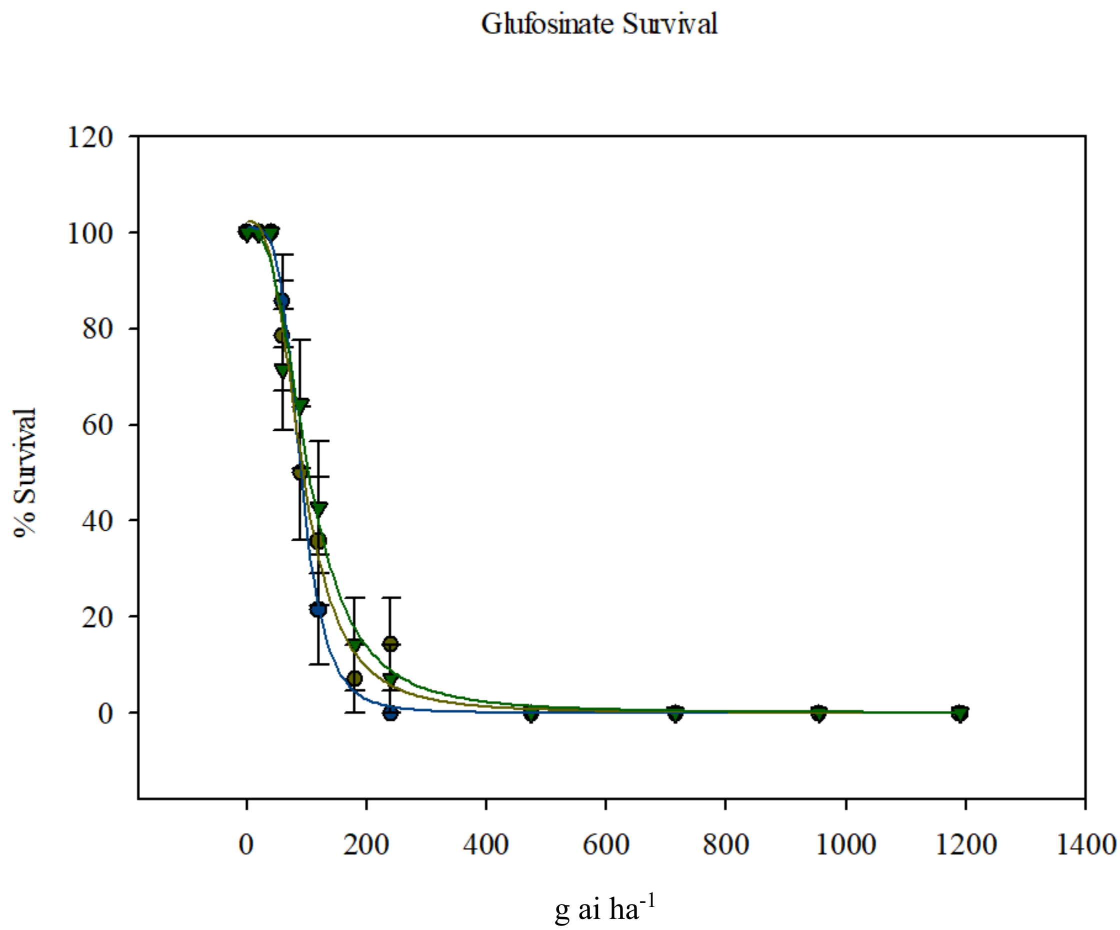

Figure 2.7 Dose-response curve fit to a three-parameter logistic regression model for plant survival of the *Amaranthus palmeri* populations (resistant: R; susceptible: Sus 1 and Sus 2) treated with glufosinate, pooled across experimental runs. Legend: R: green triangle; Sus 1: blue circle; Sus 2: dark olive circle.

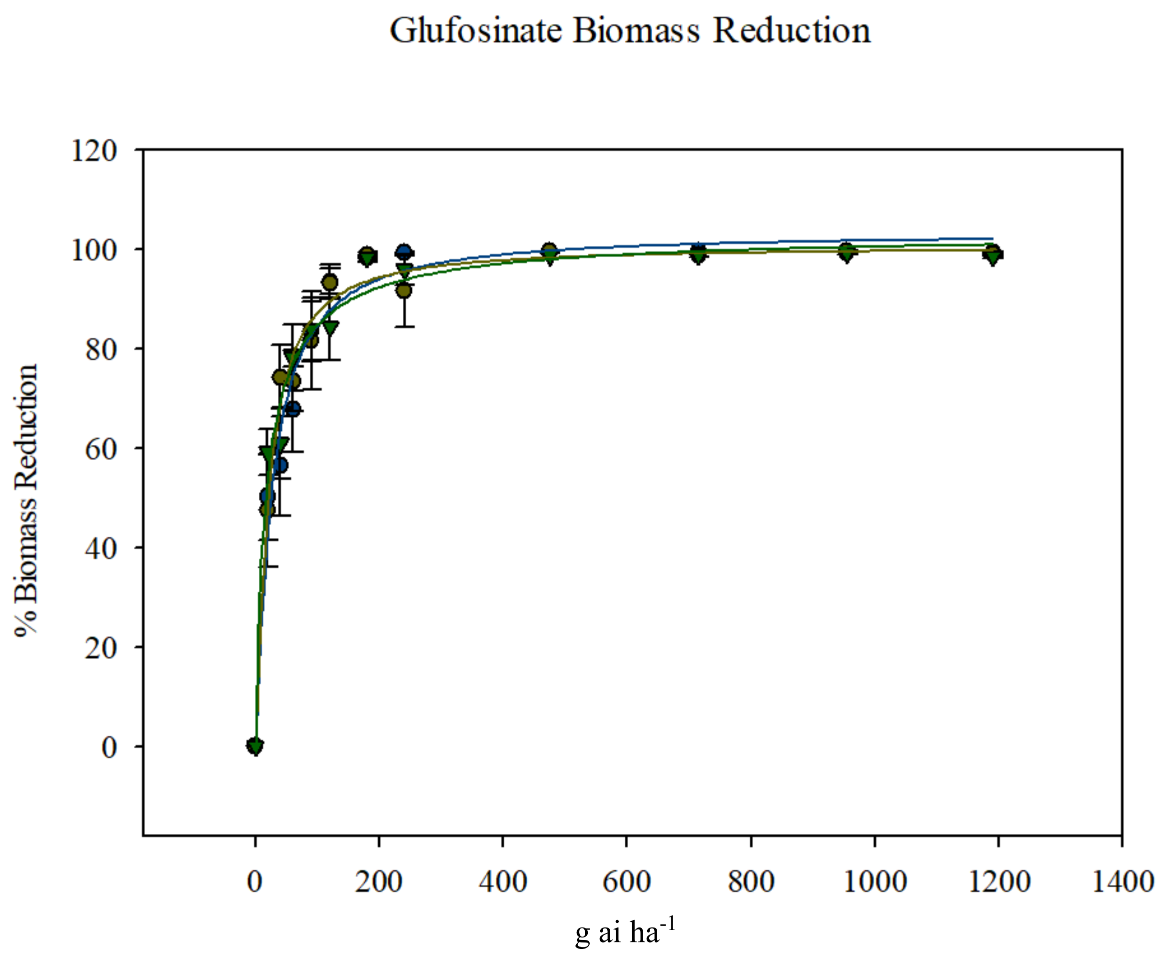

Figure 2.8 Dose-response curve fit to a three-parameter logistic regression model for biomass reduction of the *Amaranthus palmeri* populations (resistant: R; susceptible: Sus 1 and Sus 2) treated with glufosinate, pooled across experimental runs. Legend: R: green triangle; Sus 1: blue circle; Sus 2: dark olive circle.

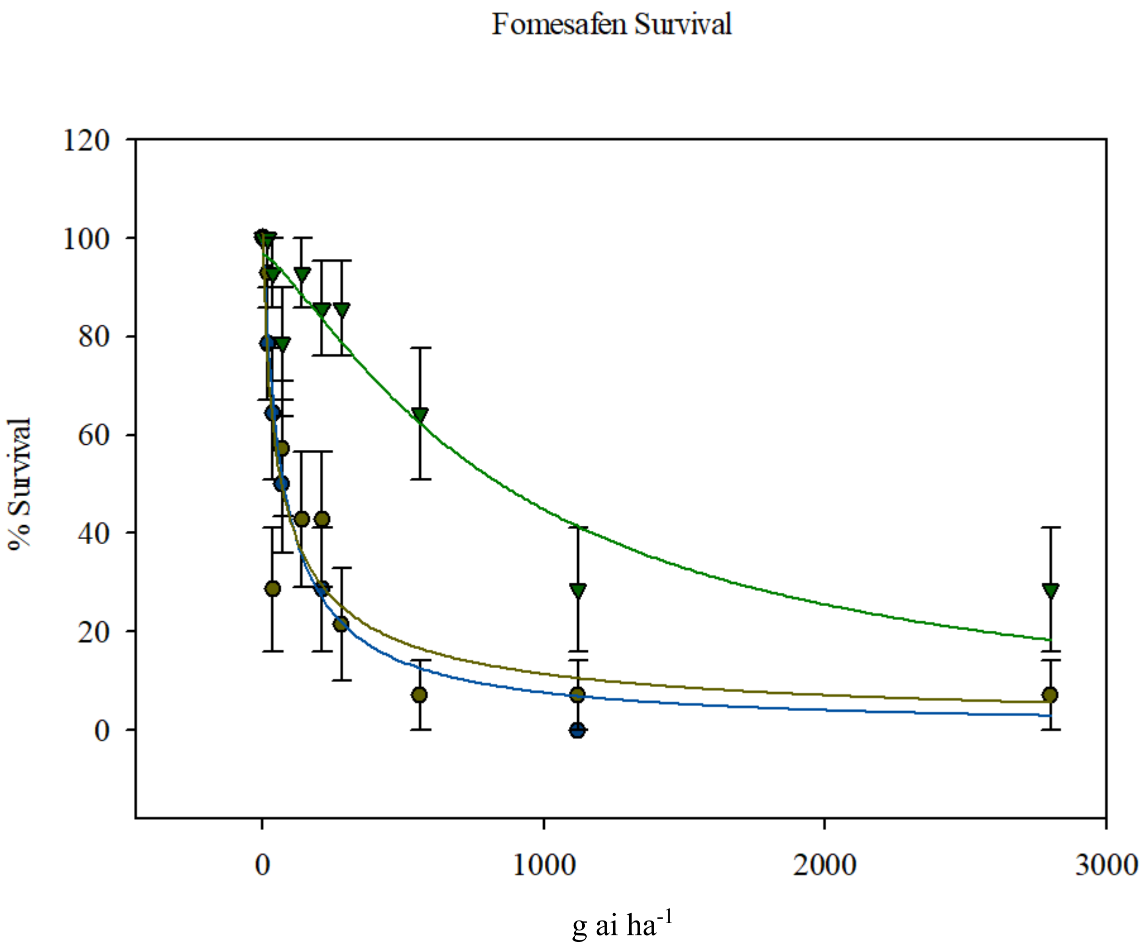

Figure 2.9 Dose-response curve fit to a three-parameter logistic regression model for plant survival of the *Amaranthus palmeri* populations (resistant: R; susceptible: Sus 1 and Sus 2) treated with fomesafen, pooled across experimental runs. Legend: R: green triangle; Sus 1: blue circle; Sus 2: dark olive circle.

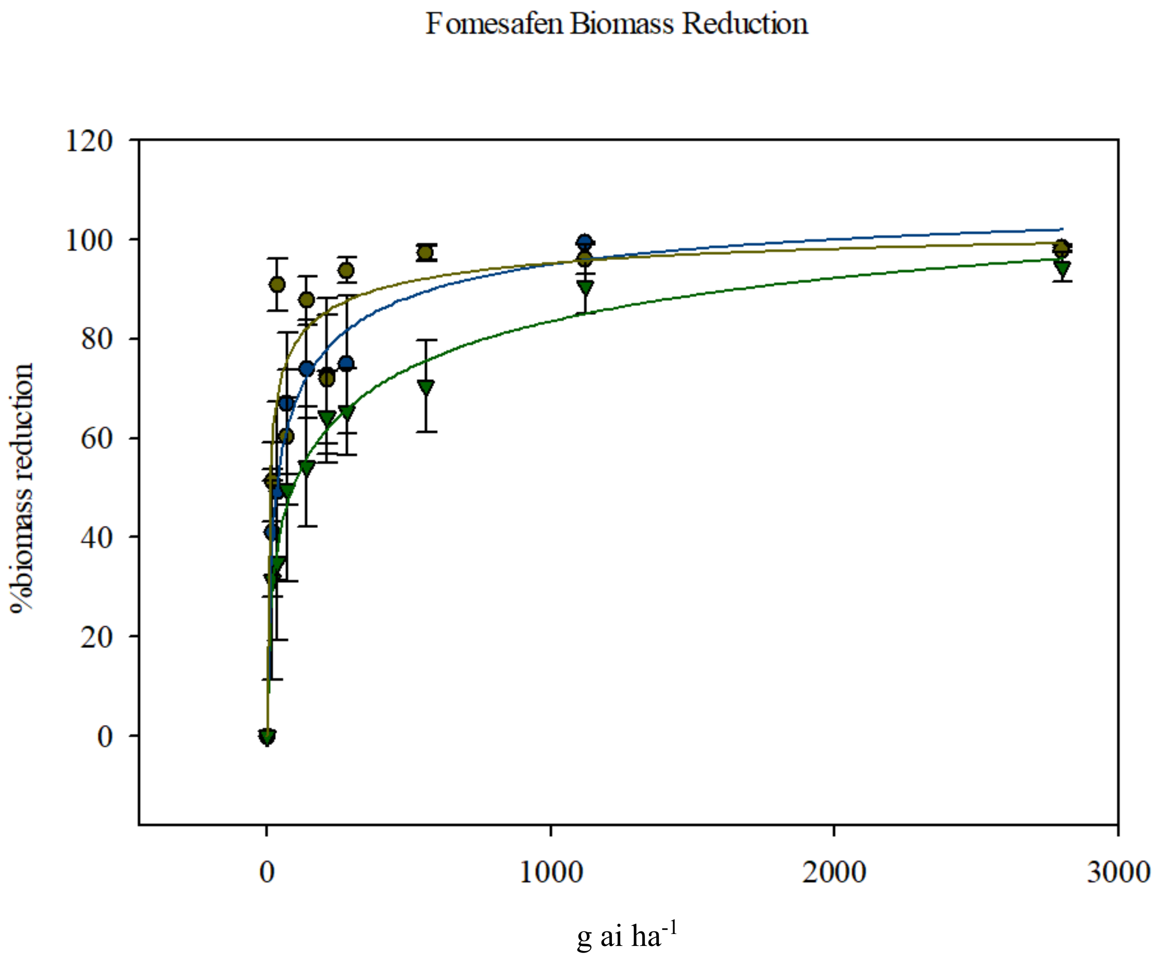

Figure 2.10 Dose-response curve fit to a three-parameter logistic regression model for biomass reduction of the *Amaranthus palmeri* populations (resistant: R; susceptible: Sus 1 and Sus 2) treated with fomesafen, pooled across experimental runs. Legend: R: green triangle; Sus 1: blue circle; Sus 2: dark olive circle.

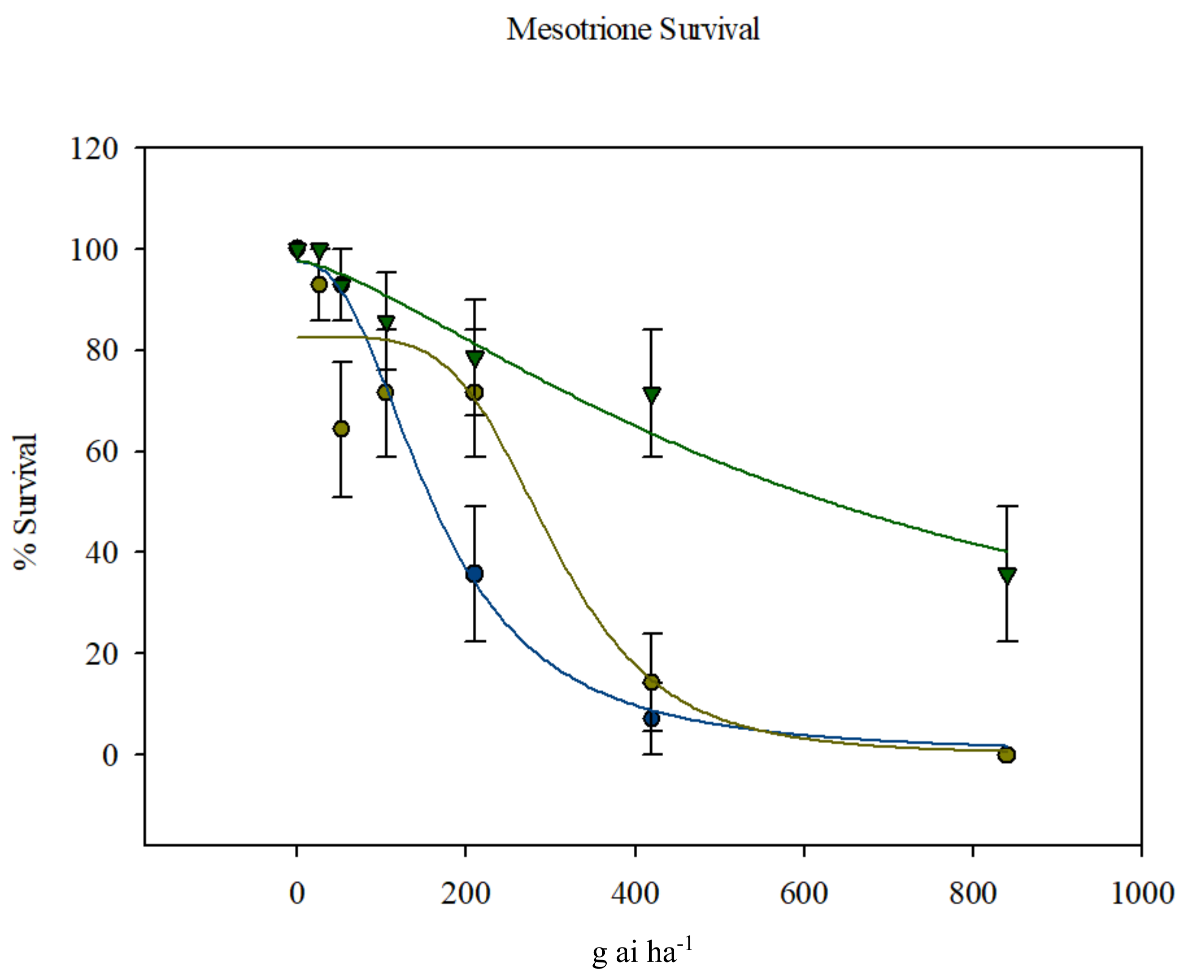

Figure 2.11 Dose-response curve fit to a three-parameter logistic regression model for plant survival of the *Amaranthus palmeri* populations (resistant: R; susceptible: Sus 1 and Sus 2) treated with mesotrione, pooled across experimental runs. Legend: R: green triangle; Sus 1: blue circle; Sus 2: dark olive circle.

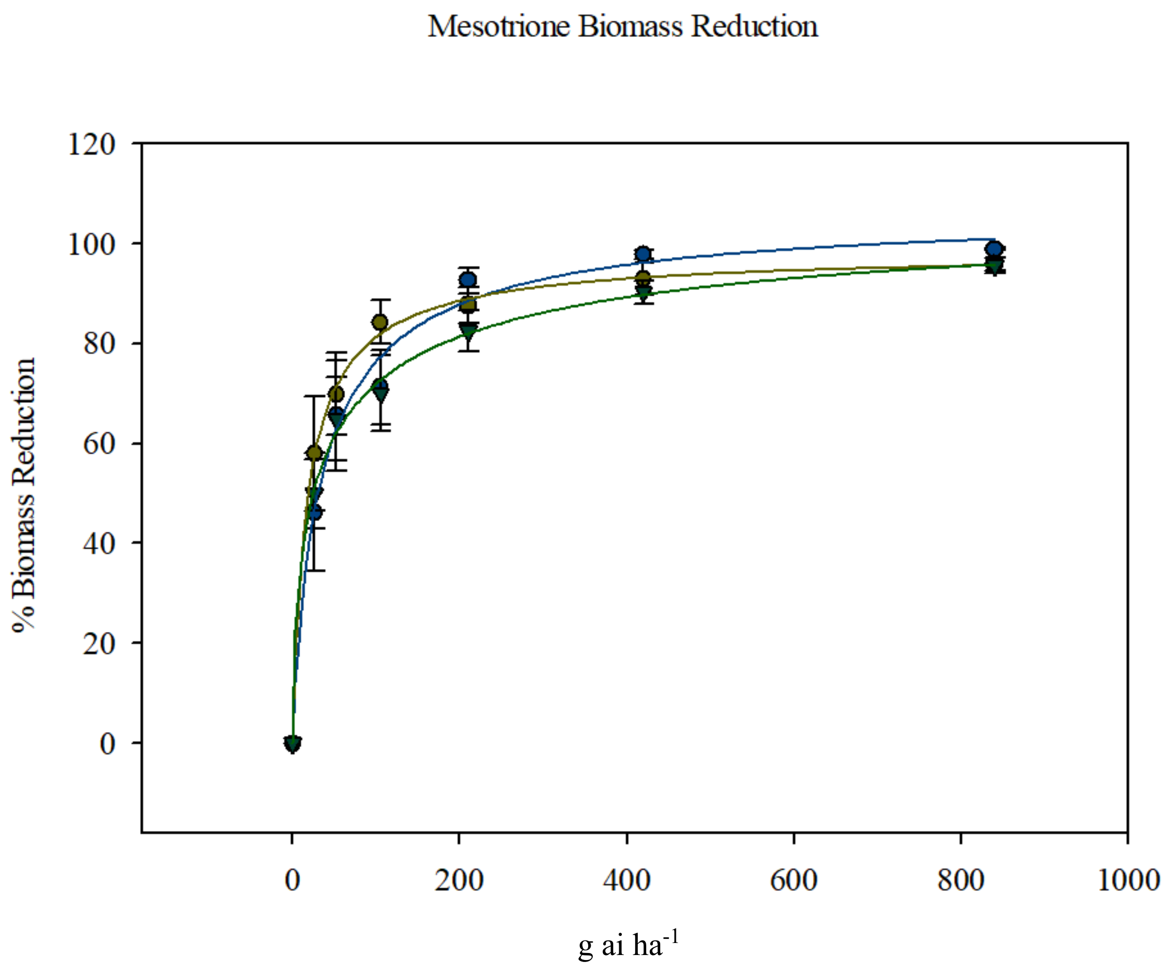

Figure 2.12 Dose-response curve fit to a three-parameter logistic regression model for biomass reduction of the *Amaranthus palmeri* populations (resistant: R; susceptible: Sus 1 and Sus 2) treated with mesotrione, pooled across experimental runs. Legend: R: green triangle; Sus 1: blue circle; Sus 2: dark olive circle.